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Trends in Biotechnology

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Review

Going Green and Cold: Biosurfactants from Low-Temperature Environments to Biotechnology Applications

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Approximately 80% of the Earth's biosphere is cold, at an average temperature of 5°C, and is populated by a diversity of microorganisms that are a precious source of molecules with high biotechnological potential. Biosurfactants from cold-adapted organisms can interact with multiple physical phases – water, ice, hydrophobic compounds, and gases – at low and freezing temperatures and be used in sustainable (green) and low-energy-impact (cold) products and processes. We review the biodiversity of microbial biosurfactants produced in cold habitats and provide a perspective on the most promising future applications in environmental and industrial technologies. Finally, we encourage exploring the cryosphere for novel types of biosurfactants via both culture screening and functional metagenomics.

Cold-Active Microorganisms for Sustainable and Energy-Saving Biotechnologies

The search for environmentally friendly biomolecules is a recent common theme in biotechnology. Biosurfactants have many desirable features: they are produced by microorganisms from renewable materials; exist in numerous chemical varieties, hence they can perform in a large range of applications; are active at very low concentrations; and are compatible with release into the environment [1,2]. Biosurfactants are considered a possible green alternative to chemical surfactants for countless commercial products, including detergents and cleaners, personal care products, cosmetics, pharmaceuticals and therapeutics, food additives, emulsifiers, and dispersants for bioremediation [3].

Another emerging requirement for biotechnological products is to be energy saving. Energy efficiency implies not only saving energy and money but also reducing the environmental impact. New strategies are being implemented at all stages of the energy chain from production and storage to consumption [4]. With the policies in this area becoming more stringent, the energy factor will be particularly important to strengthen the biotechnology market. In this context, biomolecules that both function at low temperatures and also can be produced without the need for heating hold great promise. Low-temperature biosurfactants meet both of these criteria.

Low-temperature microbiology has recently come into the scientific spotlight. The coldest regions of Earth are more easily accessible for scientific expeditions and studies are being fueled not only by pure scientific interest about life under extreme conditions but also by growing concerns about their role in the global climate change dynamics [5–7]. Cold habitats are also seen as extraordinary reservoirs of biotechnological molecules such as cold-active

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'Cold' is becoming 'cool' in microbiology. The cryosphere is becoming more easily accessible to scientific expeditions and is predicted to fuel the discovery of novel natural products.

Research on biosurfactants from psychrophilic microorganisms is just beginning but is expected to contribute to the development of sustainable (green) and energy-saving (cold) biotechnologies.

New formulations of washing products containing biosurfactants are being developed, which can enable effective detergency at lower temperatures and will help laundry practices to reduce the environmental impact.

Advances in unconventional experimental approaches such as functional metagenomics hold great promise for future discovery of entirely new biosurfactant types and producing organisms from cold and extreme environments.

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enzymes, antibiotics, and biosurfactants [8,9]. In comparison to cold-active enzymes, which represent a mainstream research field, with some already being commercialized, little information is available on biosurfactants from cold-adapted microorganisms, namely, psychrophiles. Here we first describe the different types of biosurfactants and their producing organisms isolated from cryo-habitats and discuss their ecological functions. We then present some possible applications for low-temperature-related biotechnologies such as bioremediation, gas hydrate production, antifreeze additives, and detergents. We finally address the aspect of the bioprocessing of these bioproducts and propose future strategies to increase their industrial competitiveness.

Biodiversity and Ecological Roles of Biosurfactants in Cold Soils

Cold soils such as those in the polar regions or at high altitudes are exposed to environmental extremes that include low temperature, freeze–thaw cycles, strong UV irradiation, and limited availability of liquid water and nutrients. Not surprisingly they are characterized by a generally low **biodiversity** (see Glossary) and are inhabited by organisms that have developed specific survival mechanisms (Box 1) [10]. The ability to produce biosurfactants seems a common feature among cold-adapted organisms (Table 1), with up to 50% of the members of microbial communities in polar soils and sediments screening positive [11,12]. Similar to temperate environments, certain phylotypes of biosurfactant producers, for example, *Pseudomonas*, *Burkholderia*, *Sphingomonas*, *Rhodococcus,* and *Bacillus*, also prevail in cold habitats [12–15]. Most of the isolates are able to grow at temperatures as low as 4°C but under laboratory conditions at around 20°C and above (Box 1). Less conventional organisms have been also described, for example, *Pantoea* sp. (Enterobacteriaceae family) isolated from **ornithogenic soil** in the Frazier Islands, Antarctica, that produce glycolipids over a temperature range of 5–40°C [16] and *Enterobacter* and *Luteibacter* (phylum Proteobacteria), *Pedobacter*, *Mucilaginibacter,* and *Dyadobacter* (phylum Bacteroidetes), all of which were found associated with the

Box 1. Definition of Psychrophile and Adaptation to the Cold

The concept of 'psychrophile', and therefore its definition has been always debated among microbiologists; furthermore, the misuse of terms such as 'obligate or facultative psychrophilic', 'psychrotolerant', 'psychrotrophic', 'euri- or steno-psychrophilic' has contributed over time to a substantial confusion. A first attempt to categorize cold-adapted microorganisms was made by Morita [59] who defined 'psychrophiles' as those organisms with cardinal growth temperatures (minimum, optimum, and maximum): $T_{min} < 0°C$, $T_{opt} \leq 15°C$, and $T_{max} \leq 20°C$; and 'psychrotolerant' as those characterized by higher optimum and maximum. While this definition is still in use today, Cavicchioli [60] recently raised the question of correctness of using growth rate (μ) measurements of strains under laboratory conditions as a means to assess their level of adaptation to the cold and low-temperature habitats. The concept derived mainly from laboratory practice that correlates the fastest growth (μ_{max}) with the most ideal temperature (T_{opt}) has some flaws when applied to microorganisms from cold environments. In general, they are functional in their ecosystem and highly competitive in nature at temperatures that would hardly generate growth in a flask, and there are many examples of isolates that are cultivated at temperatures well exceeding the on-site conditions [61]. In addition, studies have shown that laboratory-determined T_{opt} can be stressful for the organisms, with the heat stress cellular response being activated under the supposed optimal conditions [62]. Based on these considerations, Cavicchioli [60] suggested that the singular term 'psychrophile' is both appropriate and sufficient to describe microorganisms indigenous of cold habitats, and we will adopt this definition throughout this article.

Modern **omics** technologies have significantly advanced the current understanding of microbial cold adaptation strategies and mechanisms. The analysis of psychrophilic genomes and metagenomes revealed a generally high level of redundancy and **genome plasticity**, both often linked to cold-adaptive traits. At the molecular level, proteins and enzymes have high structural flexibility, low thermostability, and high substrate specificity, which are achieved with a preferential use of amino acids. The cellular membrane is maintained functional by changes in lipid and fatty acid composition that confer higher fluidity, and is typically encased in a thick cell envelope rich in exopolysaccharides that prevent freezing of the water in the immediate surroundings. The cytoplasm of psychrophiles accumulates numerous antifreeze proteins, compatible solutes (e.g., glycine and betaine), and cryoprotectants such as trehalose that both prevent intracellular freezing and stabilize proteins. Cold adaptation has been extensively reviewed, for example [63].

