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Review

Surface-active biopolymers from marine bacteria for potential biotechnological applications

Karina Salek^{1,2} and Tony Gutierrez^{2,*}

¹ Faculty of Chemical Technology, Poznań University of Technology, Poznań, Poland

² School of Life Sciences, Heriot-Watt University, Edinburgh EH14 4AS, UK

* **Correspondence:** Email: tony.gutierrez@hw.ac.uk; Tel: +44-(0)131-451-3315.

Abstract: Surface-active agents are amphiphilic chemicals that are used in almost every sector of modern industry, the bulk of which are produced by organo-chemical synthesis. Those produced from biological sources (biosurfactants and bioemulsifiers), however, have gained increasing interest in recent years due to their wide structural and functional diversity, lower toxicities and high biodegradability, compared to their chemically-synthesised counterparts. This review aims to present a general overview on surface-active agents, including their classification, where new types of these biomolecules may lay awaiting discovery, and some of the main bottlenecks for their industrial-scale production. In particular, the marine environment is highlighted as a largely untapped source for discovering new types of surface-active agents. Marine bacteria, especially those living associated with micro-algae (eukaryotic phytoplankton), are a highly promising source of polymeric surface-active agents with potential biotechnological applications. The high uronic acids content of these macromolecules has been linked to conferring them with amphiphilic qualities, and their high structural diversity and polyanionic nature endows them with the potential to exhibit a wide range of functional diversity. Production yields (e.g. by fermentation) for most microbial surface-active agents have often been too low to meet the volume demands of industry, and this principally remains as the most important bottleneck for their further commercial development. However, new developments in recombinant and synthetic biology approaches can offer significant promise to alleviate this bottleneck. This review highlights a particular biotope in the marine environment that offers promise for discovering novel surface-active biomolecules, and gives a general overview on

specific areas that researchers and the industry could focus work towards increasing the production yields of microbial surface-active agents.

Keywords: surface-active agents; biosurfactants; bioemulsifiers; marine biopolymers; microorganism; biotechnology

1. Introduction

Surface-active agents are a group of amphiphilic chemical compounds (i.e. having both hydrophobic and hydrophilic domains) that are distinguished for their ability to mix two immiscible substances, such as oil and water [1–3]. They form an indispensable component in almost every sector of modern industry, and their importance is evidenced from the enormous volumes that are used and the diversity of applications that include, but not limited to, food, healthcare, agriculture, public health, textiles, and in environmental pollution control (e.g. bio-remediation) [4–7]. The huge market demand for these chemicals is exemplified in their total worldwide production, which exceeds 3 million tonnes per year [1] and was worth about 1.7 billion USD in 2011 and expected to reach 2.2 billion by 2018 [8].

Most of surface-active agents for industrial applications are synthetically-manufactured from organo-chemical synthesis using petrochemicals as precursors [9]. This is problematic, not only because they are derived from a non-renewable resource, but also because of their potential toxicological effects to humans and to the environment [2,10]. Generally, synthetically-derived products are often associated with higher toxicity, poor bio-degradability, and lower functional diversity compared to their biologically-derived counterparts [4,6]. Conversely, those of biological origin (i.e. biosurfactants and bioemulsifiers) have gained increasing interest in recent years, mainly driven by changing government legislation requiring a shift toward industrial use of renewable and less toxic compounds, and an increasing consumer demand for natural and “environmentally-friendly” ingredients [11,12]. One of the key challenges for the 21st century is to reduce our dependence on finite supplies of fossil fuels (oil, coal, gas) by moving toward the use of renewable and sustainable sources to supply our energy needs and the wide range of materials and fine chemicals that are largely still derived from crude oil and its derivatives.

