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PSYCHROTOLERANT BIOSURFACTANT-PRODUCING BACTERIA FOR HYDROCARBON DEGRADATION: A MINI REVIEW

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Abstract

Biosurfactants are a structurally diverse group of surface-active substances synthesised by microorganisms. All biosurfactants have tremendous potential ranging from medicine to environmental applications especially in hydrocarbon remediation. Petroleum pollution is a major issue in both cold and temperate climate countries. These hydrocarbon pollutants have low solubility and high solid-water distribution ratios, thus limiting the interaction between microbial cells. Petroleum pollution is a major issue in both cold and temperate climate countries. In Antarctica, due to the recalcitrant nature of hydrocarbon components coupled with the region's extremely weather conditions, there were difficulties faced by bioremediation approaches. However, using biosurfactant in hydrocarbon bioremediation increases the bioavailability of hydrocarbon, thus expediting bioremediation. Few studies have reported on psychrotolerant bacterial species that are able to degrade hydrocarbon and produce biosurfactants. This review focuses on psychrotolerant bacteria with the potential to synthesise biosurfactants and degrade hydrocarbons.

INTRODUCTION

In the years ensuing the industrial revolution across the globe, many manufacturing industries settled for the use of chemicals and chemically-derived materials in the race to increase yield at a faster rate with less cost. Chemically derived surfactants have been used for more than a century and is in fact one among many other materials used by a diverse range of industries worldwide [1]. Surfactants are used in industries ranging from food, pharmaceuticals, cosmetics, petroleum and water treatment. The vast majority of chemical surfactants are derived from petrochemicals while some are synthesised from animal fats, plants and microorganisms [2, 3]. Many production markets rely heavily on petroleum-derived chemical surfactants because petroleum is considered a unique source of energy to thousands across the world [4]. With that said, the heavy consumption of petroleum and the by-products has taken a toll on the environment. Thus, in recent times, many have turned to seek for a friendlier solution as a greener alternative of the existing chemical counterparts.

Biosurfactants have been the interest for at least half a century and is still being pursued as a topic of interest to this date. The omnipresence of biosurfactants and the biologically active properties it contains have demanded widespread recognition in many applications from medicine [5, 6] to cosmetics [7, 8], gas and petroleum industries [9, 10] and contempo environmental applications [11-13]. Biosurfactants offer an alternative to chemical surfactants due to their biodegradability, low toxicity, biocompatibility and digestibility [14]. Those extracted from organic compounds such as oil possess dual-properties which are hydrophobicity and hydrophilicity [15].

Biosurfactants from extremophiles have captured the eye of the global scientific audience). They maintain endurance and effectiveness at extreme pH and temperatures [16] making them more commercially attainable especially those that can resist high temperatures, pressures and pHs [17-19]. Therefore, it is imperative to understand the biosurfactants and its characteristics with respect to the bacteria and the environment it resides in. This knowledge will spearhead research into finding an economically realistic and greener approach in mitigating hydrocarbon pollution everywhere, even in the cold ecosphere of Earth.

The cold ecospheres have been identified to be the largest extreme habitats for microbial communities. These natural reservoirs of cold-adapted microorganisms hold promise and potential for ecological studies. Here, the habitats are of the physical extremes, requiring that these microscopic forms of life develop mechanisms of adapting. This is possible only by their metabolic flexibility and ability to utilise and adapt to different carbon sources [20]. Microorganisms produce active biological compounds that act as a method of adapting to the cold [21]. As a result of gaining interest and newer technologies, the cryosphere has become more accessible to scientific expeditions that have shed a light on the potential roles of cold-adapted microorganisms in biotechnology, particularly regarding secondary metabolites [22, 23].