Glossary

Biodiversity: variety of living organisms in a certain environment on Earth, typically measured based on the variation at the genetic, species, and ecosystem levels.
Biofilm: highly organized aggregate, often formed attached to surfaces, of microorganisms embedded in a self-produced matrix of exopolysaccharides and biosurfactants.
Bioprospecting: systematic search for new biomolecules and organisms in nature with potential for biotechnological applications.
Catalysis: the process of increasing the rate of a chemical reaction by the presence of a substance (called a catalyst) that is not consumed or permanently altered during the process.
Cloud point: the temperature at which a clear fuel turns turbid as a result of crystallization and agglomeration.
Critical micelle concentration: the concentration of a surfactant above which monomers begin to assemble into aggregates called micelles.
Cryosphere: areas of Earth's biosphere where water is in the frozen state. It comprises snow cover, glaciers, ice sheets, sea ice, iceberg, permafrost, and ground ice.
Genome plasticity: feature of microbial genomes to undergo rearrangements that include acquisition of new genetic material from the environment or other microorganisms, deletion of parts of the genome, and mutations.
Hydrophilic: polar molecules that can interact with water molecules via ionic or hydrogen bonds.
Hydrophobic: non-polar molecules that do not interact with polar solvents and do not dissolve in water.
Ice-packing factor: the ratio of mass of created ice to the initial mass of water in a vessel.
Krafft temperature: the minimum temperature at which surfactants form micelles. Below the Krafft temperature, there is no value for the critical micelle concentration.
Omics: suite of technologies that focus on the study of specific molecules, for example, DNA (genomics), RNA (transcriptomics), proteins (proteomics), and

Table 1. Examples of Biosurfactants Produced by Microorganisms from Low-Temperature Environments and Main Production Conditions

Biosurfactants	Producing organisms/habitat	T (°C)	Substrate/product yields	Refs
Rhamnolipids	*Pseudomonas putida* Soil, Svalbard Islands	28	Glucose 0.15 g/L	[15]
Rhamnolipids	*Pseudomonas* sp. Marine sediment, Ross Sea	21	Tryptone + yeast extract n.d.	[32]
Glycolipids	*Pseudoalteromonas* sp. Marine sediment and water South Shetlands Islands	4–15	Tetradecane n.d.	[38]
Glycolipids	*Pantoea* sp. Soil, Frazier Islands	25	Paraffin, kerosene 0.8–1.2 g/L	[16]
Glycolipids	*Rhodococcus fascians* Soil, Wilkes Land	18–28	Kerosene, glucose n.d.	[13]
Glycolipids	*Rhodococcus* sp. Marine sediment and water South Shetlands Islands	4–15	Tetradecane, sunflower n.d.	[38]
Glycolipids	*Nocardia* sp. Soil, Antarctica	4–20	Paraffin, naphthalene n.d.	[18]
Glucotriose lipids	*Rhodococcus* sp. Deep-sea sediment, Okinawa Trough	20	Glucose + olive oil 2.51 g/L	[31]
Glucose lipids	*Alcanivorax borkumensis* Marine sediment, North Sea	4–35	Hydrocarbons n.d.	[26,27]
Mannosylerythritol lipids	*Moesziomyces antarcticus* Lake Vanda, Antarctica	20–30	Vegetable oils 100 g/L	[20,21]
Fatty acids	*Cobetia* sp. Seawater, Montemar, Chile	30	Dibenzothiophene n.d.	[29]
Lipopeptides	*Bacillus licheniformis* Sand, South Shetlands Islands	30	Glucose 0.15–0.20 g/L	[14]
Unidentified	*Bacillus* sp., *Paenibacillus* sp., *Sporosarcina* sp. Soil, King George Island	4–32	Tryptic soy broth n.d.	[12]

lichen *Peltigera membranacea* in an Icelandic soil and screened positive for biosurfactant production [17].

In many cases the synthesis of biosurfactants was found to be associated with the production of extracellular enzymes such as lipases, proteases, amylases, and in some organisms also with the ability to grow and degrade hydrocarbon contaminants including polycyclic aromatics [17,18]. Since polar environments are typically pristine, we speculate that the ecological role of biosurfactants is to support the ability of microbial communities to metabolize plant-derived material (e.g., lignin, cellulose, hemicellulose, tannins, phenols), which are naturally enriched in organic hydrocarbon and aromatic compounds. Biosurfactants are likely to act in synergy with extracellular enzymes by increasing solubilization and mobilization, and hence the bioavailability of the hydrophobic residues originating from the hydrolysis of complex biopolymers [19]. Thus, it seems plausible that biosurfactants play a primary ecological role in the turnover of carbon and nutrients in extreme cold soils.

A Model Organism for Industrial Biosurfactant Production from an Antarctic Lake

One of the current most-valued biosurfactant-producing microorganisms, *Moesziomyces antarcticus* (formerly known as *Pseudozyma*/*Candida antarctica*), was originally isolated from

metabolites (metabolomics) in single microorganisms. Meta-omic approaches are instead used to study complex microbial ecosystems as a whole.

Ornithogenic soil: soil formed in areas of penguin rookeries and particularly rich in carbon and nitrogen due to the large amounts of penguin guano.

Osmotic stress: physiological cell impairment caused by a change in the solute concentration (e.g., high salt concentration) around the cell but also by freezing as well as freeze–thaw cycles.

Permafrost: ground (soil, rock, or sediment) that remains at or below 0°C for at least two consecutive years. Permafrost covers large areas mostly in the Northern Hemisphere but can be found also in Antarctica and at lower latitudes in alpine regions (e.g., Tibetan Plateau).

Sequestrants: chemical additives contained in cleaning products that form complexes with ions (e.g., calcium) in solutions, thus preventing the precipitation of detergents and loss of effectiveness.

a polar habitat, Lake Vanda in the Wright Valley, Antarctica [20]. *M. antarcticus* is a yeast known for the production of industrially relevant molecules including extracellular enzymes (e.g., lipase) and glycolipid biosurfactants of the type mannosylerythritol lipids (MELs) [21]. MEL molecules consist of long-chain fatty acids linked to a mannopyranosyl-meso-erythritol **hydrophilic** head group, and are synthesized in various congeners, mostly diacetylated (MEL-A) and monoacetylated (MEL-B and MEL-C) and several other minor variants (e.g., triacetylated, diastereomers, with mannitol, arabitol, or ribitol replacing the erythritol group). Small differences in the chemical structure can greatly affect the molecular self-assembly in solution and consequently the interfacial properties, with MEL-A forming a sponge phase, and MEL-B and MEL-C forming giant vesicles (Figure 1). It follows that MELs exhibit a broad range of biochemical and biological properties, and thus have high versatility in terms of applications and uses. In addition, they can be produced at high yields (up to 100 g/L) from vegetable oils by intermittent feeding, which

