

Marine Derived Biosurfactants: A Vast Potential Future Resource

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Abstract

Surfactants and emulsifiers are surface-active compounds (SACs) which play an important role in various industrial processes and products due to their interfacial properties. Many of the chemical surfactants in use today are produced from non-renewable petrochemical feedstocks, while biosurfactants (BS) produced by microorganisms from renewable feedstocks are considered viable alternatives to petroleum based surfactants, due to their biodegradability and eco-friendly nature. However, some well-characterised BS producers are pathogenic and therefore, not appropriate for scaled-up production. Marine-derived BS have been found to be produced by non-pathogenic organisms making them attractive possibilities for exploitation in commercial products. Additionally, BS produced from marine bacteria may show excellent activity at extreme conditions (temperature, pH and salinity). Despite being non-pathogenic, marine-derived BS have not been exploited commercially due to their low yields, insufficient structural elucidation and uncharacterised genes. Therefore, optimization of BS production conditions in marine bacteria, characterization of the compounds produced as well as the genes involved in the biosynthesis are necessary to improve cost-efficiency and realise the industrial demands of SACs.

Keywords

Surface-active; Marine bacteria; Biosurfactant; Emulsifier; Bioremediation; Biodegradable

1 **1. Surfactant industry and problem identification**

2 Surfactants are utilized in various bulk commercial products particularly in personal care products and
3 household cleaners. To fulfil their worldwide demand, millions of tonnes of surfactants are annually produced
4 from non-renewable sources such as petrochemicals. Consumption of surfactants is probably greater than 13
5 million tonnes per year worldwide (Marchant and Banat 2012a). The most widely used synthetic surfactants such
6 as alkyl benzene sulfonates (ABS), are not readily biodegradable thus causing adverse effects on the environment.
7 U.S. detergent manufacturers therefore, replaced ABS with linear alkylbenzene sulfonate (LAS) which showed
8 no adverse impact on the environment and had the same cleaning characteristics (Cowan-Ellsberry et al. 2014).
9 However, diminishing petrochemical stocks have created a drive towards identification of novel renewable
10 bioresources for efficient surfactant production (Foley et al. 2012). In recent years researchers have been involved
11 in continued environmental research to source replacements for synthetic surfactants and other bioactives from
12 the marine environment (Kalogerakis et al. 2015). Surface-active compounds (SACs) produced by
13 microorganisms offer an ideal sustainable substitute for petrochemical based surfactants. Biologically produced
14 SACs have numerous potential applications in a wide variety of industrial sectors, due to their synthesis from
15 waste and renewable substrates such as, hydrocarbon wastes, crude oil and vegetable oils in addition to being bio-
16 compatible, non-toxic and biodegradable (Banat et al. 2010). They are usually classified based on their molecular
17 weight, those surfactant molecules with low molecular weights are known as biosurfactants (BS) whilst those with
18 high molecular weights are known as bioemulsifiers (BE).

19 BS are further categorized based upon their molecular structure e.g. glycolipids, lipopeptides,
20 phospholipids, lipoprotein, fatty acids and polymeric BS. They also have a range of different properties such as,
21 surface tension reduction, emulsification, foaming and wetting (Banat et al. 2014; Marchant and Banat
22 2012a). With over 5.73 million tonnes of oil spills in the oceans worldwide between 1970 and 2016 (ITOPF 2017),
23 there is an urgent demand for novel bioactive compounds for bioremediation. BS naturally play a major role in
24 bioremediation following oil spills and act as efficient dispersing agents facilitating microbial biodegradation (De
25 Almeida et al. 2016; Franzetti et al. 2010). Despite their versatile properties, a higher production cost compared
26 to chemical surfactants and the pathogenicity of some BS producing strains, remain a major obstacles for their
27 large-scale production (Irorere et al. 2018) and the search for non-pathogenic strains remain an important research
28 area (Elshikh et al. 2017; Funston et al. 2016). Therefore, an important question to address before they can achieve
29 widespread application is “can we develop a cost-effective and ecologically benign process for BS production?”

30

1 2. Surfactant production in marine bacteria

2 Surfactants with diverse properties and low production costs are required to increase the applications of
3 natural SACs, which gives greater incentive to develop surfactants of biological origin produced by
4 microorganisms. Marine microorganisms are ubiquitous in the marine environment as well as extreme
5 environments. The oceans have a relatively narrow range of pH, salinity and temperature while areas such as the
6 volcanic vents face extreme conditions. These microorganisms are known to be metabolically and physiologically
7 adapted to survive under extreme temperature, pressure, pH and salinity conditions (Das et al. 2010; Thavasi et
8 al. 2014). For example, members of the *Alcanivorax* genus survive at low to mild hydrostatic pressure in
9 hydrocarbon contaminated environments. The strains *A. borkumensis* SK2 and *A. dieselolei* KS 293 have
10 developed different strategies to cope with environmental stress under high pressure. While the respiration and
11 cell integrity is not affected in KS 293 at mild hydrostatic pressure, SK2 activates the production of the osmolyte
12 ectoine to cope with hydrostatic pressure (Scoma et al. 2016a; Scoma et al. 2016b). Marine bacteria secrete large
13 molecules known as exopolysaccharides (EPS) consisting of proteins, polysaccharides, lipids, nucleic acids and
14 uronic acids. EPS enhances the survival of microbial cells under changing environmental conditions through
15 various mechanisms such as, biofilm formation, enhancing substrate adhesion, protection against limited nutrient
16 availability, detoxification of metals and the presence of antibiotics (Harimawan and Ting 2016; Pal and Paul
17 2008).