The majority of biologically-derived surface-active agents used in industrial applications, however, are sourced from plants and animals, often required in large quantities in order to achieve optimal functionality. Under current climate change scenarios, even plant and animal sources used for supplying industrial materials and fine chemicals are non-sustainable since they can be seriously affected by political upheavals and meteorological events. Their high-volume demand has, in some years, seen their supply to be heavily impacted, due mainly to a shortfall in their supply from low crop yields, and increasing energy and transport costs. There is therefore increasing interest toward alternative and more reliable sources for these types of chemicals. Considering the enormous genetic diversity that microorganisms possess, they offer considerable promise in producing novel compounds for replacing some ingredients used by end-users that may not be completely sustainable

from current sources. Microbial-produced surface-active agents are a commercially-promising and sustainable alternative to those derived from plants and animals. They offer improved performance under industrial-relevant conditions (e.g. extremes of temperature, pH, pressure, salinity), even at relatively lower concentrations [4]. They are also a more attractive substitute for their chemically-synthesised counterparts due to their associated lower toxicities, higher bio-degradability, and perceived consumer-friendliness [4]. Microorganisms producing these compounds have been isolated from a wide range of environments [13] and comprising species representing many different genera of yeast/fungi and bacteria [14].

Discovering new types of surface-active agents is thus a high priority aim by industry, in particular for example the food sector to secure new ingredient additives with thickening and stabilizing abilities that may outperform gum arabic or xanthan gum. This combined with the desire to reduce dependency on plant-derived surface-active biopolymers produced by GM soybean for example, and availing of other favourable properties, including anti-adhesive, anti-oxidants, anti-microbial, and biofilm disruption capacity, has resulted in an increased interest in finding alternative natural sources for these types of biochemicals suitable for use in new and advanced formulations in food and other industries [5]. A particular feature of the high-molecular-weight surface-active agents is that they can exhibit very high surface-activities at relatively low concentrations, which may be attributed to the presence of multi-reactive chemical groups with high affinities for hydrophobic substances. For biotechnological applications, there are obvious economic advantages to the use of surface-active biopolymers exhibiting high functional activities at low concentrations.

With many years having been devoted to prospecting the biotechnological potential of terrestrial organisms (plants, animals and microbes), the marine environment has in recent times emerged as a highly promising and relatively untapped frontier for the discovery of novel natural products, including new types of the highly sought-after surface-active biopolymers [13]. This is particularly the case from microorganisms, whose collective biomass, phylogenetic and metabolic diversity in the marine biosphere far exceeds that anywhere else on Earth. Surface-active biopolymers have thus been found produced by many different types of bacteria and comprise proteins, glycoproteins, lipoproteins, polysaccharides, lipopolysaccharides or complexes containing any combination of these structural types. This paper reviews new sources of marine microorganisms that produce surface-active biopolymers for potential industrial applications and discusses some of the main bottlenecks for their industrial-scale production.

2. Classification of Surface-Active Agents

Surface-active agents are commonly classified based on their chemical charge or their molecular weight. Based on chemical charge, there are four main groups of surface-active agents—i.e. anionic, cationic, non-ionic, and Zwitterionic. These four groups are defined by the predominant charge on the surfactant molecule, which is largely conferred by the type of polar chemical group. In the case of anionic surface-active agents, the predominant polar group can be carboxylate, phosphate, sulphate, sulphonate, or a combination of these, giving the surface-active molecule a net negative charge. These are the most common surface-active agents used in various branches of industry. It has been estimated that they contribute up to 70% of the total global production of surfactants. A well-

known example of an anionic surfactant is soap—an alkylbenzene sulphonate, such as sodium stearate. Cationic surfactants, on the other hand, carry a net positive charge. The most common of these are the amine and quaternary ammonium surfactants, though their use is often limited by pH, especially for the amine-based cationic surfactants, which are used in the protonated state (i.e. at low pH). Non-ionic surfactants, however, have a net neutral charge and are the second largest group of surfactants. The polar (hydrophilic) moiety is usually composed of polyhydroxyl or polyether units. Zwitterionic surfactants are an interesting group of surface-active agents that contain both positive and negative charged moieties, thus allowing them to behave as both an acid and a base. The most common positive charge associated with this group of surfactants is ammonium, while the negative charge may be contributed by any one or more negatively-charged moieties, among which carboxylate is most common. Due to their chemical properties, zwitterionic surfactants are widely used in cosmetics and other healthcare products, as they are unlikely to cause eye and skin irritations [15].