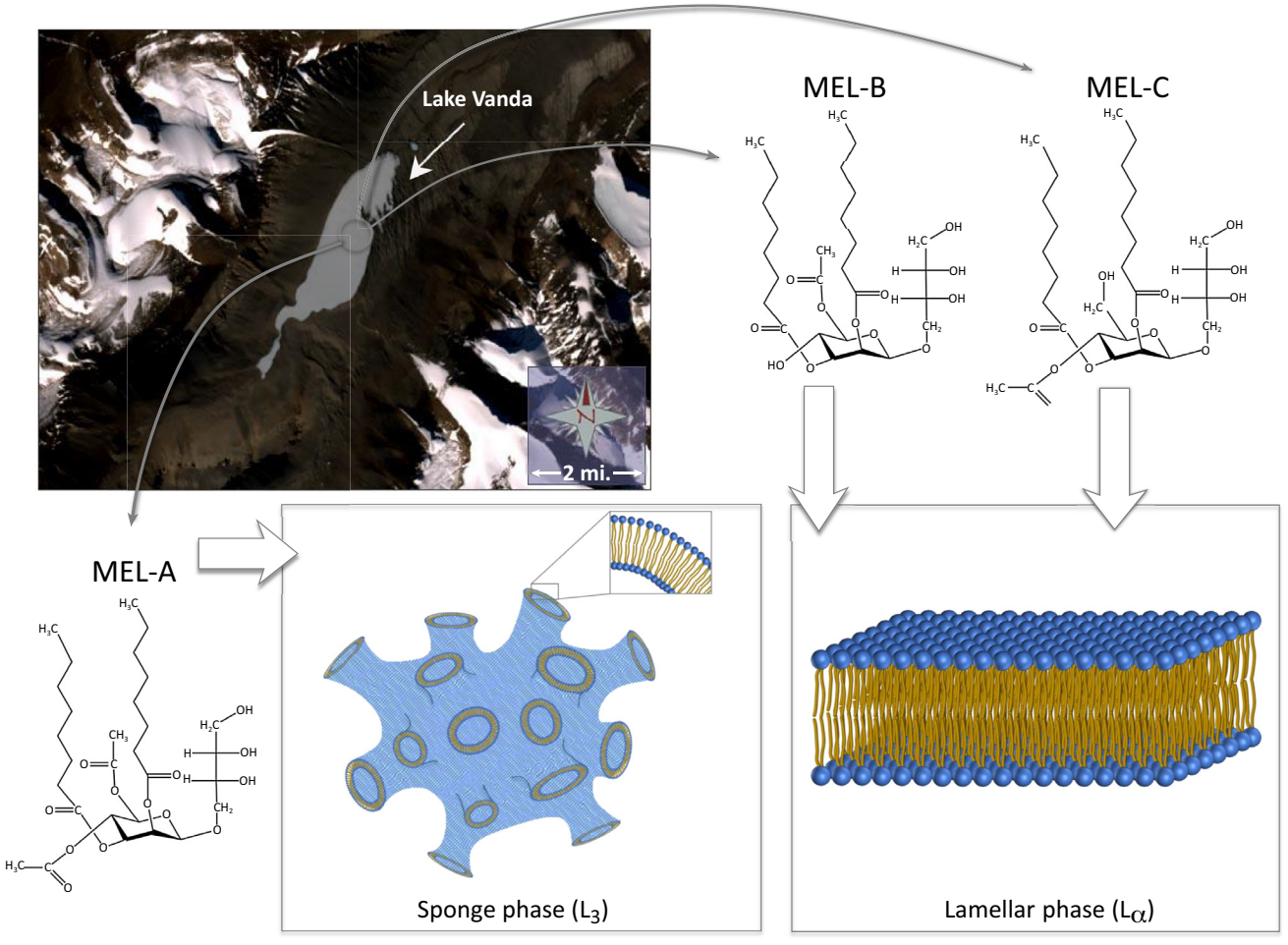

Figure 1. Lake Vanda (Wright Valley, Antarctica), the Natural Habitat of the Mannosylerythritol lipid (MEL)-Producing Yeast *Moesziomyces antarcticus*. MEL biosurfactants are produced in molecular variants, such as diacetylated (MEL-A) and monoacetylated (MEL-B and MEL-C), that differ greatly for assembly patterns (sponge and lamellar shape, respectively) and physicochemical properties in solution. MELs have high potential for biotechnology applications including at freezing temperatures as ice antiagglomerants and freezing point depressants. (Lake Vanda image was acquired by Landsat 7, credit: NASA GSFC Landsat/LDCM EPO Team.)

makes them particularly attractive for industry [21]. Despite being synthesized under moderate conditions, that is, at around 25°C or room temperature, MELs display remarkable performance also at subzero temperatures, for example, antiagglomeration of ice crystals [22] and freezing point depression [23], which will be illustrated in the 'Biosurfactant Activity and Applications at the Ice–Water Interface' section.

Considering the large distribution and biodiversity of yeasts in the **cryosphere** [24], it is reasonable to think that many more biosurfactant producers can be discovered (Box 2). For example, *Rhodotorula* is highly represented in polar and glacial environments (e.g., *R. arctica*, *R. glacialis*, *R. psychrophenolica*, *R. psychrophila*, *R. mucilaginosa*) and this genus contains several species known to be hyper-producers of sophorolipid biosurfactants. Sophorolipids have already reached an advanced stage of large-scale production and commercialization, but it would be certainly valuable to expand the collection of producing organisms with new extremophilic strains. The **bioprospecting** of cold-adapted yeasts is currently lacking research directed at biosurfactants and we believe that more attention should be given to explore the biosynthetic potential of psychrophilic eukaryotes.

Marine Biosurfactants and Bioremediation

The marine environment is extremely diverse in the same way that the terrestrial environment is, and this diversity provides a number of different potential habitats for microorganisms. The average surface temperature of the ocean is 17°C (although this is obviously lower toward the poles), while in the oceanic deep waters it is 0–3°C with a salinity of about 3.5%, which means that the freezing point is −1.9°C. One major feature of the water of the oceans is that the level of

Box 2. New Psychrophiles and Omics to Discover Novel Biosurfactants

Current knowledge and application is limited to a handful of biosurfactants and their producing organisms, while a wide diversity and large number remain undiscovered in nature. Going on an expedition to the poles is not necessary as a variety of tools, from pure culture isolates to genomic and metagenomic sequences related to low-temperature organisms, are publicly available for studies that aim at discovering new biosurfactants.

The Polar and Alpine Microbiology Collection (PAMC) is, for example, entirely dedicated to microorganisms isolated from the Arctic, Antarctic, and alpine regions. Currently, it contains approximately 6500 strains, some of which have been assessed also for the production of cold-active extracellular enzymes (e.g., lipases and proteases) and exopolysaccharides [64]. Numerous psychrophilic organisms deposited in culture collections belong to well-known biosurfactants-producing groups, for example, *Pseudomonas*, *Burkholderia*, *Bacillus*, *Rhodococcus*, *Flavobacterium,* and *Candida*. Starting with enrichments and screening for biosurfactants would be a relatively simple first step to find new producers.