18 Some microorganisms specifically produce amphiphilic EPS, particularly BS as a mechanism to increase
19 the bioavailability of hydrophobic substrates such as hydrocarbons, these BS enhance the growth of indigenous
20 bacteria capable of degrading aliphatic and aromatic hydrocarbons. BS produced from marine bacteria can
21 facilitate hydrocarbon dispersion, degradation, emulsification and bioavailability (Das et al. 2010; Mapelli et al.
22 2017). BS from cold-adapted marine microorganisms or psychrophilic organisms can work efficiently at cold and
23 freezing temperatures, and are therefore suitable in laundry detergent formulations where low temperature
24 washing conditions have become a priority for energy conservation (Perfumo et al. 2018; Marchant and Banat
25 2012b). The potential uses of BS are further improved by their low-toxicity, meaning they are applicable for large-
26 scale industrial production and subsequent environmental disposal where they can be readily biodegraded (Irorere
27 et al. 2017; Uzoigwe et al. 2015). Hence marine bacteria offer an excellent opportunity for the discovery of new
28 SAC molecules with distinctive properties. Although highly attractive, the biosynthesis of BS from marine
29 organisms has largely been overlooked. The mechanism of their regulation during synthesis is also not fully
30 understood adding further difficulties to the process for their production. Several approaches are required before

1 the widespread application of marine-derived BS can be achieved: i) Isolation and identification of novel, non-
2 pathogenic marine BS producing bacteria ii) Optimization of culture conditions to achieve sufficient yields of BS
3 and iii) Characterization of genes involved in BS production from marine organisms. These will allow the use of
4 marine strains in large scale BS production processes while improving yield and cost-efficiency of BS production.
5

6 **I. Isolation of BS producing marine strains**

7 Much of the research on BS is focused on soil isolates, including the *Pseudomonas* and *Bacillus* groups,
8 however, BS production by marine microorganisms is an area relatively unexplored since it is still considered that
9 marine microorganisms are difficult to culture in the laboratory (Stein et al. 1996; Walsh and Duffy 2013). The
10 global demand for BS has led to a number of academic research groups and manufacturers searching
11 underexploited sources such as bacteria isolated from the marine environment. Recent reports have shown the
12 successful isolation and culture of diverse BS producing microorganisms from marine habitats (Thavasi et al.
13 2009). BS production is induced in most marine bacteria in the hydrocarbon polluted environment. Marine
14 bacteria increase hydrocarbon bioavailability by the production of BS which shows surface/emulsification
15 activities and facilitates hydrocarbon degradation (Thavasi et al. 2011). The marine oil spill from deep water
16 horizon in the Gulf of Mexico caused large volumes of oil spill into the water. Several BS producing marine
17 bacteria became predominant following the spill, including *Alteromonas*, *Halomonas*, *Alcanivorax*, *Colwellia*,
18 *Cycloclasticus* and *Pseudoalteromonas* (Mapelli F et al. 2017). A number of other bacteria producing BS
19 including, *Pseudomonas*, *Bacillus*, and *Acinetobacter* were reported in oil contaminated waters worldwide
20 (Gerard et al. 1997; Hentati et al. 2016; Ortega-de la Rosa et al. 2018), although these species are not necessarily
21 restricted to the marine environment.

22 The populations of marine bacteria can be increased by the addition of inorganic nutrients which allows
23 them to use hydrocarbons as a carbon and energy source. *A. borkumensis* is found in low numbers in unpolluted
24 environments but becomes the dominant microbe in hydrocarbon polluted ocean and coastal waters. The growth
25 and multiplication of *Alcanivorax* in oil treated sea water increased up to 91% within 1-2 weeks of nutrient
26 supplementation (Syutsubo et al. 2001). It was observed that addition of inorganic nutrients improved the
27 dynamics of the bacterial community (Roling et al. 2002). Providing suitable conditions for the hydrocarbon
28 utilization can be an efficient method to achieve growth and multiplication of these organisms. In microcosm and
29 field biodegradation experiments, bacteria with 16S rRNA sequences related to *A. borkumensis* and *Pseudomonas*
30 *stutzeri* increased when supplemented with oil and inorganic nutrients (Roling et al. 2004).

1 Considering that the marine environment contains diverse marine microbes numbering approximately
2 3×10^{28} bacteria (Copley 2002), metagenomic-based approaches can be applied for the discovery of novel marine
3 microbial derived BS (Kennedy et al. 2011). Metagenomic analyses involve both sequence-based and function-
4 based strategies. Function-based strategies are used to screen metagenomics libraries constructed from marine
5 ecosystems which typically involves using *Escherichia coli* as the heterologous host and the subsequent screening
6 of this library. Sequence-based analyses, involves the identification of genes based upon homology with well
7 characterized genes that are found in sequence databases (e.g. BLAST, MEGAN, KEGG). However, the major
8 bottleneck for identifying genes from metagenomic sources is the identification of coding regions that do not have
9 homology in the sequence databases. Additionally, the metagenomic approach requires a deeper knowledge of
10 the diversity of BS molecules produced and the underlying genetic systems than we have at present.

11

12 **II. Culture conditions for production of BS from marine bacteria**

13 The quality and productivity of BS are determined by the bacterial strain and their growth conditions. The
14 optimization of growth conditions is important for the maximum production of BS. The composition and
15 productivity of BS are also influenced by carbon source, pH, temperature, salinity, nitrogen and agitation amongst
16 other factors. Marine microorganisms which require salts for growth are referred to as halophiles. Many marine
17 organisms such as moderate halophiles require 3%–15% (w/v) NaCl for optimal growth while extreme halophiles
18 grow optimally at 25% (w/v) NaCl (Margesin and Schinner 2001). *Halomonas* are known to produce different
19 types of glycolipids and glycoproteins with superior emulsifying activity than commercial emulsifiers. These
20 halophilic bacteria may play a vital role for the production of surfactants and emulsifiers in oil-polluted saline
21 environments. The growth of bacteria is influenced by salt concentration which also affects BS production. The
22 growth of halophilic bacteria at high salt concentration can reduce the contamination risks which can significantly
23 reduce upstream fermentation costs of BS production (Pepi et al. 2005; Tan et al. 2011). However, for large scale
24 industrial production growth media containing high salt concentration would be regarded as undesirable due to
25 the corrosive effects it would have on the production plant infra structure.

26 Different carbon sources in the growth medium influence the composition of BS production. Carbon
27 sources studied for BS production include crude oil, diesel, glucose, sucrose and glycerol. Several studies show
28 that marine bacteria can utilize hydrocarbons as substrate and produce BS, therefore the ability of hydrocarbon
29 degrading marine bacteria to produce BS could be utilized in the bioremediation of hydrocarbon-contaminated
30 environments. *Halomonas* sp. strain C2SS100 degraded hydrocarbons and produced BS at high salinity (Mnif et

1 al. 2009), while *Brevibacterium luteolum* synthesized BS using mineral oil as a carbon source (Vilela et al. 2014).
2 A *Brevibacillus* strain degraded phenanthrene to produce BS (Reddy et al. 2010) and *Alteromonas* sp. 17 degraded
3 hydrocarbons and produced BS using long chain alkane eicosane (Al-Mallah et al. 1990). Glycolipopeptide BS
4 was also produced by *Corynebacterium kutscheri* using cheaper carbon sources like waste motor lubricant oil and
5 peanut oil cake (Thavasi et al. 2007). It is important to bear in mind that for large-scale commercial production of
6 BS the cost of the growth medium may only constitute a smaller contribution to the total production costs when
7 the energy required to run the fermentation for a prolonged period is taken into account and since many renewable
8 plant oils are relatively cheap, readily available and of consistent composition. Therefore, the obsessive search for
9 'waste' materials as substrates towards achieving cost effective processes may be unjustified.