For classification based on molecular weight, surface-active agents are divided into two major groups: (i) low-molecular-weight (LMW) surfactants that primarily confer the ability to reduce the surface tension of, for example, two immiscible liquids, and (ii) high-molecular-weight (HMW) surfactants which are more commonly referred to as emulsifiers, or polymeric surfactants, that are able to facilitate the formation of oil-in-water (O/W) or water-in-oil (W/O) emulsions. The naturally-derived HMW surface-active agents, or surface-active biopolymers, are an important class of chemical compounds that have a number of advantages over their LMW counterparts, examples of which are: (i) a larger surface area to which a greater number of reactive groups can be expressed, effectively endowing them with structural and functional heterogeneity [16]; (ii) good texturizing and stabilizing properties that help to slow down phase separation [17,18]; (iii) tensile strength and resistance to shear [19,20]; (iv) evidence suggesting health-enhancing or nutritional qualities [21–23]; and (v) the relative ease by which they can gain health clearance for use in the food, cosmetic or pharmaceutical sector [19,20]. Gum arabic, for example, is one of the most important of these biopolymers, used extensively in a wide range of biotechnological and industrial applications, including healthcare, cleaning products, foods, drinks (an essential component in citrus beverages), pharmaceuticals and textiles [4]. It is defined as a hydrocolloid emulsifier for its ability to both emulsify and stabilize oil-in-water emulsions. However, despite this dual functionality, gum arabic has a “low yield value” [24], which means that relatively high concentrations of the polymer are needed (up to 20% w/v) in order to achieve optimal functionality [25,26].

One question that has remained contentious for some time is what defines a surfactant as “natural”, and thus be labelled a “biosurfactant”? Strictly speaking, the term covers only those surfactants obtained directly from natural/biological sources (plants, animals, microorganisms) and where no organo-chemical synthesis was involved. However, over the years a less strict meaning for “natural surfactant” has allowed for including here other types of surfactants that have not entirely been derived from biological sources. They include surfactants obtained or synthesised from natural raw materials, of which an example is alkyl polyglucosides (APGs) that are produced using renewable natural sources such as wheat, coconut, potatoes, corn etc. [27]. The general formula of APGs is often presented as C_mG_n , where “m” denotes the number of carbon atoms and “n” denotes the number of glucose units, forming the hydrophilic head group of the surfactant molecule [28].

Currently, surfactants containing only one unit of a natural origin can be classified as natural. In other words, if a surfactant contains either a hydrophilic head unit or a hydrophobic tail unit of a natural origin, it can be referred to as natural. An example of the former is N-dodecanoylarginine methyl ester, which contains a polar head group based on the amino acid arginine. An example of the latter are fatty amide ethoxylates or sterol/phytosterol ethoxylates [29].

There are, however, no defined standards of the Europe Union regulating which surfactants may be designated with the label “natural”. Even since the first detergent-related Regulation—EC No 648/2004 of the European Parliament and Council [30], and subsequent modifications since, there remains a lack of regulatory definitions for what defines a natural-based surface-active agent.

3. Marine Microbial Surface-Active Biopolymers

The world’s oceans contain a total dissolved organic carbon content that is comparable in mass to the carbon in atmospheric CO₂ [31]. Much of this dissolved organic matter (DOM) exists as biopolymers (ca. 10–25 % of total oceanic DOM) that undergo reversible transition between colloidal and dissolved phases [32,33].