Genome sequences are also a valuable support as they can reveal orthologous genes for biosurfactants across different organisms [65,66]. This approach is ideal to capture the microdiversity of biosurfactant genotypes within phylogenetically related groups of microorganisms, which may arise from the adaptation to different environmental niches, particularly extreme habitats. We expect this approach to become more dominant in the future boosted by the increasing number of microbial genome sequences deposited in gene banks. Bioinformatics tools for genome mining with respect to biosurfactants are available to identify non-ribosomal peptide synthetase gene clusters encoding lipopeptides (e.g., anti-SMASH) [67] but it is much more challenging to target yet-unknown genes (e.g., glycosyltransferases) involved in the synthesis of glycolipid biosurfactants.

Advances in knowledge of biosurfactants at the genome level will also significantly improve our ability to interpret metagenomic data. Functional metagenomics can lead to the discovery of unknown biosurfactants and is especially suited for extreme habitats where many native microorganisms may not be cultivable [68]. Although there are several technical pitfalls related to the expression in heterologous systems, the lack of reliable high-throughput screening assays, and finally the extensive analytical work required to characterize the chemical structure of new biomolecules, this approach has succeeded in identifying entirely new types of biosurfactants [69,70].

organic material is very low. Therefore, we might expect most heterotrophic microorganisms to be recoverable from sources of concentrated organic material, such as particulate detritus or immiscible organic liquids [25].

The most obvious place to commence a search for marine biosurfactant-producing microorganisms is in areas that are polluted with hydrocarbons from spills or leakages since surfactants are believed to form an important part of the system for the microbial biodegradation of hydrocarbons. *Alcanivorax borkumensis* is among the first and best-studied microorganisms of this kind. The type strain SK-2, isolated from North Sea sediment, can grow exclusively on hydrocarbons in a temperature range of 4–35°C while producing a well-characterized glucose–lipid biosurfactant [26,27]. Since then various other marine bacteria, typically *Pseudomonas*, *Pseudoalteromonas*, *Marinomonas*, *Halomonas*, *Rhodococcus,* and *Cobetia* have been described as biosurfactant producers from the polar oceans, deep sea, and marine sediments [28–32]. Kurata and colleagues [33] developed a novel approach, based on satellite remote sensing, to identify slicks of biosurfactants through their effect of surface tension reduction on the appearance of the sea surface, and demonstrated the involvement of microbial biosurfactants in the uptake of **hydrophobic** substances in the marine environment. They also confirmed that known microbial biosurfactant producers were present in the subsurface layers below these slicks.

Considerable emphasis has been placed in recent years on exploiting marine organisms to solve problems of bioremediation of pollutants [34], which has been tackled by large and multidisciplinary research projects (e.g., EU-FP7 projects 'Unravelling and exploiting Mediterranean Sea microbial diversity and ecology for xenobiotics and pollutants clean up' ULIXES and 'Integrated biotechnological solutions for combating marine oil spills' KILL-SPILL). In these projects, biosurfactants played a major part and various ways of using biosurfactants in bioremediation were explored and new surfactants were sought from marine producers [35]. One of the main routes explored in the KILL-SPILL project was the use of various formulations using oxygen-generating systems linked to nutrient supplementation and biosurfactant incorporation to stimulate the rate of hydrocarbon degradation in contaminated marine sediments.

Biosurfactant Activity and Applications at the Ice–Water Interface

In nature, cryo-environments where ice and water phases seasonally cycle and occasionally coexist are highly challenging. These environments pose serious threats to microbial life due to not only the physical disruption of cellular structures and proteins by ice crystals but also a reduced mass transfer of liquid water and nutrients [36]. Microorganisms living in such extreme settings have evolved, among other adaptations (Box 1), highly specialized cell envelopes of which biosurfactants are usually an integral part and which participate, together with other components such as exopolysaccharides, in protection against high salinity, temperature, and **osmotic stress** [37].

Trehalose lipids are the most characteristic biosurfactants in microorganisms, especially of the genus *Rhodococcus*, adapted to icy environments [38]. Compared to other sugars, trehalose has an extraordinary ability to protect biomolecules and living cells subject to freezing. Trehalose displays a dual cryoprotective action; first, it prevents water from crystallizing into ice by taking apart the tetrahedral hydrogen bond network of water molecules, and second, it forms a cage surrounding the proteins that slows the water dynamics in its proximity [39]. While it is evident that nature has tailored these biosurfactants to work under extremes of temperature and phase transition, their biotechnological exploitation remains unfortunately low. To a large

extent this is because trehalose lipids are cell bound, so they require laborious extraction and purification processes and are generally recovered only in small amounts.

Other biosurfactants have also shown good performance in ice–water systems. One example is MELs, which have been demonstrated as antiagglomerants for ice–water slurry technologies [22]. Ice slurry is a homogeneous mixture of small ice particles and water used in cold-storage systems, for example, ice storage, industrial process cooling, refrigeration, and air conditioning. Because of their high thermal energy storage density, ice slurries have considerable environmental and economic advantages, but long-term ice particle formation and growth are difficult to control. MEL-A in particular, the diacetylated form of MELs, showed a remarkable antiagglomeration activity, achieving a high **ice-packing factor** comparable to standard chemical surfactants and at a much lower concentration. It was also effective when tested further on a large-scale 300-L ice storage tank. The antiagglomeration effect of MEL-A can be explained by the ability of this biosurfactant, characterized by asymmetric molecular geometry due to the presence of two different hydrophobic alkyl chains, to adsorb to the ice surface in a highly regulated manner that contrasts with the agglomeration and growth of ice particles [22].

Biosurfactants for Gas Hydrate Technologies

Enhancing the **catalysis** and recovery of natural gas hydrates using biosurfactants is one of the newest frontiers of microbial biotechnology for energy and resources. Gas hydrates are cagelike lattices of ice inside which molecules of gas, typically methane, are trapped (Figure 2). The gas can originate either from the biological activity of microorganisms (e.g., methane produced by methanogens) or from the decomposition of fossil organic matter [40]. Gas

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Figure 2. Block of Gas Hydrate. This sample was collected from a 1200-m deep sea sediment along the subduction zone off Oregon by the research vessel RV Sonne. Overlaid is the structural representation of the ice cage that surrounds and contains molecules of gas such as methane. (Photograph by Wusel007, distributed under CC BY-SA 3.0.)

hydrates are found in nature beneath the seafloor along ocean margins and in **permafrost** polar regions, with large accumulations identified in the Gulf of Mexico, North Alaska, the Arctic region of Svalbard and the Barents Sea, and offshore Japan, India, South Korea, and China. Current estimates indicate that natural gas in hydrate deposits may account for up to 120×10^{15} m^3, thus representing a huge potential energy resource. Exploration programs as well as technologies for their recovery and exploitation are being developed in many countries worldwide [41].