10 Nitrogen is vital for microbial growth and BS production. The type and concentration of the nitrogen
11 source plays an essential role in the optimization of BS production (Davis et al. 1999). Different nitrogen sources
12 could be used for the production of BS including, yeast extract, urea, peptone, ammonium sulphate, ammonium
13 nitrate and sodium nitrate. Yeast extract was the best nitrogen source for the production of the BS in the marine
14 *Streptomyces* species B3 (Khopade et al. 2012b). Sodium nitrate and yeast extract were preferred nitrogen sources
15 for BS production by marine *B. subtilis* N3-4P (Zhu et al. 2016) while the maximum BS production was observed
16 with phenyl alanine as the nitrogen source in marine *Nocardioopsis* B4 (Khopade et al. 2012a).

17 Another important parameter is temperature that greatly influences cell growth and BS production. BS
18 from thermophilic microorganisms are industrially preferred due to their thermostability at temperatures above
19 40°C, however BS produced from mesophiles also have high levels of thermo-stability and psychrophilic marine
20 bacteria capable of producing BS have potential applications for bioremediation in cold environments. The
21 hydrocarbon-degrading bacterium, *Rhodococcus* sp. obtained from the Norwegian coastline produced BS during
22 cultivation at 20°C with kerosene, n-hexadecane or rapeseed oil as a carbon source (Dang et al. 2016). The results
23 reported above clearly indicate that conditions for growth and BS production are often organism specific and each
24 organism isolated will need to be fully investigated to optimise both medium and production conditions.

25

26 **III. Characterization of BS production in marine bacteria**

27 The identification of possible genes involved during BS synthesis is necessary in order to understand BS synthesis
28 and develop robust BS producing strains with high production capacity. The most well-characterized low-
29 molecular weight BS is rhamnolipid, produced by several species of *Pseudomonads* and *Burkholderia*. The genes
30 *rhlA*, *rhlB* and *rhlC*, which are responsible for the biosynthesis of rhamnolipids have been found in *P. aeruginosa*

1 as well as non-pathogenic *B. thailandensis*. These three genes are localized within a single gene cluster in
2 *Burkholderia* while, the *rhlC* gene is located at two different, remote position within the *P. aeruginosa* genome
3 (Dubeau et al. 2009; Perfumo et al. 2013). Recently, homologues to *P. aeruginosa* rhamnolipid genes *rhlA* and
4 *rhlB* were identified in a non-pathogenic marine *Pseudomonas* species MCTG214(3b1) (Twigg et al. 2018). Most
5 of the genetic studies of BS production are limited to well-characterised BS molecules, but the expression of genes
6 involved in BS synthesis is not well studied in marine bacteria.

7 The genes and regulatory pathways are not necessarily identical in different BS producers. Different
8 species can produce a BS with totally different chemical structure and even small variations in the congener
9 composition of a surfactant can greatly affect its functional property. To fully understand how SAC synthesis is
10 regulated in these marine strains, it is important to characterise the chemical structure and necessary genes required
11 for BS synthesis. The chemical composition of the SAC molecules can be determined by electrospray ionization
12 mass spectrometry (ESI-MS), high performance liquid chromatography mass spectrometry (HPLC-MS) and
13 nuclear magnetic resonance (NMR) techniques (Smyth et al. 2010).

14 Few reports have been published regarding BS synthesis during hydrocarbon degradation in marine
15 bacteria. It was reported that *P. aeruginosa* JP-11 isolated from the marine environment utilized $98.8\% \pm 2.3\%$ of
16 biphenyl within 72h from contaminated sites. Although *P. aeruginosa* cannot be considered a true marine
17 bacterium, it is a common organism isolated from the marine environment. The production of BS was confirmed
18 by the expression of the rhamnolipid synthesizing genes *rhlAB* (Chakraborty and Das 2016). *Bacillus* species are
19 known to produce BS such as lichenysin, surfactin, fengycin, pumilacidin, iturin and bacillomycin (Vater et al.
20 2002). BS production was seen during anthracene degradation by a marine alkaliphile *Bacillus*
21 *licheniformis* (MTCC 5514). The strain degraded >95% of 300 ppm anthracene and showed tolerance up to
22 500 ppm of anthracene concentration. The gene involved in the BS lichenysin production was *licA3*, followed by
23 degradation through the catabolic degradative enzyme, catechol 2,3 dioxygenase (C23O) (Swaathy et al. 2014).

24 *Acinetobacter* species are also known to produce high-molecular weight emulsifiers (Ortega-de la Rosa
25 et al. 2018). In *Acinetobacter lwoffii* RAG-1, the genes encoding the biosynthesis of emulsan (a polysaccharide
26 BE) were reported to be clustered within a 27-kbp region termed, wee cluster (Nakar and Gutnick 2001). The
27 bioemulsan alasan produced by *A. radioresistens* is a complex mixture of anionic polysaccharides and protein.
28 The *alnA* gene which codes for the surface-active protein of alasan was cloned, sequenced, and expressed in *E.*
29 *coli*. Significant sequence similarity (21%) between the recombinant emulsifier protein AlnA of *A. radioresistens*
30 and OmpA of *E. coli* was seen. However, no emulsifying or hydrocarbon solubilizing activities have been

