

Lipopeptides: Biosynthesis and Applications

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Abstract

Microbial surfactants are amphiphilic molecules. They possess a strong tendency to reduce the interfacial tension among compounds. Biosurfactants possess diverse chemical structures and are nontoxic and hence are biodegradable. They have many applications in bioremediation, enhanced oil recovery, pharmaceutical and in food industry. In addition, biosurfactants have antifungal, antibacterial and antiviral properties. They are also found to have many therapeutic applications which as a result are very helpful for mankind in eradicating many pathogenic diseases. This review depicts the applications of lipopeptide biosurfactants in various fields.

Keywords: Bio surfactants; Applications; Biosynthesis; Lipopeptides

Introduction

Today environmental degradation is a major concern of policy makers and governments worldwide. Sustainable development is a challenging concept, with theories shaped by people's and organizations different worldviews, which in turn influence how issues are formulated and actions proposed. It is usually presented as the intersection between environment, society and economy, which are conceived of as separate although connected entities. We would argue that these are not unified entities: rather they are fractured and multi-layered and can be considered at different spatial levels. The priority is always given to economy in policies and the environment is viewed as apart from humans. They are interconnected, with the economy dependent on society and the environment while human existence and society are dependent on, and within the environment. The separation of environment, society and economy often leads to a narrow technoscientific approach, while issues to do with society that are most likely to challenge the present socio-economic structure are often marginalized, in particular the sustainability of communities and the maintenance of cultural diversity. It is rightly said that "necessity is the mother of invention," so biosurfactants have got more emphasis than their synthetic counterparts because they are eco-friendly, biodegradable, less toxic and biocompatible than the synthetic surfactants. Biosurfactants are surfactant active compounds which are produced by microorganisms. These are amphiphilic molecules and possess a spectacular tendency to reduce the interfacial tension of aqueous media. They contain both hydrophobic and hydrophilic moieties. They vary in their molecular size, structure, chemical properties and composition. Biosurfactants possess antifungal, antibacterial and antiviral quantities. Moreover, they also have anti-adhesive action against many pathogenic microorganisms. Biosurfactants are found to have many applications in enhanced oil recovery, bioremediation of heavy metals, pharmaceuticals, food and cosmetic industry. In addition, biosurfactants also have anti-microbial and anti-fouling properties. Microbial surfactants also play a vital role in swarming motility of microorganisms. There is a tremendous increase in global biosurfactants market in the last few years. According to Transperacy Market Research, both the household detergents and personal care segment will be the main focus that will contribute more than 56.8% of the global biosurfactants market in 2018. In addition, according to their survey Europe is at the top worldwide in production and consumption of biosurfactants, followed by North America. Another report given by Markets and Markets, the average of biosurfactant market in the whole world is expected to be 476,512.2 tons by 2018. Out of this total, 21% of volume consumption will come from developing regions such

as Asia, Africa and Latin America. In 2013, the global biosurfactants market was 344,068.40 tons and is expected to reach 461,991.67 tons by 2020, growing at a CAGR of 4.3% from 2014 to 2020. The patents on biosurfactants are continuously increasing worldwide.

This review represents the biosynthesis of lipopeptide biosurfactants and their applications in various fields.

Biosurfactants: An Overview

The first rhamnase containing biosurfactant was produced by *Pseudomonas aeruginosa* and it was first elucidated by Jarvis and Johnson in 1949 while the biotechnological concern in biosurfactants started in the 1980s, but at that time the use of biosurfactants was only restricted to oil recovery and bioremediation [1]. Initially, hydrocarbons were used as the main C-source for the biosurfactant production [2,3]. The main focus of this research was to identify or find the microbial species which can be able to produce biosurfactants, [4] explanation of their chemical structure [5,6] and to study their surface active properties [6]. In 1980s, the biosurfactants became very interesting class of compounds but were much expensive than their synthetic counterparts [7]. The main cause of this economic drawback was the high substrate cost i.e. pure hydrocarbons. Moreover, microbial strains were pathogenic or due to lack of technology, it was difficult to handle at a large scale [8]. Even though, biosurfactants possess surfactant properties and show similar features with those of their chemically or petrochemically derived counterparts, their commercial interest in late 80s was only restricted to produce valuable sugars like L-rhamnase [1] for nutrition and pharmaceutical applications [9]. Currently, biosurfactants are used in wider way like in cosmetics [10], in food industry [11] bioremediation (Kosaric, Makkar and Cameotra,) [12,13] due the use of new cheaper and renewable resources as substrates [14] in comparison to the pure hydrocarbons. Nowadays, biosurfactants have many applications in medicine and agricultural sector [15,16].