Several studies have shown that rhamnolipids and lipopeptides, as well as other biosurfactants, can catalyze the formation of gas hydrates [42–44]. Biosurfactants can both accelerate the induction and initiation of the hydrates (when hydrate nuclei and then a critical crystal cluster begin to form) and increase the formation rate after crystal initiation. When biosurfactant micelles begin to form above the **critical micelle concentration**, hydrocarbon gas molecules that interact with the hydrophobic tails remain enclosed in the inner core. In addition, typically for catalysts, biosurfactants can function over time without being consumed [43]. Culture-independent studies on the microbial communities associated with gas hydrates and marine sediments have demonstrated the presence of *Pseudomonas* and *Bacillus* [45,46] and some were also isolated [47]. A strain of *Bacillus subtilis*, cultivated anaerobically at 20°C to simulate the *in situ* environmental conditions, could produce surfactin with a strong catalytic effect on gas hydrate formation [43].

Despite the growing scientific evidence, our understanding of the possible synthesis of biosurfactants at gas hydrate sites is still preliminary. The difficulty of accessing natural samples and to reproduce faithfully in the laboratory *in situ* environmental conditions together with the lack of cultivation-independent techniques to detect biosurfactant producers have so far significantly slowed down this emerging new field of research.

Enhanced Cold Flow Properties of Biodiesel

Biodiesel is a renewable green fuel that can be manufactured from low-cost and waste materials (e.g., vegetable oils, animal fats and recycled cooking oil, and grease) and, compared to petroleum fuels, has similar physical properties but is cleaner burning. Biodiesel utilization is, however, hampered by its poor cold thermal fluidity, which results in molecular aggregation and solid gel formation at low temperatures. Biosurfactants of the MEL type have been successfully tested as fuel additives to improve the flow properties at freezing temperatures [23]. Biodiesel phase transition to gel typically begins at around 1.4°C but when supplemented with MEL-A it significantly decreased to −7.3°C. The performance of MEL-A used in low concentration (0.3% v/v) was equivalent to traditional chemical pour point depressants that are in general added in much higher amounts (5% v/v concentration). Furthermore MEL-A was able to decrease the **cloud point** of biodiesel from 22.5°C to 19.3°C. It is likely that the ability of MEL-A molecules to interact with the fatty acid methyl esters, the main components of biodiesel, is the underlying mechanism that prevents agglomeration [23].

Cold-Active Detergents

Environmental issues such as climate change and global warming caused by increased greenhouse gas emissions call for a low-carbon and resource sustainability economy. In recent years, companies have been adopting the life-cycle analysis approach to assess the environmental impact of their products. In the specific case of washing detergents, the impact of the consumer use of the product is also taken into account, and the washing temperature is an important factor (Box 3). The International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.) promoted in 2013 a ‘Low Temperature Washing Campaign’ initiative

Box 3. The Downside of Cold Washing

While laundry at lower temperatures certainly has great merit in societal progress related to environmental impact and sustainability, the downside is that many bacteria, fungi, and also viruses, typically killed at 60°C, can persist at 30°C, thus raising the issue: how clean, really, are our clothes? Reduced washing temperatures, together with the absence of bleach and disinfectants in laundry products, provide optimal conditions for microorganisms, including potential pathogens, to both persist in garments and colonize various compartments of the washing machine (e.g., detergent drawer, rubber ring of the door, drum) [71,72]. The microbial community is very diverse, typically originating from the human skin microbiota but including also members of known cold-adapted biosurfactant producers (e.g., *Pseudomonas*, *Bacillus*, *Flavobacterium,* and *Rhodotorula*). They are often organized in **biofilms** that are particularly resistant to standard chemical detergents [71]. However, this problem could be solved by exploiting the innate antimicrobial, antiadhesive, and biofilm-degrading activities of many biosurfactants [73]. As such, when incorporated in laundry product formulations biosurfactants would have a dual action, as both detergents and bactericides.

to encourage low-temperature washing practices and the use of fewer petrochemically derived surfactants as primary detergents, in efforts to reduce carbon footprint and move toward more sustainable formulation components. For example, the SUSCLEAN project initiated by Unilever (2007–2010) focused on discovering and selecting new enzymes and biosurfactants for optimum synergy in the absence of inorganic **sequestrants** and buffers to reduce the level of inorganic materials within laundry detergents, thus chemical loads, volumes, and wash temperatures.

Using lower wash water temperatures affect detergency effectiveness and require the detergent to maintain the same cleaning results (Figure 3) [48]. Below the **Krafft temperature** some surfactants crystallize and this leads to loss of surface activities such as dispersion, emulsification, and critical micelle-formation abilities [49]. For many ionic and non-ionic surfactants, however, the Krafft point becomes elevated at higher salt concentrations. All of these effects are important and should be considered with any new cold-temperature detergent, including biosurfactants. Many biosurfactants are available in liquid formulations, which are likely to reduce their Krafft point in detergency applications. In addition, they are stable and function across a broad range of temperature (4–55°C), pH, and salinity [50,51]. In the current work, rhamnolipid-containing mixtures seem to provide a greater degree of tolerance to a temperature reduction from 25°C to 10°C than chemical surfactants alone, which suggests that the incorporation of rhamnolipids in detergent formulations would probably improve their operating range at lower temperatures. In addition, monorhamnolipids consistently increased adsorption/performance at lower temperatures compared with dirhamnolipids (unpublished data). To assist in the behavior study of biosurfactants in solution, deuterium-labeled rhamnolipids produced in adapted strains of *Pseudomonas aeruginosa* are of great help [52]. The structural difference between the two forms (Figure 3), which consists of the additional rhamnose sugar in dirhamnolipids, leads to an increase of molecular weight from 504 to 650 and to a different degree of hydrophobicity. It is possible that smaller monorhamnolipid molecules are better at micelle formation or stronger at partitioning to the surface, while bigger dirhamnolipids have packing constraints [53]. It may also be that monorhamnolipids allow a different more favorable self-assembly structure with some of the chemical detergent components in a synergistic way, all of which remains to be further investigated and confirmed.