1 observed with *E. coli* OmpA (Toren et al. 2002). It has also been reported that the marine hydrocarbonoclastic
2 bacterium *Alcanivorax borkumensis* synthesizes glycolipids for hydrocarbon degradation (Abraham et al. 1998;
3 Yakimov et al. 1998). The genome sequence of *A. borkumensis* SK2 revealed its capacity for BS production. The
4 glycosyltransferase (ABO_1783) similar to RhlB from *P. aeruginosa* and glycosyltransferase protein family 9
5 (ABO_2215) were found to be potentially involved in BS production. The *A. borkumensis* SK2 genome encodes
6 other proteins involved in emulsifier production namely, OmpA (ABO_0822), OprG/OmpW (ABO_1922) and
7 OmpH (ABO_1152) (Schneiker et al. 2006). Similarly, genes involved in BS production were reported in the
8 marine bacterium *Achromobacter* sp. HZ01. The genome of strain HZ01 harbours OmpH (gene_1336) and OmpA
9 (gene_2469) which are both related to emulsifier production (Hong et al. 2017). Similar genes involved in
10 biosynthesis of BS were found in the genome of *Cobetia* sp. MM1IDA2H-1 (Ibacache-Quiroga et al. 2017). A
11 significant problem in using genetic information from one organism to another is the lack of sequence homology
12 between genes which may lead to the production of similar products. The rhamnolipid production genes in *P.*
13 *aeruginosa* PAO1 and *B. thailandensis* have only about 50% sequence homology (Dubeau et al. 2009). While in
14 a marine *Pseudomonas* species MCTG214(3b1), unrelated to *P. aeruginosa*, which produces di-rhamnolipids
15 identical in structure to *P. aeruginosa*, the *rhlA* and *rhlB* genes have very high sequence homology while it has so
16 far been impossible to find an *rhlC* homologue which indicates the presence of second novel rhamnosyltransferase
17 (Twigg et al. 2018). This suggests that it will be necessary to search for specific domains within the genes rather
18 than whole gene sequences when investigating such capabilities.

19

20 **3. Marine-derived BS in bioremediation**

21 The release of petroleum hydrocarbons in marine environments due to oil spill and chronic pollution is a serious
22 major concern. Chemical dispersants are effectively utilized worldwide to minimize oil spill damage. Dispersants
23 are mixtures of one or more surfactants and solvents that enhance dispersion of oil into droplets leading to
24 increased mobility and bioavailability of hydrocarbons. The dispersed oils are solubilized in water and degraded
25 by microorganisms. However, chemical dispersants are toxic to aquatic species and replacing them with biological
26 non-toxic alternatives would be very advantageous and highly sought after. Various marine γ -proteobacteria are
27 known to secrete cell surface amphiphilic substances (BS or BE) that allow the solubilization of aromatic
28 hydrocarbons. During growth on hydrocarbons, microbial cells attach to oil droplets by secreting BS to increase
29 the bioavailability of hydrocarbons. BS production by these bacteria increases dispersion of hydrocarbons thereby
30 enhancing their degradation by non-BS-producing microorganisms (McGenity et al. 2012; Perfumo et al. 2010).

1 Marine microorganisms such as *Halomonas*, *Marinobacter*, *Myroides* as well as the tropical marine yeast
2 *Yarrowia lipolytica* can play an important role in the ultimate removal of hydrocarbon compounds from
3 contaminated sites by the production of BE. Due to their diverse structural and functional property, these BE have
4 potential applications in bioremediation processes (Table 1). The *Halomonas* sp. participate in the removal of
5 spilled oil by synthesizing surface-active emulsifiers. Glycolipid molecules produced by *Halomonas* sp. on their
6 cell surface enhance the solubility of hydrocarbon, and thus increase their bioavailability for degradation. The
7 emulsifying BS produced by *Halomonas* sp. could be used for enhanced oil recovery processes in extreme
8 environments (Dhasayan et al. 2014), since the emulsifier produced by this bacterium at low temperature could
9 enhance the bioavailability of hydrocarbons in cold environments (Pepi et al. 2005).

10 Ornithine lipids, another type of BE produced by *Myroides* sp. SM1 have strong emulsification ability
11 for crude oil and stability in a wide range of temperatures and pH. It was reported to show better surface activity
12 than synthetic detergents and surfactin (Maneerat et al. 2006). Emulsan, the most powerful emulsion stabilizer
13 produced by *Acinetobacter calcoaceticus* RAG-1 was reported to have potential applications in microbial
14 enhanced oil recovery (MEOR) and cleaning oil spills (Belsky et al. 1979; Rosenberg et al. 1979). Other
15 biosurfactants have also been reported to enhance oil/hydrocarbon bioremediation activity (Banat et al. 2000;
16 Franzetti et al. 2011) The bioemulsifier produced by *Alteromonas* sp. 17 (now known as *Marinobacter*) could also
17 be used for the efficient degradation of hydrocarbons (Al-Mallah et al. 1990). In another report, a *Marinobacter*
18 species produced a phospholipopeptide class of BS capable of emulsification. The emulsions showed low
19 ecotoxicity and were able to disperse crude oil in artificial marine water suggesting their application for
20 bioremediation purposes (Raddadi et al. 2017). The glycolipid BS produced from *Marinobacter*
21 *hydrocarbonoclasticus* strain SdK644 showed 2-fold greater solubilisation of crude oil than tween 80 hence
22 showing potential in marine based bioremediation (Zenati et al. 2018). Another emulsifier “Yansan” produced by
23 *Yarrowia lipolytica*, an aerobic yeast showed high emulsification activity and stability over a pH range of 3–9 and
24 potential applications in the formulation of perfluorocarbon (PFC) based emulsions and degradation of
25 hydrocarbons (Amaral et al. 2006). Biosurfactants produced by *Candida lipolytica* yeast strain was also used
26 formulating a commercial related product for oil bioremediation (Santous et al. 2017).

27 28 **4. Marine-derived BS as antimicrobial and therapeutic agents**

29 The indiscriminate use of antibiotics leading to drug resistance among pathogenic organisms is responsible for
30 the rise of many life threatening diseases. Marine bacteria produce BS under fluctuating oceanic conditions such

1 as oil-contaminated waters as well as secondary metabolites for their defence and survival against other micro-
2 organisms. BS derived from the marine environment have been reported to inhibit cell adhesion and biofilm
3 formation (Das et al. 2009a; Kiran et al. 2010a). They effectively show antimicrobial, anti-adhesive and biofilm
4 disrupting activities against pathogenic micro-organisms. In addition, some have been found to display anti-
5 tumour/anti-cancer activity and, as a result can be a potential source for future drugs (Table 2). Marine derived
6 glycolipid BS show potential for the development of novel antibiofilm drugs. For instance, the marine
7 actinobacterium *Brevibacterium casei* MSA19 produces glycolipid BS which significantly disrupts the biofilm
8 formation in both mixed culture and individual strains at 30µg glycolipid/ml (Kiran et al. 2010a). Another
9 glycolipid BS from a marine *Staphylococcus saprophyticus* SBPS 15 showed antimicrobial activity against
10 different human pathogenic clinical isolates. The BS was stable at a broad range of pH (3–9) and temperature (up
11 to 80°C) (Mani et al. 2016).