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Lipopeptides

Historical perspectives: The group of lipopeptides isolated during 1950s and 1960s from *Bacillus* sp. These contain more than 20 different peptides linked to various fatty acid chains. At present, more than a 100 different compounds can so be described.

Surfactin: It was 1968 when Arima et al. [18] from the supernatant of culture of *Bacillus subtilis* isolated an exocellular compound having an excellent biosurfactant activity. This compound was named surfactin and its structure was explicated as that of a lipopeptide [17]. It was exemplified as a valuable inhibitor of fibrin clot formation, an antibacterial, anti tumour and hypocholesterolemic agent.

Iturins: It is mentioned in the literature in 1949 (Walton and Woodruff) that Mycosubtilin was the first antifungal compound from *Bacillus subtilis*. Declambe in 1950 illustrated a second similar compound named iturin. Its name is associated to ituri, a region from Congo, where the compound was isolated from a soil sample. Iturin was first found to be a strong anti fungal agent with constrained anti bacterial activity against *micrococcus* and *sarcina* strains. During the 1970s and 1980s the accurate structure of these two compounds and similar compounds from the same species was described: mycosubtilin [18], bacillomycin L [19] identical to bacillomycin Lc or bacillopeptin [20], iturin A [21], iturin C [21], bacillomycins D and F [22]. Mycocerein, an anti fungal peptide produced by *Bacillus cereus* was partially described and could belong to the iturin family [23].

Fengycin and plipastatin: Discovery of Fengycin and plipastatin was concomitant. In 1986, German [24] and Japanese teams [25] discovered simultaneously a third family of lipopeptides: fengycin produced by *Bacillus subtilis* and plipastatin produced from *Bacillus cereus*. The first lipopeptide was determined to be an anti fungal agent and later as a phospholipase A2 inhibitor. There was only a small structural difference between these two compounds and a doubt still exists today about them as well as their biological activities. Production of fengycin was also determined by *Bacillus thuringiensis* [26].

Kurstakins: A new family of lipopeptides from *Bacillus* sp. [27] with the use of Matrix assisted laser desorption/ionization (MALDI) so as to characterize and differentiate between different species or strains of *Bacillus* spores discovered a new family of lipopeptides produced by *Bacillus thuringiensis* subsp. kurstaki and consequently called kurstakin. These compounds display anti fungal activity against *Stachybotrys charatum* and are mainly absorbed to the spore surfaces.

Lipopeptides structure: Lipopeptides are mainly produced by *Bacillus* species. These are low molecular weight compounds. Lipopeptides lowers the surface and interfacial tension more efficiently than glycolipids. Lipopeptides which are synthesized by *Bacillus* sp. have a good heterogeneity in accordance with the type and sequence of amino acid moiety and nature, length and branching of fatty acid chain and their moiety [28]. Lipopeptides are mainly classified into: Iturin biosurfactants, Surfactin biosurfactants and Fengycin biosurfactants.