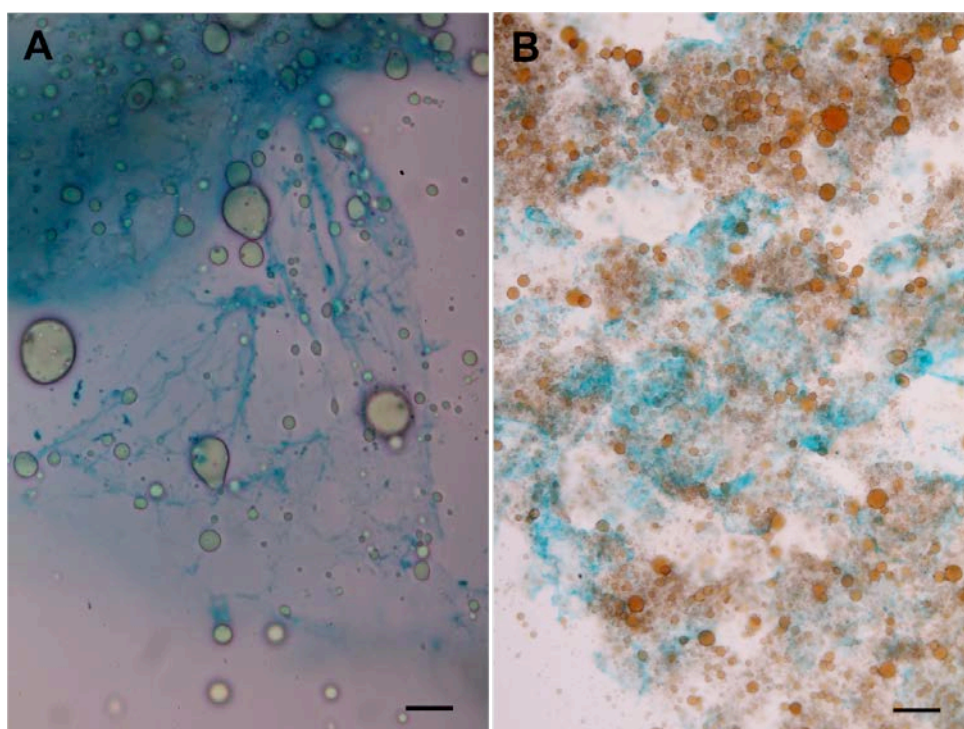


Figure 1. Glycoprotein surface-active biopolymers produced by hydrocarbon-degrading bacteria isolated from a sea surface oil-slick water sample during the Deepwater Horizon oil spill in the Gulf of Mexico (A), and emulsification of the Macondo crude oil by these bacterial surface-active biopolymers (B). The biopolymers shown in both panels were stained with the amino acid-specific dye coomassie brilliant blue G, showing that they are partially composed of protein. The orange-brown spheres in panels A and B are emulsified oil droplets.

A major source of this material derives from the synthesis and release of extracellular polysaccharides (EPS) by bacteria (Figure 1A) that contributes to the total DOM pool in the ocean [34]. EPS can serve a variety of functions, including biofilm formation, the solubilisation of hydrophobic organic chemicals, and in the binding and fate of cationic species [35,36].

A key property of many chemically characterized marine bacterial biopolymers is that they have a net negative charge, attributable to any number of anionic groups (e.g. COO^- , C-O^- , SO_4^{2-}). Compared to EPS produced by marine eukaryotic phytoplankton [37] and non-marine bacteria [38], marine bacterial EPS characteristically contains higher levels of uronic acids, notably D-glucuronic and D-galacturonic acid [39]. This renders these macromolecules highly polyanionic (negatively charged) and thereby quite reactive [39].

Ecologically, surface-active biopolymers serve important functions in marine environments where they may be involved in microbial adhesion to solid surfaces and biofilm formation [40], the emulsification of hydrocarbon oils to enhance biodegradation [41] (Figure 1B), or mediating the fate and mobility of heavy metals and trace metal nutrients in biogeochemical cycles [37,42,43]. This wide spectrum of functional activity is reflected not merely in the complex chemistry of these molecules, but also in the diversity of bacterial genera found producing them [44]. A high uronic acid content of marine bacterial biopolymers is quite interesting commercially.

Recent evidence implicates uronic acids in conferring these soluble biopolymers with an ability to interface with hydrophobic organic chemicals, such as food oils and petrochemical hydrocarbons [43,45]. Hence, these polyanionic macromolecules can exhibit surface-active properties that allow them to interact with oily substrates [45], in some cases enhancing the dissolution of the oils by the process of emulsification [41,45]. Their amphipathic nature is often attributed to the presence of a hydrophobic component(s) attached to the polysaccharide backbone, such as the fatty acids of RAG-1 emulsan [46], or protein of some glycoprotein emulsifiers [47,48]. In the case of emulsan, for example, its anionic nature has been shown to chelate cations, thus demonstrating its potential for use in the remediation of environmental sites contaminated with toxic metals [49–51]. Amino acids and peptides are also often found associated with marine bacterial biopolymers, and have been shown to confer amphiphilic characteristics to these macromolecules [45,52]. Uronic acids, amino acids/peptides can, therefore, render biopolymers highly reactive at surfaces and represent a potential source of commercially valuable surface-active biopolymers. The polyanionic property of these biopolymers has also been shown to chelate cations, including heavy metals [42,43,53], thus demonstrating their potential for use in the remediation of environmental sites contaminated with toxic metals.