Bioprocessing at Industrial Scale

Psychrophilic microorganisms are generally seen as slow growers, hence not ideal for production at industrial scale. However, in psychrophiles, growth and activity respond differently to temperature, and maximum production of biomass and therefore of biotechnological products is typically reached at temperatures lower than those at which growth is fastest [54]. Thus, to compensate for the low growth rates dictated by low temperatures in nature, psychrophilic organisms maximize or maintain their growth yields at temperatures below optimum [55]. This

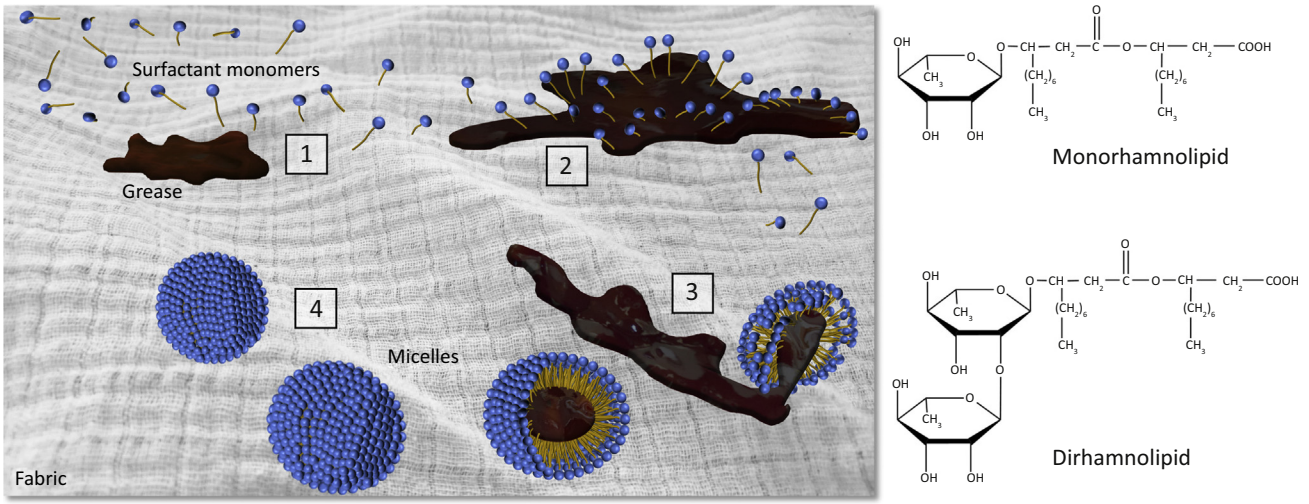

Figure 3. Detergency Action of Biosurfactants. Monorhamnolipids and dirhamnolipids produced by *Pseudomonas* spp. can be used as effective detergents in cold-wash laundry products. Biosurfactants dissolve in water (1) and attach via their hydrophobic tail to greasy stains on fabric (2). Biosurfactant monomers begin to assemble as micelles around the grease and mechanical agitation helps to lift the stain (3). With the process continuing, the stains are fully removed from the fabric encapsulated inside surfactants micelles (4) and washed off.

physiological aspect should be taken into account when optimizing production conditions for low-temperature biotechnological products. However, the growing market of cold-active enzymes clearly indicates that bioprocessing at industrial scale using psychrophilic strains is well established and profitable.

The capability of cold-adapted microorganisms to synthesize biosurfactants has only recently begun to be appreciated, and for understanding their application potential as well as their qualities as highly sustainable bioproducts, more work is necessary to scale up to industrial level. Current information is rather preliminary and fragmentary; for example, product yields have only rarely been determined and no production optimization has ever been attempted (Table 1). In addition, in some cases either the organism has not been rigorously identified, or the chemical analytical methods were not appropriate to definitely classify the product. This is a general problem for publications in the field of microbial biosurfactants that we have recently raised [56]. We suggest a more systematic approach starting with a thorough direct quantification of biosurfactant yields on purified or crude extracts. Low titer is a major hurdle, and comparable to biosurfactants from mesophilic strains, low-temperature biosurfactants synthesized in batch cultures are also in the order of a couple of grams per liter at best (Table 1). High yields (>100 g/L) suitable for large-scale production can be currently attained only in eukaryotic organisms such as *M. antarcticus*, which synthesizes MELs [21]. At present, only natural hyperproducer yeasts and fungi meet these requirements (e.g., high titers, easy recovery via filtration, cheap substrates) for bioprocessing at industrial scale [2]. In this case, strain engineering routes to produce customized products, for example, via the control of specific structural isoforms, are also becoming available [57]. We expect yeast and fungi from cold habitats to be similarly competitive to mesophilic strains and bring the additional advantage of fermentation processes effective at room temperature, thus reducing both production costs and environmental impact.

Bacterial biosurfactants such as rhamnolipids, despite being synthesized in much lower yields, are also produced at industrial scale by various companies worldwide [3]. Here, the latest trend to increase productivity is to bypass the complex regulation of rhamnolipid biosynthetic pathway and reroute the carbon flux of central metabolism to increase rhamnolipid precursors supply [58]. These strategies can be tested also on cold-adapted strains once the fundamentals of biosurfactant type, genetic organization, and regulatory factors have been elucidated.

Finally, the possibility to combine the production of cold-active enzymes and biosurfactants may be highly attractive for industrial bioprocessing. Many psychrophilic isolates [17,18] have shown concurrent capabilities, but more effort is now needed to characterize the synergistic interactions of the two processes also as a function of temperature and growth conditions.

Concluding Remarks

Extreme environments and extremophilic microorganisms are extraordinary sources of novel biomolecules with unique properties, but unexpectedly, they have so far attracted little attention in the quest for biosurfactants. Biosurfactants play key ecological roles in many cold habitats improving their habitability. They participate in carbon-cycling processes by enhancing the bioavailability of poorly soluble compounds, including pollutants, in cold soils and marine environments; for example, trehalose lipids can prevent ice crystallization in the immediate surroundings of the microbial cells. These natural capabilities can be used to develop new biotechnological products and processes that have low energy demand and operate under low-temperature regimes. There is also growing interest in the potential role and application of microbial biosurfactants in the catalysis and recovery of natural gas hydrates from deep sea or permafrost environments. However, there are aspects of biosurfactants related to both fundamental research and experimental approaches that need to be tackled with greater effort (see Outstanding Questions) to advance further with their use and applications. Undoubtedly, the discovery of new types of biosurfactants from psychrophilic organisms or directly from the cryosphere will boost biosurfactant biotechnological potential and support the spread of sustainable and energy-saving practices.

Outstanding Questions

Are there unique chemical and/or structural features of biosurfactants produced by psychrophiles that make them particularly suited to function at low and freezing temperatures?

How can we advance our understanding of biosurfactant-coding genes?

How can functional metagenomic pipelines be adapted and strengthened in the search for novel biosurfactants?

Can we develop reliable high-throughput screening methods for biosurfactants to apply to both cultural isolates and clonal samples?

What other types of biosurfactants should we expect to find besides the known glycolipids and lipopeptides?

Can we find natural isolates or genetically modify them to produce concurrently biosurfactants and cold-active enzymes? In general, to what extent can me manipulate psychrophiles to obtain customized strains for industrial applications?

Are (relatively slow) metabolic rates *in situ* under environmental conditions an impediment for the exploitation of native biosurfactants producing microbial communities in environmental technologies? Is it possible to stimulate the synthesis of biosurfactants directly in nature?

Since biosurfactant producers play a key role in the biodegradation of hydrophobic substances (both pollutants and non-pollutants), are there any co-occurrence patterns with other microbial groups and can we identify preferential interactions within the metabolic network? Can we use this information to develop effective consortia to apply to environmental technologies?