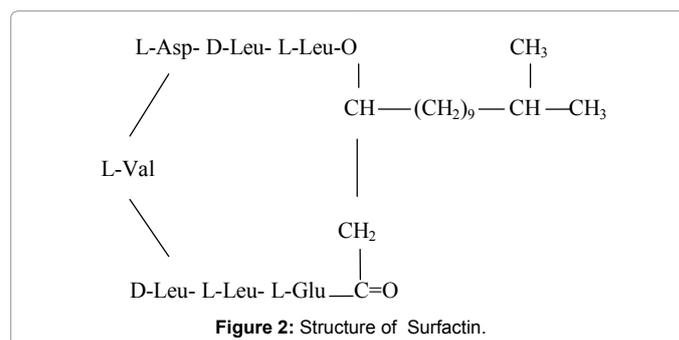
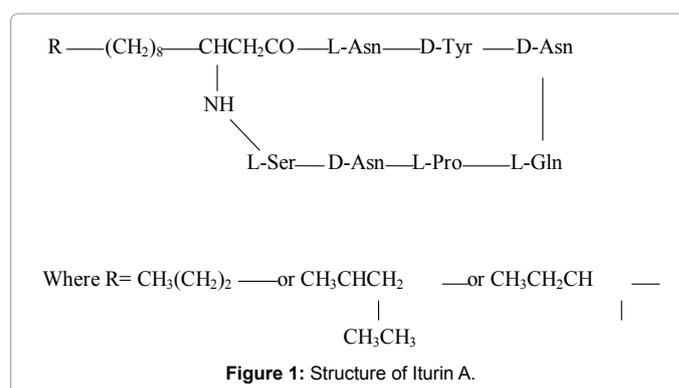
Iturin biosurfactants: These are heptapeptides linked to β -amino fatty acid chain with a length of C14 to C17. There are many types of iturin: Iturin A, C, bacillomycin D, F, L, Lc and mycosubtilin. These are produced by *B. subtilis* strains. Iturin A is the only lipopeptide which is produced by all *B. subtilis* strains. Iturin possess a very high antifungal activity against many yeast and fungal species whereas they have very limited antibacterial activity but no antiviral activity. Their fungi toxicity and their interaction are influenced because of their membrane permeabilization properties.

Surfactin biosurfactants: Surfactin was first discovered in the culture broth of *B. subtilis* in 1968 by Arima et al. [18]. It was named surfactin because of its exceptional surfactant activity [29]. They are cyclic lipopeptides and are heptapeptides interlinked with a β -hydroxy fatty acid. These are a mixture of isoforms A, B, C and D and are classified in accordance with the difference in their fatty acid sequence [30]. Surfactin lipopeptides are extensively studied. They possess a very potent haemolytic, antiviral, antimicrobial activity. Surfactin has a wide range of therapeutic applications than other biosurfactants.

Fengycin biosurfactants: These are deca-peptides with an internal lactone ring in the peptidic moiety and also with a β -hydroxy fatty acid chain (C14- C18). Fengycins are classified into Fengycins A and B. These are also known as plisplastatins. Fengycins are found to have a strong antifungal activity but are less haemolytic than iturins and surfactins. They really interact to lipid layers and to a certain extent have the ability to change cell membrane structure and permeability in a dose dependent way [28] (Figures 1 and 2).

Lipopeptide biosynthesis

B. subtilis lipopeptides are synthesized by non-ribosomal peptide synthetase (NRPSs) or hybrid polyketide synthases and NRPSs (PKSs/NRPSs). Biosynthesis of several hundred bioactive compounds is due to these molecular proteins. NRPS module contains about 1000 amino acids and is responsible for activation as an adenylate (A-domain), PCP domain (C-domain). Modules are mega enzymes organized in iterative functional units and perform the catalization of different reactions which leads to peptide transformation. Further, each module is sub-divided into several catalytic domains which are responsible for performing each biochemical reaction. SrfA-A, SrfA-B, SrfA-C are the three large open reading frame codes for surfactin synthesis. Products of Srf A-A and Srf A-B contains three modules while the last in Srf A-C. The fatty acid chain gets attached to the amino acid activated in the first module and synthesized product is released from the enzymatic



template due to the last activation of PCR domain. Further, the biosynthesis is initiated by second-thioesterase/acyltransferase which are encoded by a fourth gene of *srfA-D*. Fengycins are synthesized in the same way by NRPSs encoded by five open reading frames *fen A-E*. The two modules are contained by first three enzymes while the fourth enzyme contains three modules and the last enzyme contains one module. On the contrary, the iturin derivatives are synthesized by a hybrid complex (PKS-NRPSs). *Itu d*, *Itu A*, *Itu B* and *Itu C* are the four ORFs in the operon. The last three genes codes for first residue i.e. for *itu A*, the subsequent four residues for *itu B* and the last two residues for *itu C*. *Itu D* encodes malonyl-CoA transacylase, moreover *itu A* contains genes related to PKSs and these genes are responsible for the last elongation and β -amination, i.e., the last steps for the biosynthesis of fatty chain before it is transferred to the amino acid of peptide moiety.