Most bacterial bioemulsifiers are glycoproteins [4,54] and recent work has shown that marine bacteria are a viable source of glycoprotein bioemulsifiers. In fact, a large fraction of bacterial-derived EPS/biopolymers in the ocean is of glycoprotein composition [52,55]. These protein-polysaccharide conjugates are highly attractive as ingredients in, for example, food and drink formulations because they offer improved emulsifying and emulsion stability properties compared to their artificially constructed counterparts [56]. The uronic acid moieties of these macromolecules is likely to confer them with an ability to interface with hydrophobic organic chemicals, such as food oils and petrochemical hydrocarbons [43,45]. Similarly, the amino acid and/or peptide groups of

these exopolymers have also been shown to confer amphiphilic characteristics to these macromolecules [45,52].

Surface-active bacterial glycoproteins contain O-glycosidic links that act as the predominant bonding mechanism of the glycan to the protein via acidic amino acids or -OH groups [57]. The enhanced functionality of these biopolymers is attributed to the hydrophobic regions of the protein constituents, and possibly also uronic acids, serving here as anchoring points to help attach the large bulky hydrophilic carbohydrate regions to emulsion oil droplet surfaces. This mediates the formation of a very thick layer of adsorbed biopolymer molecules around emulsion droplets. Hence, these biopolymers are extremely efficient as emulsion stabiliser, preventing droplet coalescence and emulsion breakdown.

Whilst the marine environment is a highly promising and a relatively underexploited source for discovering new types of surface-active biopolymers, no methods currently exist that can match any type of surface-active biopolymer in an environmental sample to its biological source. Hence, traditional methods of screening, which are based on cultivation-dependent techniques, continue to be the principal method to ascertain whether a microbial species produces these types of biopolymers. A major shortcoming with this, however, is that it is laborious and time consuming because it requires the isolation of bacterial strains in pure culture prior to then testing them for the production of biosurfactants and/or bioemulsifiers. Furthermore, strains destined for these screening programmes quite often had been isolated from water column or sediment samples collected during opportune field trips or from serendipitous samples taken during research cruises at sea. An alternative to this “shotgun” approach to screening hundreds of isolated strains for surface-active biopolymers is to focus on particular niches in the marine environment where the prospect for discovering strains that produce these chemicals is highly likely. Hydrothermal vents, for example, have been described as largely unexplored “hot spots” for discovering new types of industrial-relevant biomolecules, including surface-active biopolymers [58]. Considering the global ocean comprises about 70% of Earth’s surface area, with vast reaches that have not yet been explored, it remains as the most promising horizon to discover new types of surface-active biopolymers for potential biotechnological applications.

4. Marine Micro-Algae: a Frontier for Discovering Novel Surface-Active Biopolymers

Hydrocarbon-degrading bacteria are commonly associated with the production of surface-active agents (biosurfactants or bioemulsifiers). As hydrocarbons are poorly soluble in aqueous media, surface-active agents help increase the solubility of hydrocarbons and thereby increase their bioavailability to the bacteria for biodegradation. Microorganisms that produce these chemicals hence play an important role in the degradation and ultimate removal of petrochemical pollutants from the marine environment.

Over 20 genera, distributed across the major bacterial Classes (*Alpha-*, *Beta-*, and *Gammaproteobacteria*; *Actinomycetes*; *Flexibacter-Cytophaga-Bacteroides*), comprise representatives of hydrocarbon-degrading bacterial species. Some of these organisms comprise members that “specialize” in the degradation of linear or branched saturated hydrocarbons (*Alcanivorax*, *Oleiphilus*, *Oleispira* and *Thalassolitus*), or of aromatic hydrocarbons (*Cycloclasticus*

and *Neptunomonas*)—i.e. these specialist bacteria use these hydrocarbons almost exclusively as a sole source of carbon and energy. These fastidious organisms are strongly selected for in oil-impacted environments, where they successively increase in numbers from near undetectable levels to constituting up to 70–90% of the total bacterial population—for example, after the onset of an oil-spill event [59].