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References

1. Marchant, R. and Banat, I.M. (2012) Microbial biosurfactants: challenges and opportunities for future exploitation. *Trends Biotechnol.* 30, 558–565
2. Banat, I.M. *et al.* (2014) Cost effective technologies and renewable substrates for biosurfactants' production. *Front. Microbiol.* 5, 697
3. Sekhon Randhawa, K.K. and Rahman, P.K.S.M. (2014) Rhamnolipid biosurfactants – past, present, and future scenario of global market. *Front. Microbiol.* 5, 454
4. Hermann, B.G. *et al.* (2007) Producing bio-based bulk chemicals using industrial biotechnology saves energy and combats climate change. *Environ. Sci. Technol.* 41, 7915–7921
5. Cowan, D.A. *et al.* (2014) Microbial ecology and biogeochemistry of continental Antarctic soils. *Front. Microbiol.* 5, 154
6. Jansson, J.K. and Tas, N. (2014) The microbial ecology of permafrost. *Nat. Rev. Microbiol.* 12, 414–425
7. Verde, C. *et al.* (2016) Polar marine microorganisms and climate change. *Adv. Microb. Physiol.* 69, 187–215
8. Cavicchioli, R. *et al.* (2011) Biotechnological uses of enzymes from psychrophiles. *Microb. Biotechnol.* 4, 449–460
9. Borchert, E. *et al.* (2017) Psychrophiles as a source of novel antimicrobials. In *Psychrophiles: From Biodiversity to Biotechnology* (Margesin, R., ed.), pp. 527–540, Springer International Publishing
10. Nikrad, M.P. *et al.* (2016) The subzero microbiome: microbial activity in frozen and thawing soils. *FEMS Microbiol. Ecol.* 92, fiw081
11. Vasileva-Tonkova, E. and Gesheva, V. (2004) Potential for biodegradation of hydrocarbons by microorganisms isolated from Antarctic soils. *Z. Naturforsch. C* 59, 140–145
12. Vollú, R.E. *et al.* (2014) Aerobic endospore-forming bacteria isolated from Antarctic soils as producers of bioactive compounds of industrial interest. *Polar Biol.* 37, 1121–1131
13. Gesheva, V. *et al.* (2010) Biosurfactant production by halotolerant *Rhodococcus fascians* from Casey Station, Wilkes Land, Antarctica. *Curr. Microbiol.* 61, 112–117
14. Coronel-León, J. *et al.* (2015) Biosurfactant production by AL 1.1, a *Bacillus licheniformis* strain isolated from Antarctica: production, chemical characterization and properties. *Ann. Microbiol.* 65, 2065–2078

15. Janek, T. *et al.* (2013) Identification and characterization of biosurfactants produced by the Arctic bacterium *Pseudomonas putida* BD2. *Colloids Surf. B Biointerfaces* 110, 379–386
16. Vasileva-Tonkova, E. and Gesheva, V. (2007) Biosurfactant production by antarctic facultative anaerobe *Pantoea* sp. during growth on hydrocarbons. *Curr. Microbiol.* 54, 136–141
17. Sigurbjörnsdóttir, M.A. and Vilhelmsson, O. (2016) Selective isolation of potentially phosphate-mobilizing, biosurfactant-producing and biodegradative bacteria associated with a sub-Arctic, terricolous lichen, *Peltigera membranacea*. *FEMS Microbiol. Ecol.* 92, fiw090
18. Gesheva, V. and Negoita, T. (2011) Psychrotrophic microorganism communities in soils of Haswell Island, Antarctica, and their biosynthetic potential. *Polar Biol.* 35, 291–297
19. Perfumo, A. *et al.* (2017) Biodiversity of biosurfactants and roles in enhancing the (bio)availability of hydrophobic substrates. In *Cellular Ecophysiology of Microbe* (Krell, T., ed.), pp. 1–29, Springer International Publishing
20. Goto, S. *et al.* (1969) A taxonomic study of Antarctic yeasts. *Mycologia* 61, 748–774
21. Morita, T. *et al.* (2013) Production of mannosylerythritol lipids and their application in cosmetics. *Appl. Microbiol. Biotechnol.* 97, 4691–4700
22. Kitamoto, D. *et al.* (2001) Remarkable antiagglomeration effect of a yeast biosurfactant, diacylmannosylerythritol, on ice-water slurry for cold thermal storage. *Biotechnol. Prog.* 17, 362–365
23. Madihalli, C. *et al.* (2016) Mannosylerythritol lipid-A as a pour point depressant for enhancing the low-temperature fluidity of biodiesel and hydrocarbon fuels. *Energy Fuels* 30, 4118–4125
24. Buzzini, P. *et al.* (2012) Psychrophilic yeasts from worldwide glacial habitats: diversity, adaptation strategies and biotechnological potential. *FEMS Microbiol. Ecol.* 82, 217–241
25. Decho, A.W. and Gutierrez, T. (2017) Microbial extracellular polymeric substances (EPSs) in ocean systems. *Front. Microbiol.* 8, 922
26. Abraham, W.-R. *et al.* (1998) Novel glycine containing glucolipids from the alkane using bacterium *Alcanivorax borkumensis*. *Biochim. Biophys. Acta* 1393, 57–62
27. Yakimov, M.M. *et al.* (1998) *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. *Int. J. Syst. Bacteriol.* 48, 339–348
28. Pini, F. *et al.* (2007) Molecular and physiological characterisation of psychrotrophic hydrocarbon-degrading bacteria isolated from Terra Nova Bay (Antarctica). *Eur. J. Soil Biol.* 43, 368–379
29. Ibacache-Quiroga, C. *et al.* (2013) The hydrocarbon-degrading marine bacterium *Cobetia* sp. strain MM1IDA2H-1 produces a biosurfactant that interferes with quorum sensing of fish pathogens by signal hijacking. *Microb. Biotechnol.* 6, 394–405
30. Cai, Q. *et al.* (2014) Screening of biosurfactant producers from petroleum hydrocarbon contaminated sources in cold marine environments. *Mar. Pollut. Bull.* 86, 402–410
31. Konishi, M. *et al.* (2014) Deep-sea *Rhodococcus* sp. BS-15, lacking the phytopathogenic fas genes, produces a novel glucotriose lipid biosurfactant. *Mar. Biotechnol.* 6, 484–493
32. Tedesco, P. *et al.* (2016) Antimicrobial activity of monoramnholipids produced by bacterial strains isolated from the Ross Sea (Antarctica). *Mar. Drugs* 15, 83
33. Kurata, N. *et al.* (2016) Surfactant-associated bacteria in the near-surface layer of the ocean. *Sci. Rep.* 6, 19123
34. Mapelli, F. *et al.* (2017) Biotechnologies for marine oil spill cleanup: indissoluble ties with microorganisms. *Trends Biotechnol.* 35, 860–870
35. Antoniou, E. *et al.* (2015) Biosurfactant production from marine hydrocarbon-degrading consortia and pure bacterial strains using crude oil as carbon source. *Front. Microbiol* 6, 274
36. Bakermans, C. (2017) Determining the limits of microbial life at subzero temperatures. In *Psychrophiles: From Biodiversity to Biotechnology* (Margesin, R., ed.), pp. 21–38, Springer International Publishing
37. Ewert, M. and Deming, J.W. (2013) Sea ice microorganisms: environmental constraints and extracellular responses. *Biology (Basel)* 2, 603–628
38. Malavenda, R. *et al.* (2015) Biosurfactant production by Arctic and Antarctic bacteria growing on hydrocarbons. *Polar Biol.* 38, 1565–1574
39. Corradini, D. *et al.* (2013) Microscopic mechanism of protein cryopreservation in an aqueous solution with trehalose. *Sci. Rep.* 3, 1218
40. Hester, K.C. and Brewer, P.G. (2009) Clathrate hydrates in nature. *Annu. Rev. Mar. Sci.* 1, 303–327
41. Chong, Z.R. *et al.* (2016) Review of natural gas hydrates as an energy resource: prospects and challenges. *Appl. Energy* 162, 1633–1652
42. Rogers, R.E. *et al.* (2003) Catalysis of gas hydrates by biosurfactants in seawater-saturated sand/clay. *Can. J. Chem. Eng.* 81, 973–980
43. Zhang, G. *et al.* (2007) Investigation of microbial influences on seafloor gas-hydrate formations. *Mar. Chem.* 103, 359–369
44. Arora, A. *et al.* (2016) Biosurfactant as a promoter of methane hydrate formation: thermodynamic and kinetic studies. *Sci. Rep.* 6, 20893
45. Lanoil, B.D. *et al.* (2001) Bacteria and Archaea physically associated with Gulf of Mexico gas hydrates. *Appl. Environ. Microbiol.* 67, 5143–5153
46. Marchesi, J.R. *et al.* (2001) Methanogen and bacterial diversity and distribution in deep gas hydrate sediments from the Cascadia Margin as revealed by 16S rRNA molecular analysis. *FEMS Microbiol. Ecol.* 34, 221–228
47. Parkes, R.J. *et al.* (2009) Culturable prokaryotic diversity of deep, gas hydrate sediments: first use of a continuous high-pressure, anaerobic, enrichment and isolation system for subseafloor sediments (DeepIsoBUG). *Environ. Microbiol.* 11, 3140–3153
48. Laitala, K. and Mollan Jensen, H. (2010) Cleaning effect of household laundry detergents at low temperatures. *Tenside Surfactants Deterg.* 47, 413–420
49. Tsujii, K. and Mino, J. (1978) Krafft point depression of some zwitterionic surfactants by inorganic salts. *J. Phys. Chem.* 82, 1610–1614
50. Bello, X.V. *et al.* (2012) Study of the synergistic effects of salinity, pH, and temperature on the surface-active properties of biosurfactants produced by *Lactobacillus pentosus*. *J. Agric. Food. Chem.* 60, 1258–1265
51. Rizzo, C. *et al.* (2014) Influence of salinity and temperature on the activity of biosurfactants by polychaete-associated isolates. *Environ. Sci. Pollut. Res. Int.* 21, 2988–3004
52. Smyth, T.J. *et al.* (2010) Directed microbial biosynthesis of deuterated biosurfactants and potential future application to other bioactive molecules. *Appl. Microbiol. Biotechnol.* 87, 1347–1354
53. Chen, M. *et al.* (2013) Influence of calcium ions on rhamnolipid and rhamnolipid/anionic surfactant adsorption and self-assembly. *Langmuir* 29, 3912–3923
54. Margesin, R. (2009) Effect of temperature on growth parameters of psychrophilic bacteria and yeasts. *Extremophiles* 13, 257–262
55. Bakermans, C. and Nealson, K.H. (2004) Relationship of critical temperature to macromolecular synthesis and growth yield in *Psychrobacter cryopegella*. *J. Bacteriol.* 186, 2340–2345
56. Irorere, V.U. *et al.* (2017) Microbial rhamnolipid production: a critical re-evaluation of published data and suggested future publication criteria. *Appl. Microbiol. Biotechnol.* 101, 3941–3951
57. Baccile, N. *et al.* (2017) Development of a cradle-to-grave approach for acetylated acidic sophorolipid biosurfactants. *ACS Sustain. Chem. Eng.* 5, 1186–1198
58. Tiso, T. *et al.* (2016) Creating metabolic demand as an engineering strategy in *Pseudomonas putida* – rhamnolipid synthesis as an example. *Metab. Eng. Commun.* 3, 234–244
59. Morita, R.Y. (1975) Psychrophilic bacteria. *Bacteriol. Rev.* 39, 144–167