Lipopeptides: Applications

Lipopeptides as anti microbial agents: The literature shows that lipopeptides show very strong anti-fungal, anti-microbial, anti tumour, anti-viral and immune modulator properties. Plaza et al. [31] have showed that surfactin, a type of lipopeptide produced from *Bacillus subtilis*, growing on molasses is profound to have anti-fungal and anti-bacterial properties. They concluded that surfactin from *Bacillus* species growing on molasses inhibited the growth of four fungi out of ten tested. The results showed the fungi inhibited to the highest percentage was *Botrytis cinerea* A258 (about 50% inhibition) followed by *Sclerotinia sclerotiorum* K2291 (about 50 inhibition), *Collectotrichum sclerotiorum* A259 (about 40 % inhibition), *Phoma complanata* A233 (about 38% inhibition), lastly, *Phoma exigua* var. *exigua* A175 (about 20% inhibition). Further, for testing anti bacterial activity *E. coli* inhibition was demonstrated by agar plate inhibition assays and high growth was noted among 76% *E. coli* isolates.

Nielson et al. [32], found out a new cyclic depsipeptide from *Pseudomonas fluorescens* DR54, named viscosinamide which showed anti fungal properties against *Phythium ultimum* and *Rhizoctonia solani*. They concluded that the new CLPs possess both biosurfactant and antibiotic properties, thereby can be used as a potent biological control agent. Nielson et al. [33] reported a new cyclic compound tensin from *Pseudomonas fluorescens* strain 96.578 having anti-fungal activity against plant pathogenic fungus *Rhizoctonia solani*. Kruijt et al. [34] found a putisolvin like cyclic lipopeptide biosurfactant produced from *P. putida* 267 having zoosporicidal and anti-fungal activity. Moreover, the CLPs are also found to be vital in swarming motility and biofilm formation. The CLPs were found to be potent against fungitoxicity *Phytophthora damping-off* of cucumber.

Lipopeptides as immunomodulators: Infante-Duarte et al. [35] demonstrated that the lipopeptides derived from *B. burgdorferi* stimulate the IL-17 expression (Cytokine produced by activated murine cytotoxic T-cells) in both murine and human T_H cells. They induce the IL-17 expression together with TNF- α , so are coexpressed with other proinflammatory cytokines. Das et al. [36] demonstrated the anti microbial activity of a lipopeptide biosurfactant produced from a marine *Bacillus circulans*. The produced biosurfactant showed a potent anti microbial activity against both gram positive and gram negative pathogenic and semi pathogenic microbial strains. Moreover, this biosurfactant was also found to be non haemolytic.

Lipopeptides in laundry and enhanced oil recovery: A.K. Mukherjee, [37] studied that Crude cyclic lipopeptide biosurfactant synthesized from two *B. subtilis* strains (DM-03 and DM-04). They

showed that (CLP) is stable and compatible to use as laundry detergents. Kiran et al. [38] found a new lipopeptide biosurfactant produced from strain *B. aureum*. The biosurfactant produced can be employed successfully in enhanced oil recovery process in marine environments.

Sriram et al. [39] found a lipopeptide from *E. fergusonii* KLU01. The biosurfactant found to be resistant to manganese, iron, lead, nickel, copper and zinc. So, this biosurfactant possess a potential in hydrocarbon degradation and heavy metal bioremediation. Pathak and Kaharia, [40] studied the extracellular lipopeptide produced from endophytic *Bacillus subtilis* K1 which was taken from aerial of banyan. The strain has high heterogeneity with secreted mixtures of surfactins, iturins and fengycins. The lipopeptide biosurfactant resulted in about 43% enhanced oil recovery on laboratory scale sand pack column with four stroke engine oil.

Suwat Soonglerdsongpha [41] isolated a lipopeptide biosurfactant from *Bacillus* sp. GY19. The results showed that 1 - 3% lipopeptide solutions had 80-100% oil displacement efficiency with Oman light oil, heavy oil, diesel oil and Arabian light oil. The activities were comparable to a commercial dispersant (Slickgone NS) and much higher than a synthetic surfactant (Tween 20). In addition, the lipopeptide solutions were stable at extreme salinity, pH, and temperature. Consequently, foamate and powder containing lipopeptides could be used directly as dispersants for oil spill remediation.