Recent evidence highlights the cell surface, or “phycosphere”, of micro-algae as a new and largely underexplored biotope in the ocean that harbours novel taxa of hydrocarbon-degrading bacteria, including those that “specialise” in the degradation of hydrocarbons. This algal-bacterial association may be attributed to the potential for some micro-algae to actively synthesize hydrocarbon molecules [60,61] and translocate them to the algal cell wall [61–64], or to passively adsorb them from the surrounding seawater [65,66]. Either through biogenic synthesis or adsorption of hydrocarbon molecules, the algal cell surface is a potential “hot spot” where novel hydrocarbon-degrading bacteria may be found to produce new types of surface-active agents. As illustrated in Figure 2, members of the *Roseobacter* clade, which are recognized for encoding the protocatechuate pathway involved in aromatic hydrocarbon degradation [67–69], are found inhabiting the phycosphere of the dinoflagellate *Pseudonitzschia*.

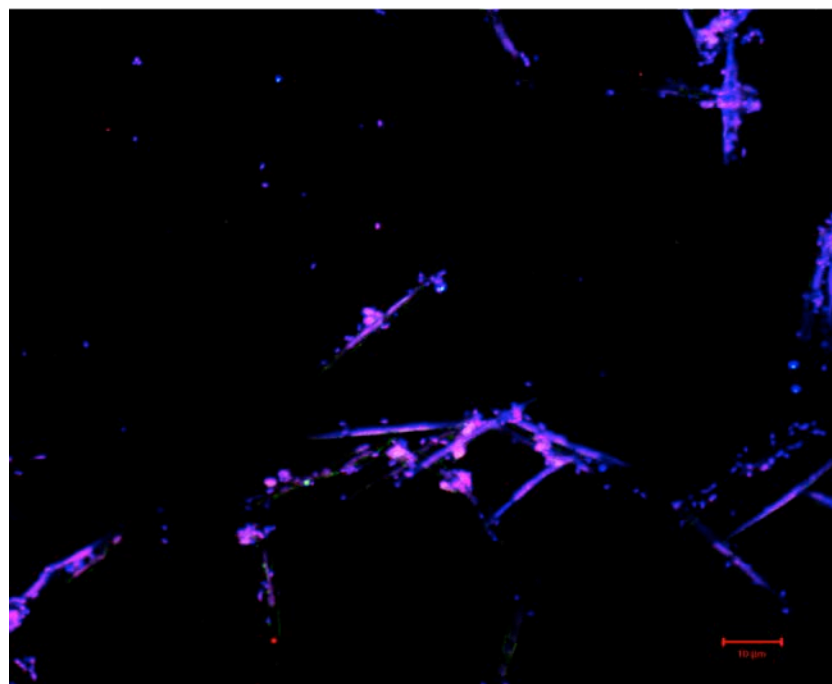


Figure 2. Image of a laboratory culture of *Pseudonitzschia* (CCAP1061/25) shown with associated members of the *Roseobacter* clade that were targeted using Cy3-labelled ROS537 probe by fluorescence in situ hybridisation.

5. Bottlenecks to Commercialising Microbial Biosurfactants

The high-volume demand for a product or process ingredient can impact heavily on manufacturing and end-product costs, as well as the source of its supply. This has been evident in

recent years, notably for the emulsifiers/stabilizers gum arabic and locust bean gum, which have been the subject to a number of price fluctuations, due mainly to a shortfall in their supply from low crop yields, and increasing energy and transport costs. Projected impacts of climate change scenarios produced by the HadCM3 global climate model under the IPCC (Intergovernmental Panel on Climate Change) Special Report on Emissions Scenarios (SRES) predicts significant decreases in both regional and global crop yields within the next 70 years. In order to prepare for future shortfalls in the supply chain of bio-based surface-active agents associated with projected climate change scenarios, there is increasing interest directed toward alternative and more reliable sources for these types of ingredients.

For commercial exploitation, microorganisms offer a reliable and sustainable alternative for producing SAs compared to organo-chemical synthesis, or from the derivation of these chemicals from plant or animal sources [70]. Availability and cost performance are today the main disadvantages for large volume applications and wider use of SAs [9,71]. Despite the potential advantages of microbial surface-active agents, commercial production is often stifled because of typically low yields and high production costs [72]. In the case of specialty surfactants, which may not be required in very large volumes, although may be prone to higher prices, this can be compensated by their environmental profile and performance benefits [73]. Optimisation of the fermentation conditions is an important step to maximising the production yields of microbial surface-active biopolymers.