12 The biological activities of lipopeptides have been reported in marine bacteria. Marine derived
13 lipopeptide, surfactin from *B. circulans* DMS-2 has potential antitumor activity against cancer cell lines HCT-15
14 (IC₅₀ 80 µg ml⁻¹) and HT-29 (IC₅₀ 120 µg ml⁻¹) (Sivapathasekaran et al. 2010). The lipopeptide BS produced by
15 the marine *B. circulans* has anti-adhesive activity against several potential pathogenic strains. It was seen that BS-
16 mediated surface conditioning significantly decreased bacterial adhesion of pathogenic strains like *E. coli*,
17 *Micrococcus flavus* and *Proteus vulgaris* up to 89% at a concentration as low as 0.1 g/l showing its potential in
18 biomedical applications (Das et al. 2009a). In another study a marine-derived *B. subtilis* SDNS produced ε-poly-
19 L-lysine (ε-PL) which showed anti-cancer activity against human Hela S3 cell line (El-Sersy et al. 2012).

20 The molecular structure of a particular lipopeptide variant defines its biological activity. For example,
21 the length of carbon-chain affects the antifungal activity in iturinic lipopeptides. Due to their hydrophobic nature
22 the longer fatty acid chain interact effectively with the cell membrane. As reported, among three different
23 lipopeptides isolated from marine derived *B. mojavensis*, the antifungal activity of fengycins (C₁₆ and C₁₇) were
24 stronger than mojavensin A (C₁₅) (Ma and Hu 2014; Ma et al. 2012). Likewise, the longer fatty acid chain isoform
25 C₁₆ of iturin A from marine *Bacillus megaterium* inhibited the proliferation of tumour cells by disrupting the Akt
26 pathway leading to apoptosis (Dey et al. 2015). In another report, fengycin fractions produced by *B. circulans*
27 strain, where out of four different fractions, antimicrobial activity was observed only with variants of C₁₆ and C₁₇
28 (Sivapathasekaran et al. 2009). The longer fatty acid chain of BS may offer advantages in increasing the surface-
29 activity of these molecules.

1 These properties suggest that marine-derived BS can serve as a source of new biomolecules for the
2 discovery of novel drugs. Other possible applications of BS can be the synthesis of metallic nanoparticles using
3 marine-derived surfactants. Metallic nanoparticles are receiving great interest in the field of biomedicine for
4 example, tissue engineering, drug delivery, detection of pathogens, detection of tumours, biological markers etc.
5 (Salata 2004). Recently BS have been used both in nanoparticle synthesis and stabilization (Plaza et al. 2014).
6 Microorganisms have developed the capability to grow and survive at high metal concentrations by various
7 mechanisms such as, impermeable cell membrane, efflux of toxic ions, oxidation or reduction of ions and
8 production of EPS (Decho 1990). Marine bacteria can bind a wide range of heavy metals through production of
9 BS and contribute to organic carbon cycling (Das et al. 2009b). Therefore, utilization of marine derived BS is a
10 safer route for environmental friendly synthesis of nanoparticles. In a study, a glycolipid BS was synthesized from
11 sponge-associated marine *Brevibacterium casei* MSA19 under solid state fermentation. The glycolipid helped in
12 stabilization of nanoparticles and prevented aggregation (Kiran et al. 2010b). Biosynthesis of metallic
13 nanoparticles using bacteria and fungi has gained more attention while, synthesis of metallic nanoparticles using
14 marine bacteria is an area yet to be fully explored (Plaza et al. 2014).

15

16 **5. Marine-derived BS in food and cosmetic formulations**

17 Due to increased use of stabilizing and thickening agents in food products, these industries are looking for
18 ingredients which can improve food quality and properties. Gum arabic, xanthan gum and lecithin are widely used
19 hydrocolloid emulsifiers which efficiently emulsify and stabilize oil-in-water emulsions. In Quorn products
20 (quorn.co.uk), dried fungus culture from *Fusarium venenatum* is used together with egg albumen as a binder.
21 While, in vegan food products, potato protein is added in place of egg albumen. However, climate change and
22 weather fluctuations such as drought will produce defoliation and affect the production of plant based gums. In
23 order, to reduce the dependency on synthetic emulsifiers and plant based emulsifiers there is increased interest in
24 finding new ingredients. Contrary to chemical surfactants, marine derived BS are non-toxic and/or less toxic with
25 high stability at extreme temperature, pH and salinity.

26 Not many studies on the applications of marine-derived BS in food and cosmetic formulations have been
27 reported so far. The only commercialized microbial emulsifier is Emulsan produced from *A. calcoaceticus*. BS,
28 which due to its emulsion stabilizing property, can improve consistency, texture and solubilisation of fat globules,
29 and aroma in food products. Incorporation of BE has been found to improve the rheology of dough, increasing
30 the volume and emulsification of fat and thus finds useful applications in the bakery and meat processing

1 industries. For example, a glycoprotein emulsion stabilizer produced by marine *Antarctobacter* sp. TG22 can
2 produce stable oil-in-water emulsions with commercial food-grade oils (Gutierrez et al. 2007a). Similarly two
3 other glycoprotein emulsifiers from marine *Halomonas* species TG39 and TG67 show superior emulsifying
4 properties when compared to commercial emulsifiers with stable activity under acidic conditions and high
5 temperatures (Gutierrez et al. 2007b).

6 The lipopeptide MSA31 from a marine *Nesterenkonia* species is an effective emulsifier, with good
7 antioxidant activity and its addition to muffins improved softness and retained food quality. The lipopeptide
8 MSA31 effectively reduced 90% of biofilm formed by *Staphylococcus aureus* and was non-toxic to the brine
9 shrimp nauplii (up to 200µg/ml) (Kiran et al. 2017). Another bioemulsifier produced by a marine bacterium
10 *Enterobacter cloacae* was reported to promote the viscosity of acidic food products (Iyer et al. 2006). In the food
11 industry, there is wide scope for marine derived-BS as emulsifiers, stabilizing agents, antimicrobial and
12 antiadhesives/antibiofilm agents (Table 3).