Sriram et al. [42] found a lipopeptide biosurfactant produced from, a propitious bacterial strain isolated from oil contaminated soil *Escherichia fergusonii* KLU01. The strain emerges as a new class of biosurfactant producer with potential environmental and industrial applications, especially in hydrocarbon degradation and heavy metal bioremediation. (Table 1)

Lipopeptides in biomedical applications: Kuiper et al. [43], found two lipopeptides putisolvin I and II from *P. putida* strain PCL 1445. These are the first reported lipopeptides having 12 amino acids. Putisolvin I and II inhibit the biofilm formation of *Pseudomonas* on polyvinylchloride and they can also break down the biofilms of many *Pseudomonas* sp. which are formed on PVC. Rivardo et al. [44], characterized two biosurfactant producing strains *B. subtilis* and *B. licheniformis*. The two lipopeptides showed good anti-adhesion activity able to significantly inhibit the biofilm formation of two pathogenic strains i.e. *E. coli* CFT073 and *S. aureus* ATCC 29213 up to 97% and 90%. It was observed that V9T14 biosurfactant was active on gram negative strain but ineffective against gram positive strain and vice versa for V19T21. Sriram et al. [45], identified the strain *Bacillus cereus* NK1 which produced a lipopeptide biosurfactant. The lipopeptide produced thus showed anti microbial activity and also inhibits the biofilm formation. The strain *B. cereus* NK1 (GU 167978) was isolated from soil sample. The resulting biosurfactant was able to inhibit the biofilm formation by *P. aeruginosa* and *S. epidermidis*. The maximum biofilm inhibition was found against *S. epidermidis* with 57% of inhibition percentage. The lipopeptide at conc. 15mg/ml performed the maximum biofilm inhibition of both the test organism. The strain was also found to be resistant against ferrous sulphate, lead acetate and zinc sulphate.

Pradhan et al. [46] isolated a biosurfactant producing halophile from Lake Chilika, Odisha, India. The halophile was identified as *Bacillus tequilensis* CH. The microbial surfactant produced by the halophile was lipopeptide. The biosurfactant was found to inhibit the biofilm of *E. coli* and *Streptococcus* mutans at the minimum concentration of 50 μ g/ml on both hydrophilic and hydrophobic surface.

Lipopeptides in nanotechnology: According to Plaza et al. [47] Lipopeptide biosurfactants can also be used in the synthesis of nanoparticles. According to Reddy et al. [48] Surfactin can be used as both the template and stabilizing agent in the synthesis of silver and gold nanoparticles. The stable gold nanoparticles were synthesized by reducing aqueous AuCl₄ using sodium borohydrate in the presence of surfactin which was produced by *Bacillus subtilis*.

Reddy et al. [49] used Surfactin as a templating-agent stabilizer to make nanostructured zinc oxide rose-like arrangements using the precipitation method. The rose-like structures were found to be 9 to 13 nm thick.

Singh et al. [50] used surfactin from *Bacillus amyloliquifaciens* for the synthesis of stable cadmium sulfide nanoparticles (CdS-NPs). The yield of the surfactin was found to be 160 mg/L. CdS nanoparticles were synthesized directly from this crude surfactin biosurfactant. The produced nanoparticles covered with surfactin were found to be stable for up to six months without any apparent change in their structure.

Conclusion

Initially, the use of lipopeptides was limited only to the enhanced oil recovery and to bioremediation. However, with the changing scenario over the last decade, lipopeptides have proved to be eco-friendly, biodegradable, less toxic and more biocompatible than the chemical surfactants, their use in pharmaceuticals, food and cosmetic industry has been increased to a great extent. Due to their anti-fungal, anti-bacterial, anti tumour and anti-viral properties they are now showing their potential for use as major immunomodulatory molecules and anti-adhesive agents. They are also being used for gene transfection, as ligands for binding immunoglobulins. They proved to be very useful in eradicating nosocomial infections caused by different microorganisms by inhibiting their biofilms. Microbial surfactants may also be incorporated into probiotic preparations to fight urogenital tract infections and pulmonary immunotherapy. Biosurfactants can also be used in production of green electricity as biosurfactants increase the surface area and emulsify hydrophobic compounds like oil (O/water and W/O emulsions). This could eventually lead to increase production of the products (Biofuel).

Therefore we can see, that over the period of time, lipopeptide biosurfactants have traveled a long way from being just seen as some chemical agents having their applications in environmental biotechnology to being seen as vital chemical moieties having major promising roles in human healthcare as well.

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