Although a plethora of reports during the past two decades have prophesied potential commercial application of microbial-produced surface-active agents, very few have reached a commercial endpoint. This is due to the fact that very few research programmes take the bio-surfactant/bio-emulsifier discovery pipeline all the way through to pilot-scale optimisation, production and final end-user formulation and commercialisation.

The influence of abiotic parameters (e.g. pH, temperature, agitation, salinity) and different nutritional conditions or type of feedstock have been shown to influence production yields and physico-chemical characteristics of microbial surface-active agents [74]. However, even this is not always adequate as often the producing strains will still not produce sufficient yields to meet end-user demands. The fact that manipulation of culture conditions is not always enough to increase production yields of these chemicals to meet the volume demands of industry is testament to why very few microbially-produced surface-active biopolymers have reached commercial production. An example of a microbial surface-active biopolymer that has reached a commercial endpoint is xanthan gum (Figure 3A)—a hydrocolloid produced by the plant-pathogenic bacterium *Xanthomonas campestris*. In addition to possessing interfacial properties, xanthan gum is also a viscosity builder, which makes it an important component of many healthcare products and food processing formulations [75]. Emulsan is another example of a surface-active biopolymer with excellent emulsifying properties that has reached commercial production (Figure 3B). Produced by *Acinetobacter venetianus* RAG-1 (previously *A. calcoaceticus* RAG-1), it contains hydrophobic moieties, such as fatty acids, that are attached to its polysaccharide backbone conferring it with amphiphilic qualities. Of the LMW surface-active agents, rhamnolipids (Figure 3C) and sphorolipids (Figure 3D) have already found commercial outlets in applications that include products for cleaning applications [76].