Properties of biosurfactants

The terms biosurfactant and bioemulsifiers, have been used interchangeably along with surface-active compounds in addition to biologically active compounds. There are marked differences between a biosurfactant and a bioemulsifier. All surface-active compounds share mutual properties in the instance where the structures have lipophilic and hydrophilic moieties making them amphiphilic [3, 24, 25]. The lipophilic moiety has been suggested to be a protein or a peptide which has a high number of hydrophobic side chains. Structures like these must have hydrocarbon chain of a fatty acid with 10-18 carbon atoms although more have been reported. The hydrophobic tail usually consists of linear alkyl groups or branched tails which makes it more difficult to biodegrade. With a hydrophilic headgroup and a hydrophobic tail, this creates the basis for the classification of biosurfactants into anionic, cationic, non-ionic and zwitterionic surfactants [26]. This partitioning also allows the formation of spherical micelles in water above the critical micelle concentration. While both are surface-active compounds and amphiphilic, biosurfactants are classified according to their biochemical nature which are glycolipids, lipopeptides/lipoproteins or fatty acid/polymer phospholipids and saponins [26, 27].

Biosurfactants are produced mainly from aerobic microorganisms that apply carbon as an energy source [3, 28]. The carbon sources can vary, bearing in mind that the type of carbon influences the chemical structure of biosurfactants [29, 30]. These carbon sources can range from carbohydrates, lipids, oils, to hydrocarbons from fuel and mixtures although, the type of carbon does have an influence of the chemical structure of biosurfactants [30, 31]. Thus, the spectrum of activity depends on the chemical composition of the biosurfactants [32].

Biosurfactants are amphipathic molecules that partitions preferentially at the interface between fluid phases with different degrees of polarity such as oil/water or water/oil and air/water interfaces [33]. This important character of surface-active compounds such as biosurfactant gives it the ability to reduce interfacial and surface tension to form microemulsion. This is where hydrocarbons can solubilise in water and vice versa [34]. A desirable biosurfactant must be efficient in reducing surface tension of water and have low critical micelle concentrations [35]. A low critical micelle concentration generally means that it has the maximum concentration of surfactant monomers in water which is subjected to pH, ionic strength and solution [36, 37]. Generally, the lower the critical micelle concentration is, the better the surfactants to self-associate to form micelles which are needed and useful at solubilising hydrophobic compounds.

Where surfactants dominate the markets, biosurfactants have been steady but not explosive in activity. There are many more gaps in knowledge that has yet to be surmised. The challenges in optimizing the production of biosurfactants was highlighted in a review by Parkinson [38], and was concluded that "biosurfactants have been shown to be as effective, if not more so, than many conventional synthetic surfactants and their future utilisation may depend ultimately upon the prevailing economics for their production." who said that biosurfactants have shown more effectiveness than synthetic surfactants however, the production of biosurfactants itself depend on the economy of that time. In the intervening 34 years thereafter, large number of reports has devoted attention to important fundamental matters regarding biosurfactants, such as production from different microorganisms and molecular basis for biosurfactant sysnthesis. Although the chemical and physical properties of some if not all biosurfactant classes have been well-investigated, there is still the need to characterise the type of biosurfactant in regard to the substrate, such as those produced during hydrocarbon degradation [39]. Another niche which is also sparse in the field but is gaining some due acknowledgement is the molecular details of their interactions with biological components [26].

Biosurfactant from psychrotolerant bacteria

The cryosphere is the frozen part of the Earth and makes up over 70% of habitats and niches with near- or below- freezing temperatures. The cold soils belonging to the Polar Regions or at high altitudes are most often than not exposed to environmental extremes. This includes low temperature, freeze–thaw cycles, strong UV irradiation, and limited availability of liquid water and nutrients [21, 40]. In polar soils and sediments, 50% of the microbial communities are found to be Gram positive [41, 42]. The term psychrophile is not uncommon to scientific reports these days due to the increasing number of studies on psychrotrophs being conducted. The concept and usage of terms such as psychrotolerant, psychrophilic and psychrotrophic posed much debate and have contributed to substantial confusion. Morita [43] first attempted to classify psychrophiles as the organisms with fundamentally minimum growth temperatures of 0°C, optimum at 15°C, and maximum of 20°C. These microorganisms also thrive in the polar regions of the planet, both in the Arctic Circle and the Antarctic continent. Bacteria species that are common to temperate environments include *Pseudomonas, Burkholderia, Sphingomonas, Rhodococcus* and the *Bacillus* also prevails in these cold habitats [42, 44-46].