13 Chemicals used in cosmetic formulations often cause skin irritations and allergies. There is significant
14 interest in natural cosmetic products among consumers. Replacement of SACs in these products with BS can
15 reduce such harmful effects. The surface-active properties are essential to determine the type and amount of BS
16 in detergents, cosmetic, pharmaceuticals and various other industries. The type of BS compound to be
17 incorporated in the formulations can be selected based on their emulsifying ability and/or surface-activity such
18 as, hydrophilic–lipophilic balance (HLB) and critical micelle concentration (CMC), respectively. The HLB
19 defines the polarity of the BS, which gives an indication of its solubility in different systems. BS with a high HLB
20 value indicates that it is highly hydrophilic while, a low HLB value shows a high lipophilic character. Based on
21 the HLB values, a BS will be an emulsifier, antifoaming agent and wetting agent which are desirable properties
22 in cosmetic products.

23 CMC is the minimum concentration of BS required to lower the ST of water. At the CMC surfactant
24 molecules form micelles to reduce surface tension and interfacial tension. The surface-active properties of a BS
25 are determined by its side chain length, unsaturated bonds and the size of hydrophilic group. With increasing
26 hydrophobicity, the CMC of BS molecules tends to decrease. That means, a lower concentration of BS is required
27 for micelle formation. It is reported, *Burkholderia thailandensis* producing rhamnolipids Rha-Rha-C₁₄-C₁₄ has a
28 CMC of 225 mg L⁻¹ compared to *P. aeruginosa* PG201 producing Rha-Rha-C₁₀-C₁₀ of 600 mg/L. Due to the
29 hydrophobicity of the longer fatty acid chains, *Burkholderia* BS has a lower CMC compared to *P. aeruginosa*
30 (Dubeau et al. 2009).

1 Considering the foaming and emulsifying properties, BS can be used for different applications in health
2 care products including, cleansers, moisturizers, toothpaste and personal care products. Marine-derived BS
3 effectively show antimicrobial, anti-adhesive and biofilm disrupting activities against pathogenic
4 microorganisms. This property can be utilised in cosmetic and skin care products. Similar to skin cell membranes,
5 the fatty acid chain in BS can prevent generation of free radicals from UV radiation. Therefore, BS can be applied
6 as antioxidants in skin care products (Vecino et al. 2017).

7 Before introducing BS in industrial formulations, it is necessary to determine the toxicity of these SACs
8 on cells or animal models. For example, the toxicity of glycolipid BS (BS-SLSZ2) produced by a marine epizootic
9 bacterium *Staphylococcus lentus* towards eukaryotic model organism was determined. The glycolipid BS-SLSZ2
10 efficiently inhibited biofilm formation in *Vibrio harveyi* and *P. aeruginosa*. In vivo experiments showed that BS-
11 SLSZ2 was non-toxic towards *Artemia salina* and was effective in protecting *A. salina* against *V. harveyi* and *P.*
12 *aeruginosa* infections (Hamza et al. 2017). In another report, marine *Pseudomonas* sp. MK90e8 and MK91CC8
13 was reported to produce massetolide A, novel cyclic depsipeptide and viscosin, respectively. Massetolide A and
14 viscosin exhibited in vitro antimicrobial activity against *Mycobacterium tuberculosis* and *Mycobacterium avium-*
15 *intracellulare*. The effect of massetolide A was found to be non-toxic to mice at a dose of 10 mg/kg thus showing
16 potential in treating infections against *Mycobacterium* (Gerard et al. 1997).

17 The activity of Pseudofactin II (PFII), a lipopeptide BS isolated from the Arctic strain of *P. fluorescens*
18 BD5 was compared with normal human dermal fibroblast (NHDF). PFII induced apoptosis of melanoma skin
19 cancer cells while NHDF were less affected under same conditions. The mechanism of melanoma cell death may
20 be due to increased plasma membrane permeability by BS micelles. The activity of PFII was most effective above
21 CMC (130–140 μ M) (Janek et al. 2013). The toxicity of BS from marine bacteria *Nocardiopsis VITSISB* was
22 evaluated in toothpaste formulation. BS was found to more efficient and less toxic surfactant compared to
23 chemical surfactant sodium lauryl sulphate (Das et al. 2013). Further investigations should be done to supply safe
24 and effective products to satisfy consumers' demands.

25 26 **6. Recommendation and conclusion**

27 Due to their interesting biological properties, BS from extremophiles have great potentials with a broad range of
28 applications for industrial and consumer products. Considering their properties such as, emulsifiers, thickeners,
29 anti-oxidants, extreme tolerance to pH or temperature as well as antifungal and antimicrobial activities we look
30 forward to potentially new products with marine-derived BS as their surface-active ingredients. Although highly

1 desirable they also have their disadvantages such as the production of relatively low yield of BS and high
2 production costs. In some studies, either the product yield is very low or have not been quantified. Additionally,
3 in some studies, the taxonomic identification of strains has not been carried out using reliable methods. As
4 suggested by Irorere et al. (2017) criteria should be followed before claims for a BS producing strain and the
5 identity of the product are made. In comparison to BS produced from mesophilic microorganisms, not much work
6 has been done on marine BS. Fermentation optimisation by varying physiochemical factors including,
7 temperature, pH, aeration and agitation speed and nitrogen would be beneficial to increase the product yield. In
8 order to develop robust BS producing strains it is important to identify the genes involved in SACs biosynthesis
9 and to investigate the regulatory mechanisms involved in BS synthesis by targeting those regulatory genes.

10 Close examination of the information presented in Tables 1, 2 and 3 reveals the deficiencies in the data
11 currently available from the published literature. Although many of the organisms have been reliably identified
12 using 16S rRNA sequence data, other identifications have relied on morphological, physiological and biochemical
13 tests. This lack of specific taxonomic data makes extrapolations from one study to another significantly more
14 difficult. The other glaring lack of information concerns the yield of BS produced either as a crude yield or as a
15 purified material. As we have already pointed out one important issue dictating whether BS will become a
16 common constituent of consumer products or will be used in bulk bioremediations is cost. We can see from the
17 few yield figures given that in general they are exceptionally low, of the order of mg/L, rather than g/L. In the
18 case of one microbial biosurfactant that has reached industrial use, the sophorolipid from *Starmerella bombicola*,
19 yields are of the order of hundreds of g/L. Until the production from marine organisms can be increased, either
20 through the manipulation of the growth conditions or through genetic manipulation, there is little prospect of
21 major commercial interest.