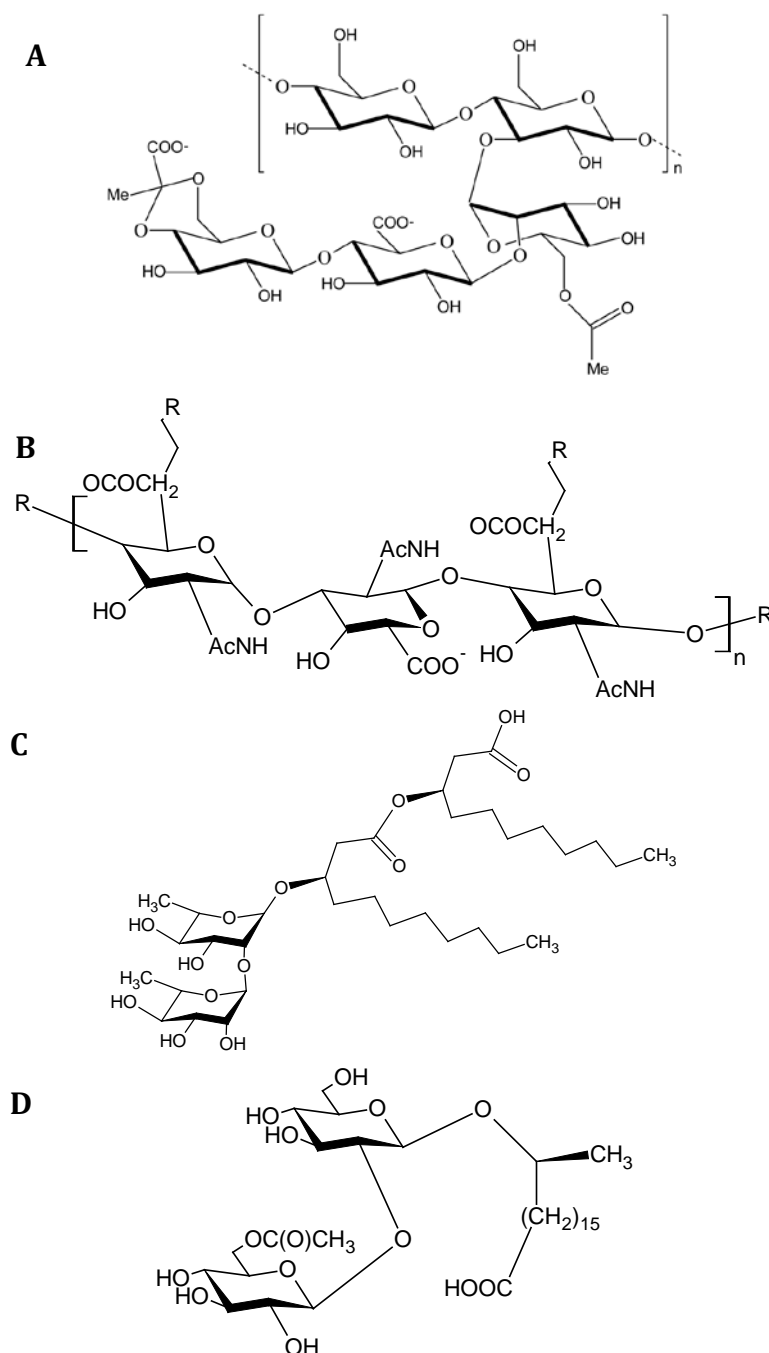


Figure 3. Chemical structures of four important natural surface-active agents. (A) Xanthan gum, as adapted from [80]; (B) Emulsan, as adapted from [81]; (C) Rhamnolipid (first identified rhamnolipid called α -L-rhamnopyranosyl- α -L-rhamnopyranosyl- β -hydroxydecanoyl- β -hydroxydecanoate, or Rha-Rha-C₁₀-C₁₀), based on [82]; (D) Sophorolipid, according to [83].

Quite often, however, microbial strains are found to produce surface-active agents in low quantities and, for this reason, are often given little attention or discarded as uninteresting candidates for biotechnological development. Discovering new microbial polysaccharides and surfactants is a high priority aim for many food industries to secure new ingredient additives with thickening and

stabilizing abilities similar to xanthan gum. This combined with the desire to reduce dependency on plant emulsifiers produced by GM soybean for example, and availing of other favourable properties, including anti-adhesive, anti-oxidant, anti-microbial, and biofilm disruption capacity has resulted in an increased interest in finding alternative natural sources for amphiphilic molecules suitable for use in new and advanced formulations in food and other industries [77].

One method that offers high promise to optimising the production, and thus sustainable supply and demand, of surface-active agents from microorganisms is in the design of over-producing strains and in the selective-tailoring of these biochemicals, such as through the use of recombinant DNA technology to express them *in vitro* using alternative host systems [78]. The combination of systems and synthetic biological approaches offers a way forward for the cost-effective, sustainable and selective-tailored production of microbial biosurfactants/bioemulsifiers by using carefully-designed over-producing microbial expression hosts, or “chassis”, systems. For example, the three genes (*sfp*, *sfpO*, *srfA*) encoding the synthesis of the lipopeptide biosurfactant by *Bacillus licheniformis* NIOT-AMKV06 were cloned and expressed in *Escherichia coli*, resulting in increased production of the lipopeptide from 3 g/L to 11.7 g/L [79]. Such methods are promising, yet they are still reliant on the genetic and biochemical limitations of the recombinant producing strain for boosting production yields and providing the means to tailor the functionality of these biochemicals.

6. Conclusion

Surface-active agents derived from biological sources (i.e. biosurfactants and bioemulsifiers) have gained high interest in recent years, due largely to consumer demand for natural ingredients and by companies in search of chemical ingredients conferring improved functional properties and that can be derived from sustainable and ‘green’ sources. The marine environment, which is recognised to harbour the largest biosphere and microbial diversity on Earth, offers great potential in the discovery of novel types of surface-active agents for commercial development. Though still largely unexplored in this respect, certain niches or biotopes in the ocean, such as deep-sea hydrothermal vents and, more recently the discovery of hydrocarbon-degrading bacteria associated on the ‘phycosphere’ of marine micro-algae, has been shown to be new sources for the discovery of novel types of surface-active agents. Whilst the discovery of new types of these chemicals from bacteria and other microorganisms continues to populate the literature every year, a major bottleneck to their development and reaching a commercial end-point is the mere fact that sufficient production yields are often unachievable. With the recent and significant advancements in genetic engineering techniques, combined to improved fermentation technologies, the generation of biosurfactant/bioemulsifier overproducing strains may be a possibility in the very near future.

Conflict of interest

All authors declare no conflict of interest in this paper.

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