Microorganisms inhabiting such extreme places have evolved to include highly specialised cell envelopes that contain biosurfactants. Being an integral part of the cell structure, biosurfactants act in synergy with exopolysaccharides and extracellular enzymes to protect the cell against high salinity, temperature and osmotic stress [47]. Many of the reported cases highlight the production of biosurfactants to be associated with extracellular enzymes such as amylase, lipase and protease [48, 49]. Among the many isolates, *Pseudomonas* and *Rhodococcus* are the well-known biosurfactant producers [50-52], but such capability has been rarely reported for cold-adapted isolates [53-55].

In cold environments, biosurfactants with low Krafft temperature with respect to the Krafft temperature of the environment are desired to avoid crystallisation of the biosurfactants, ultimately denying the surface-active function [56]. Krafft temperature also called as Krafft point is the minimum temperature where micelles are formed, in this case, where a surfactant can form micelles. Consequently, psychotolerant microorganisms would ultimately be capable of producing biosurfactants with low Krafft temperatures [55]. Nature has tailored biosurfactants to work under extremes of temperature and phase transitions, yet their biotechnological exploitation unfortunately remains low. Among the classes of biosurfactants, trehalose lipids are the most distinguished microbial biosurfactants especially in the genus *Rhodococcus* that is adapted to icy environments [25]. Compared to other sugars, trehalose has an extraordinary ability to protect biomolecules and living cells which are subjected to freezing. Trehalose displays dual-cryoprotective action; first, it prevents water form crystallising into ice and second, it slows down water dynamics in the proximity of proteins thus protecting biomolecules from freezing. Mannosylerythritol lipids (MELs) is another type of biosurfactant that shows excellent performance in ice-water systems that act as an anti-agglomerant. Even though MELs is produced by yeast, *Moesziomyces antarctica* isolated from Wright Alley, Antarctica, it promises enormous potential to be exploited in biotechnologies. Reports on biosurfactants produced by psychophiles are still sparse [21]. Those that have been discovered are shown in **Table 1.**

Biosurfactant by cold-adapted bacteria in hydrocarbon bioremediation

Bioremediation fully utilises the mechanism of microorganisms to remove toxic pollutants from the environments. Temperatures and pH are the crucial elements in this field as they are needed by the microorganism to grow rapidly, thrive and utilise a vast range of carbon sources. Bacteria have always been dominating the studies regarding hydrocarbon degradation [62, 63]. Coming from Antarctica, the biotechnological potential of isolated microorganisms has been recognized, especially regarding secondary metabolites produced by bacteria [22, 23].

The production of biosurfactants occurs predominantly on hydrophobic substrates as shown in **Table 2** including petroleum hydrocarbons [64] since petroleum contaminated environments have a higher potential of providing favourable conditions for biosurfactants produce [65-67].

Biosurfactant producing bacteria have been known to originate from both Gram positive and negative [24, 74, 75]. These compounds are secreted during the growth of aerobic microorganisms [48, 49] and facilitate the transport and translocation of insoluble substrates across the cell membranes. Biosurfactants have been associated with the bacterial ability to grow and degrade hydrocarbon molecules that persist as environmental contaminants, which also include polycyclic aromatics [48, 49, 76]. Poly-aromatic hydrocarbons (PAHs) are structurally stable. Due to this, they are considered as recalcitrant, environmental pollutants because of their extreme resistance to various methods of bioconversion [77]. In hydrocarbon degradation, some PAHs have low water solubility that limits their bioavailability to microorganisms (this can be improved by the addition of biosurfactants due to their amphipathic structure by several folds) [78-80]. Monoaromatic hydrocarbons display higher solubility in cold seawater than in temperate, which is contrary to diaromatic hydrocarbons that show lower solubility [81, 82]. In the environment, most hydrocarbon pollutants are introduced into soil through oil spills and forms strong adsorption making their effectiveness of removal to be limited, which can be overcome using biosurfactants [83, 84].