22 The final point we can take from data in the tables is the lack of consistency and reliability of the methods
23 used to identify the products. In some instances, no real attempts have been made to specifically identify the
24 products and in others general analytical methods have been used which only provide indicative information
25 concerning the structures. In many of the applications under considered for BS, particularly by the pharmaceutical
26 and cosmetic industries, specific knowledge of the compounds being produced is imperative. In many cases
27 microbial BS are typically a mixture of different congeners and where bioactivity is being investigated it is critical
28 that pure samples of individual congeners are used in order to be able to assign a specific bioactivity to a molecule.
29 Mixtures of different molecules may have competing activities which may cancel each other out.

1 Some studies have been done on potential rhamnolipid producing strains including *P. aeruginosa* and
2 *Burkholderia* species to enhance rhamnolipid production. One such approach is cloning the rhamnolipid
3 biosynthesis genes *rhlA* and *rhlB* into non- rhamnolipid producing strains, *E. coli* BL21(DE3) and *P. aeruginosa*
4 PAO1 *ΔrhlA*. However, the BS yield from the recombinant strains was too low in comparison to the wild type
5 strains (Wang et al. 2007). Another approach to increase the BS yield is to silence the genes competing with the
6 BS production pathway. For example, Funston et al. (2017) knocked-out the polyhydroxyalkanoate (PHA)
7 pathway synthesis genes for enhanced rhamnolipid production. The halophile *Halomonas* species are ubiquitous
8 in marine environments and produces BE which effectively emulsifies hydrocarbons and other petroleum
9 contaminants. These species are known BS producers as well as PHA producers. However, the effect of PHA
10 deficient mutation on the yield of its SACs is not known. It might be interesting to follow a similar strategy in
11 *Halomonas* species to reduce the carbon flux towards other metabolic pathways and increase the BS production.

12 The chemical composition and toxicity must be known before commercialisation of the SACs. The
13 developed techniques such as high-performance liquid chromatography (HPLC) and ultra-performance liquid
14 chromatography tandem mass spectrometry (UPLC-MS/MS) can be used to analyse BS congeners (Rudden et al.
15 2015). The analysis and purification of a particular BS fraction displaying bioactivity is a prerequisite for
16 subsequent biomedical applications. Reverse-phase high-performance liquid chromatography (RP-HPLC) has
17 recently been used to fractionate and purify glycolipid and lipopeptide compounds (Sivapathasekaran et al. 2009).
18 The separation and purification of BS can enhance the level of bioactivity of BS isoforms. The antimicrobial
19 activity of the lipopeptide was reported to increase further after purification (Mukherjee et al. 2009). While, the
20 toxicity of BS can be tested in eukaryotic models such as, *Caenorhabditis elegans* and *Artemia* sp. The toxicity
21 of the BS producing strain can be tested in the *Galleria mellonella* model. The *Galleria* model was used to test the
22 pathogenicity in rhamnolipid BS producing strains and it was found that *B. thailandensis* was significantly less
23 pathogenic than *P. aeruginosa* (Irorere et al. 2018) and that a marine Pseudomonad was also non-pathogenic
24 (Twigg et al. 2018).

25 Marine-derived BS effectively emulsify hydrocarbons therefore, are suitable for marine-based
26 bioremediation. The improved biodegradation levels obtained with marine-derived BS indicate that they represent
27 the most efficient accelerators for hydrocarbon biodegradation through increasing the bioavailability of oil. Use
28 of extremophiles such as, *Acinetobacter*, *Halomonas*, *Marinobacter* which effectively produce BS and participate
29 in marine bioremediation should be preferred over mesophilic microorganisms for remediation of oil spills.
30 Psychrophilic bacteria are capable of degrading crude oil efficiently at low temperatures. Therefore, BS produced

1 from psychrophilic bacteria are suitable for bioremediation of hydrocarbon contaminated sites in cold
2 environments. For bioremediation purposes, the impurities within the BS mixture act as a co-substrate and
3 enhance the degradation of pollutants (Mata-Sandoval et al. 2001). Considering the downstream processing costs
4 further purification of BS is not required for bioremediation purposes.

5

6 **Acknowledgements**

7 The authors LT, RM and IMB acknowledge the financial support from the MARISURF Project funded by the
8 European Union Framework Programme for Research and Innovation, Horizon 2020 under grant agreement no.
9 635340. VUI acknowledges Ulster University Vice Chancellor's Research Scholarship.

10

11 **Compliance with ethical standards**

12 **Conflict of interest**

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

14

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Table 1. Summary of biosurfactant/bioemulsifier suggested for bioremediation applications produced by different marine microorganisms

Organism	Organism identification method(s)	Biosurfactant type	Biosurfactant Property	BS/BE yield	Characterisation method	Reference
<i>Halomonas</i> sp. MB-30	16S rRNA	Glycolipid	MEOR	NA	FTIR, NMR	(Dhasayan et al. 2014)
<i>Brevibacterium luteolum</i>	16S rRNA	Lipopeptide	Bioremediation	NA	FTIR	(Vilela et al. 2014)
<i>Myroides</i> sp. SM1	16S rRNA	L-ornithine lipid	Emulsify crude oil	2.6 g/10 l	MS, NMR, FTIR, GC-MS	(Maneerat et al. 2006)
<i>Acinetobacter calcoaceticus</i> RAG-1	Enrichment culture technique	Emulsan: heteropolysaccharide protein	Bioremediation, MEOR	1.5 g/27 l (heptane extraction), 2.1g/10 l (ammonium sulphate precipitation)	IR, NMR, GLC	(Belsky et al. 1979; Reisfeld et al. 1972; Rosenberg et al. 1979)
<i>Marinobacter</i> sp.	Identified according to Bergey's manual of systematic bacteriology	Carbohydrates: lipids complex	Emulsify HCs	NA	TLC, GC	(Al-Mallah et al. 1990)
<i>Marinobacter</i> sp.	16S rRNA	Phospholipopeptide	Biodispersant	NA	FTIR	(Raddadi et al. 2017)
<i>Yarrowia lipolytica</i>	NA	Glycoprotein	Water-in-oil emulsions with HCs and with PFCs	NA	FTIR, Raman and NMR	(Amaral et al. 2006)
<i>B. stratosphericus</i> FLU5	16S rRNA	NA	Bioremediation	NA	NA	(Hentati et al. 2016)
<i>Achromobacter</i> sp. HZ01	16S rRNA	Lipopeptide	Emulsify HCs	6.8 g/l	FTIR, GC-MS, HPLC-LTQ-Orbitrap MS	(Deng et al. 2016)
<i>Nocardiopsis</i> VITSISB (KC958579)	16S rRNA	Rhamnolipid	Bioremediation of oil spill	NA	FTIR, GC-MS	(Roy et al. 2015)

