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## Marine Microbial Surfactants: Searching for Needles in a Haystack

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Surfactants are a group of amphiphilic chemical compounds (i.e. having both hydrophobic and hydrophilic domains) that are distinguished for their ability to mix non-aqueous and aqueous substances together [1-3]. They form an indispensable component in almost every sector of modern industry. Their significance is evidenced from the enormous volumes that are used and wide diversity of applications they are used in, ranging from food and beverage, agriculture, public health, healthcare, textiles, bioremediation etc [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]. Compared to emulsifiers, which are also surface-active biomolecules but generally of a high molecular weight and have emulsifying properties, surfactants are low-molecular-weight compounds that primarily act in reducing the surface and/or interfacial tension of, for example, two immiscible liquids.

A major drive in recent decades has been toward the discovery of surfactants from biological/natural sources - namely, the bio-surfactants. Most surfactants that are used today for industrial applications are synthetically-manufactured via organo-chemical synthesis using petrochemicals as precursors [9]. This is problematic, not only because they are derived from non-renewable resources, but also because of their environmental incompatibility and potential toxicological effects to humans and other organisms [2,10]. Generally, synthetically-derived surfactants are often associated with poor bio-degradability, higher toxicity and lower functional diversity compared to their biologically-derived counterparts [4,6]. Bio-surfactants have thus 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]. This is timely as one of today's key challenges is to reduce our reliance on fossil fuels (oil, coal, gas) and to move toward using renewable and sustainable sources. Considering the enormous genetic diversity that microorganisms possess, they offer considerable promise in producing novel types of bio-surfactants for replacing those that are produced from organo-chemical synthesis. Microorganisms producing these types of molecules have been isolated from a wide range of environments [13] and comprising species representing many different genera of yeast/fungi and bacteria [14]. Where a huge potential exists for discovery of new bio-surfactants is the ocean [15].

The total biomass of microorganisms in the global ocean, as well as their phylogenetic and metabolic diversity, far exceeds that anywhere else on Earth. Considering the global ocean comprises about 70% of Earth's surface area, with vast reaches that have not yet been explored, it is thus a frontier for discovering new types of bio-surfactants for potential biotechnological applications. Traditional methods of screening for these types of biomolecules, which are based on cultivation-dependent techniques, continue to be the principal method in bio-surfactant discovery pipelines. But this poses a major shortcoming in bio-surfactant discovery, as isolation of microorganisms is a laborious and time consuming exercise that involves the isolation of organisms in pure culture prior to then screening them. Furthermore, since at least 99% of the ocean's microbial diversity is not amenable to cultivation in the laboratory using conventional methods, it is a

huge window of the microbial diversity in the oceans that we are unable to tap into for discovering these types of molecules - the 'needles in the ocean haystack'. New technologies are needed to tap into this window and attempt to uncover the potentially huge diversity of bio-surfactants, and other bio-molecules, that lie invisible to our current screening, identification or prospecting capabilities.

Few bio-surfactants from microorganisms, however, have reached a commercial end-point. This is because of the need to overcome some major bottlenecks surrounding the microbial production of bio-surfactants, principally the high costs involved and the relatively low yields that are often achieved from microorganisms. In order to overcome these limitations, more research effort should focus on the following areas: i) The implementation of low-cost methods for the downstream extraction and purification of bio-surfactants; ii) The use of low-cost, alternative and sustainable substrates/feedstocks for bio-surfactant production in fermentation processes; iii) Exploring genetic engineering approaches to improve rates and yields of bio-surfactant production; iv) Expand bio-prospecting studies, especially at underexploited or extreme environments (e.g. the oceans and especially the deep-sea), to identify novel microbial strains that produce novel bio-surfactants. At the outset of any bio-surfactant discovery pipeline, rather than waste time and resources with 'coarse fishing expeditions', it is advantageous to employ a targeted approach. For example, hydrocarbon-degrading bacteria are commonly associated with the production of bio-surfactants. Hence, such organisms could be targeted for isolation by enrichment with hydrocarbons. One frontier with great promise for bio-surfactant discovery, and which remains largely underexploited in this respect, is the 'phycosphere' of marine eukaryotic phytoplankton - i.e. the cell surface of micro-algae. This niche environment, or biotope, in the ocean has been shown to harbour a diversity of hydrocarbon-degrading bacteria [16,17] and which has shown great promise for discovering novel bacterial species producing bio-surfactants (and bio-emulsifiers) with commercial potential (<http://www.marisurf.eu/>).

Bio-surfactant prospecting from the environment (terrestrial and marine) has been the focus by many laboratories worldwide for the past 50 years. However, the use of these bio-molecules in the commercial sector continues to be heavily overshadowed by those produced by organo-chemical synthesis. It is clear that several bottlenecks need to be addressed to help facilitate the entry of bio-surfactants into the market. To this end, funding and collaborations between surfactant end-users and academic researchers, both domestic and internationally, needs to be expanded. There will be many challenges along the way, but to reach a successful implementation of bio-surfactants in industry, there will need to be a requirement for close engagement between both sectors.

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