Biosurfactants are likely to act together with extracellular enzymes by increasing solubilisation and mobilisation, hence increasing the bioavailability of the hydrophobic residues from the hydrolysis of complex biopolymers [76]. In cold temperatures, bacterial growth and activity are slow in the presence of high concentrations pollutants, recalcitrant behaving pollutants. Biosurfactants are added to stimulate the bioremediation process [85]. The suitability of biosurfactants to be applied in oil-related industries has long been recognised and commercially applied [85, 86]. All surface-active compounds share mutual properties; their structures have lipophilic and hydrophilic moieties making them ampiphilic [3, 24-25]. Additionally, the application of biosurfactants in bioremediation has further expanded into not just hydrocarbons, but other toxic pollutants including heavy metals thus further supporting the variable of biosurfactants in bioremediation [87, 88]. Bioremediation is just as desirable in extremely cold climates as in temperate climates, thus increasing the necessity for biosurfactant producing psychrophiles that can degrade toxic pollutants.

Psychrotolerant, biosurfactant-producing hydrocarbon degraders isolated from water

Nearly 75% of the Earth's surface establishes the marine environment and is a robust reservoir of diverse microflora of biosurfactant producers [80]. Marine microorganisms have extraordinary metabolic and physiological capabilities rarely found in their terrestrial counterparts [55, 89]. In recent years, there have been considerable emphasises on exploiting marine organisms to solve the problems of bioremediation of pollutants [55, 90]. Large and multidisciplinary research projects (e.g., EU-FP7 projects, ULIXES and KILL-SPILL) have made successful attempts where biosurfactants played major roles. New and countless methods including biosurfactants in bioremediation were explored and new surfactants were sought from marine producers [73].

Alcanivoracaceae was the most abundant bacterial family with *A. borkumensis* SK2 strain as the dominant strain. This specific strain is very well described in the literature and is one of the major players in hydrocarbon degradation in the water column being commonly found in enrichment cultures and contaminated areas [53, 91, 92]. Members of the *Rhodobacteraceae, Rhodospirillaceae, Halomonadaceae, Oceanospirillaceae, Pseudomonadaceae,* and *Shewanellaceae* families have been also reported to be included as soil-degraders and biosurfactant producers [59, 93-98]. *Alcanivorax, Marinobacter,* and *Cycloclasticus* genera are commonly isolated from marine hydrocarbon-degrading consortia as they are obligate oil-degraders [99]. There are only few reports available on the production of biosurfactant by bacterial consortiums isolated from hydrocarbon contaminated soil or marine water column [100-102] and even fewer that use crude oil as the carbon source at the same time.

Over the years, the discoveries of new groups of marine hydrocarbon-degrading bacteria have highlighted how important these microorganisms are in solving bioremediation problems in the current marine environment [70, 103-107]. Furthermore, only several in the last recent years have had a hand in studying microbial hydrocarbon biodegradation in cold marine environment [108-111].