4 MEOR: microbial enhanced oil recovery, NA: not available, TLC: thin-layer chromatography, FTIR: Fourier transform infrared spectrometry, NMR: nuclear magnetic
5 resonance, MS: Mass spectrometry, IR: Infrared, HPLC-LTQ-orbitrap: high performance liquid chromatography coupled with linear ion trap-orbitrap mass spectrometry, GC:
6 Gas Chromatography, GLC: Gas Liquid Chromatography, HCs: hydrocarbons, PFC: perfluorocarbons

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1 Table 2. Summary of biosurfactant/bioemulsifier suggested for biomedical applications produced by different marine microorganisms

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Organism	Organism identification method(s)	Biosurfactant type	Biosurfactant Property	BS/BE yield	Characterisation method	Reference
<i>Brevibacterium casei</i> MSA19	Morphological, biochemical and phylogenetic analysis	Glycolipid	Anti-biofilm, nanoparticle formation	18 g/l	Orcinol, TLC, FTIR, HPLC, GC-MS	(Kiran et al. 2010a; 2010b)
<i>Staphylococcus saprophyticus</i> SBPS 15	16S rRNA	Glycolipid	Antimicrobial	1.345 ± 0.06 g/l	TLC, FTIR, MALDI-ToF-MS	(Mani et al. 2016)
<i>B. circulans</i> DMS-2	Identified at MTCC	Lipopeptide	Anti-tumor	1.64 ± 0.1	HPTLC, FTIR, MALDI-ToF	(Sivapathasekaran et al. 2010)
<i>Bacillus circulans</i>	Biochemical microbial identification method	Lipopeptide	Antimicrobial, anti-adhesion	1 g/l	UV-visible spectroscopy, TLC, HPLC, FTIR, MALDI-ToF	(Das et al. 2009a; Mukherjee et al. 2009; Sivapathasekaran et al. 2009)
<i>B. subtilis</i> SDNS	16s rRNA	ε-poly-L-lysine	Anti-cancer	76.3 mg/l	HPLC, TLC	(El-Sersy et al. 2012)
<i>B. mojavensis</i> B0621A	16S rRNA	Lipopeptide	Cytotoxic activity, antifungal	15.5 g/24 l	HPLC, NMR, RP-HPLC, GC-MS	(Ma and Hu 2014; Ma et al. 2012)
<i>Bacillus megaterium</i>	Morphological, physiological and biochemical tests	Lipopeptide	Anti-cancer	NA	RP-HPLC	(Dey et al. 2015)
<i>Aneurinibacillus aneurinilyticus</i> SBP-11	16S rRNA	Lipopeptide	Antimicrobial activity and crude oil recovery	NA	TLC, GC-MS, MALDI-TOF-MS, FT-IR, NMR	(Balan et al. 2017)
<i>Brevibacillus laterosporus</i> PNG-276	16S rRNA	Lipopeptide	Antimicrobial	NA	HPLC, MS, NMR	(Desjardine et al. 2007)
<i>Staphylococcus lentus</i>	16S rRNA	Glycolipid	Anti-adhesive, anti-biofilm	NA	Orcinol, GC-MS, FTIR, TLC, HRMS	(Hamza et al. 2017)

3 NA: not available, TLC: thin-layer chromatography, FTIR: Fourier transform infrared spectrometry, NMR: nuclear magnetic resonance, HPLC: high performance liquid
 4 chromatography, RP-HPLC: Reversed phase-high performance liquid chromatography, MALDI-ToF MS: matrix assisted laser desorption ionization-time of flight mass
 5 spectrometry, GC: Gas Chromatography, HPTLC: High Performance Thin Layer Chromatography, HRMS: high-resolution mass spectrometer, MTCC: Microbial Type Culture
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Table 3. Summary of biosurfactant/bioemulsifier suggested for food/cosmetic applications produced by different marine microorganisms

Organism	Organism identification method(s)	Biosurfactant type	Biosurfactant Property	Biosurfactant yield	Characterisation method	Reference
<i>Antarctobacter</i> sp. TG22	16S rRNA	Glycoprotein	Emulsion-stabilizing agent	21.1 mg/l	HPLC, GC, Size-exclusion chromatography, NMR	(Gutierrez et al. 2007a)
<i>Halomonas</i> species TG39 and TG67	16S rRNA	Glycoprotein	Emulsification and stabilisation of food oils	131.0 ± 0.07 mg/l, 28.0 ± 0.01 mg/l	HPLC, NMR, GC	(Gutierrez et al. 2007b)
<i>Nesterenkonia</i> species MSA31	16S rRNA	Lipopeptide	Emulsifier-stabilizing agent in food, anti-biofilm, antioxidant	NA	GC-MS, FTIR, TLC, NMR	(Kiran et al. 2017)
<i>Enterobacter cloacae</i>	API system	EPS	Emulsifier-stabilizing agent in food	NA	NA	(Iyer et al. 2006)
<i>Nocardiopsis</i> VITSISB	NA	NA	Emulsifying and foaming agent in toothpaste	NA	NA	(Das et al. 2013)
<i>Pseudomonas fluorescens</i> BD5	16S rRNA	Lipopeptide	Anti-melanoma compound	10 mg/l	RP-HPLC, MALDI-ToF-MS, MS/MS	(Janek et al. 2010; 2013)

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NA: not available, API: analytical profile index, TLC: thin-layer chromatography, FTIR: Fourier transform infrared spectrometry, NMR: nuclear magnetic resonance, MS/MS: Tandem mass spectrometry, RP-HPLC: Reversed phase-high performance liquid chromatography, MALDI-TOF MS: matrix assisted laser desorption ionization-time of flight mass spectrometry, GC: Gas Chromatography