60. Cavicchioli, R. (2016) On the concept of a psychrophile. *ISME J.* 10, 793–795

61. Williams, T.J. *et al.* (2017) Cold adaptation of the Antarctic haloarchaea *Halohasta litchfieldiae* and *Halorubrum lacusprofundi*. *Environ. Microbiol.* 19, 2210–2227

62. Williams, T.J. *et al.* (2011) Defining the response of a microorganism to temperatures that span its complete growth temperature range (−2°C to 28°C) using multiplex quantitative proteomics. *Environ. Microbiol.* 13, 2186–2203

63. De Maayer, P. *et al.* (2014) Some like it cold: understanding the survival strategies of psychrophiles. *EMBO Rep.* 15, 508–517

64. Lee, Y.M. *et al.* (2012) Polar and Alpine Microbial Collection (PAMC): a culture collection dedicated to polar and alpine microorganisms. *Polar Biol.* 35, 1433–1438

65. de Bruijn, I. *et al.* (2007) Genome-based discovery, structure prediction and functional analysis of cyclic lipopeptide antibiotics in *Pseudomonas* species. *Mol. Microbiol.* 63, 417–428

66. Dubeau, D. *et al.* (2009) *Burkholderia thailandensis* harbors two identical *rhl* gene clusters responsible for the biosynthesis of rhamnolipids. *BMC Microbiol.* 9, 263

67. Aleti, G. *et al.* (2015) Genome mining: prediction of lipopeptides and polyketides from *Bacillus* and related *Firmicutes*. *Comput. Struct. Biotechnol. J.* 13, 192–203

68. Cowan, D.A. *et al.* (2015) Metagenomics of extreme environments. *Curr. Opin. Microbiol.* 25, 97–102

69. Jackson, S.A. *et al.* (2015) Metagenomics for the discovery of novel biosurfactants of environmental interest from marine ecosystems. *Curr. Opin. Biotechnol.* 33, 176–182

70. Thies, S. *et al.* (2016) Metagenomic discovery of novel enzymes and biosurfactants in a slaughterhouse biofilm microbial community. *Sci. Rep.* 6, 27035

71. Gattlen, J. *et al.* (2010) Biofilms isolated from washing machines from three continents and their tolerance to a standard detergent. *Biofouling* 26, 873–882

72. Callewaert, C. *et al.* (2015) Bacterial exchange in household washing machines. *Front. Microbiol.* 6, 1381

73. Diaz De Rienzo, M.A. *et al.* (2016) Antibacterial properties of biosurfactants against selected Gram-positive and -negative bacteria. *FEMS Microbiol. Lett.* 363, fnv224