Table 1. Psychrotolerant bacteria species that produce biosurfactants

Type of biosurfactant	Location	Producing organisms	Temperature (°C)	Substrate	References
Glycolipids	Soil, Frazier Islands, Antarctica	*Pantoea* sp.	18-28	Paraffin, kerosene	[57]
Glycolipids	Soil, Wilkes Land	*Rhodococcus fascians*	4-15	Kerosene, glucose	[44]
Glycolipids	Soil, Haswell Island, Antarctica	*Nocardia* sp.	20	Paraffin, naphthalene	[48]
Mano-sylerythritol lipids	Lake Vanda, Antarctica	*Moesziomyces antarcticus*	30	Vegetable oils	[8, 58]
Fatty acids	Seawater, Montemar, Chile	*Cobetia* sp.	30	Dibenzothiophene	[59]
Unidentified	Soil, King George Island	*Bacillus* sp., *Paenibacillus* sp., *Sporosarcina* sp.	4-32	Tryptic soy broth n.d.	[42]
Lipopeptides	Sand, South Shetlands Islands	*Bacillus licheniformis*	30	Glucose 0.15–0.20 g/L	[46]
Glycolipoprotein	Antarctica	*Oceanobacillus* sp.	27	Sugarcane juice	[60]
Rhamnolipids	Marine sediment, Ross Sea	*Pseudomonas* sp.	21	Tryptone + yeast extract	[61]

Table 2. Potential psychrotolerant, hydrocarbon-degrading, biosurfactant-producing bacteria

Biosurfactant class	Location	Bacteria species	Temperature (°C)	Hydrocarbon source	References
Unidentified	Antarctica soil	*Arthrobacter protophormiae*	10	n-hexadecane	[68]
Glucose lipids	Marine sediment, North Sea	*Alcanivorax borkumensis*	20-30	Hydrocarbons	[69, 70]
Glycolipids		*Rhodococcus* sp.	4-30	Chloronated-benzene and n-alkane	[71]
Glycolipoprotein	Antarctica	*Oceanobacillus* sp.	30	Lubricant oil, crude oil, diesel and kerosene	[72]
Unidentified		*Alcanivorax, Exiguobacterium, Halomonas, Rhodococcus, Bacillus, Acinetobacter, Pseudomonas* and *Streptomyces*	30	n-hexadecane, diesel	[55]
Glycolipids	Marine sediment and water South Shetlands Islands	*Pseudoalteromonas* sp.	25	Tetradecane	[25]
Rhamnolipids, Shoporolipids	Marine sediment, Aegean Sea	*Rhodobacteraceae, Rhodospirillaceae, Shewanellaceae, Alcanivoracaceae, Halomonadaceae, Oceanospirillaceae* and *Pseudomonadaceae* families	14	Crude Oil	[73]

As stated by Yakimov et al. [99], in the marine environment, hydrocarbon-degrading bacteria are present at low or undetectable levels. When a pollution event occurs, the introduction of these oil constituents into the ocean causes successive blooms of limited number of these indigenous marine bacteria, also called as obligate hydrocarbonoclastic bacteria (OHGB). The exploration of marine biosurfactant producers is relatively rare. There have only been a few discoveries of cold-adapted marine bacteria that can produce biosurfactants [89, 112], which were isolated form seawater [54, 69, 113]. Nevertheless, in 2014, several biosurfactant-producing bacteria were isolated from oily seawater and sediment in the North Atlantic Canada [55]. This makes it among the very few literatures that have discovered biosurfactant-producing bacteria from low temperature locations over the span of decades. There are extraordinary metabolic and physiological abilities of marine microorganisms that are uncommonly found in their terrestrial counterparts [55, 89]. The gap in knowledge to find more marine bacteria that can produce biosurfactants is warranted.

CONCLUSION

Biosurfactants are a unique group of surface-active agents, which have diverse applications. They offer safer and greener alternatives to chemical surfactants but more importantly, they play a pivotal role in bioremediation. There is certainly more room to discover biosurfactants from psychrotolerant hydrocarbon-degrading bacteria not just from terrestrial, but also marine origins. Biosurfactants have yet to find a proper hold in other industrial niches, but as the global population moves towards economical, greener approaches, the key to achieve this lies in biosurfactant producing bacteria, capable of tolerating low temperatures and degrade a vast range of hydrocarbons and other environmental pollutants.

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