Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 80 (3) 279–304 (2015) UDC **Pseudomonas aeruginosa*:577.115+547.441:
JSCS–4716 661.185.000.57+66.0
Review

REVIEW

Rhamnolipid biosurfactant from *Pseudomonas aeruginosa* – from discovery to application in contemporary technology

MILENA G. RIKALOVIĆ[1], MIROSLAV M. VRVIĆ[1] and IVANKA M. KARADŽIĆ[2]*

[1]*Faculty of Chemistry, University of Belgrade, Studentski trg 12–16, Belgrade and* [2]*Department of Chemistry, Faculty of Medicine, University of Belgrade, Visegradska 26, Belgrade, Serbia*

(Received 27 June, revised 19 September, accepted 20 September 2014)

Abstract: The rhamnolipids will most likely be the next generation of biosurfactants to reach the market. They should follow closely after alkyl polyglycosides, already established in the biosurfactants market, and sophorolipids, which can be found in several cleaning agents. However, the greatest numbers of recent publications and patents among glycolipid biosurfactants have been dedicated to rhamnolipids. Produced mainly by *Pseudomonas aeruginosa*, rhamnolipids are mixtures of different rhamnolipid congeners, which show physico-chemical properties that differ from those of single congeners, with the most abundant structure in the mixture having the largest impact on the overall characteristics of the total mixture. Characteristics of biodegradability, low toxicity, production from renewable sources and antimicrobial (particularly antifungal) activity together make rhamnolipid biosurfactants particularly promising for broad commercial application. Although to date, bioremediation has been the major topic filed for patents utilizing rhamnolipids, the increasing number of patents for applications in cosmetics, agronomy and food industries, formulation of cleaners and nanotechnology indicates their future implementation in these fields.

Keywords: rhamnolipids; *Pseudomonas aeruginosa*; methods; production; application.

CONTENTS

* Corresponding author. E-mail: ikaradzic@med.bg.ac.rs
doi: 10.2298/JSC140627096R

1. INTRODUCTION

Research related to the characterization of microbial surfactants and their applications has been rapidly growing in recent years. The reasons for the increased attention in these naturally derived compounds are: diversity, environmentally friendly nature, biodegradability, low toxicity, excellent foaming properties, high selectivity and activity at extremes of temperature, pH and salinity, plus the fact that they are able to be produced from industrial wastes and by-products.[1] Biosurfactants have shown great potential for applications in environmental protection, crude oil recovery, health care and food-processing industries.[1–6]

Microbial surface active compounds are important for the physiology of the cells that produce them, as they are involved in cell motility (gliding and swarming motility as well as de-adhesion from surfaces), cell–cell interactions (biofilm formation, maintenance and maturation, quorum sensing, amensalism and pathogenicity), cellular differentiation, substrate accession (*via* direct interfacial contact and pseudosolubilization of substrates), as well as avoidance of toxic elements and compounds.[6]

Biosurfactants are divided into low-molecular-mass molecules, which efficiently lower surface and interfacial tension, and high-molecular-mass polymers, which are more effective as emulsion stabilizing agents.[6] The major classes of low-mass surfactants include glycolipids, lipopeptides and phospholipids, whereas high-mass surfactants include polymeric and particulate (vesicles and whole cells) surfactants.[6] *Pseudomonas* spp. are considered to be the most common producers of surface active compounds, synthesizing both classes of biosurfactants,[6,7] with *P. aeruginosa* being the preeminent rhamnolipid (RL) biosurfactant-producing bacteria species.[6,8]

After alkyl polyglycosides, bio-based surfactants that are already accepted on the market, and sophorolipids, which are in several cleaning agents formulations, RLs will probably be the next generation of biosurfactants to reach the market.[9] The properties of RLs, including biodegradability, low toxicity, the possibility for production from renewable sources, together with their antimicrobial and antifungal activity, make them particularly interesting for commercial application.[9]

Aside from the environmental use of RLs,[4,10,11] an increasing number of patents[9] indicate the potential successful application of rhamnolipids in cosmetics, agronomy, the food industry, cleaner formulations and nanotechnology. In this review, special attention is paid to RLs produced by *P. aeruginosa*, their properties and the potential for their use in different technological fields.

2. GLYCOLIPID BIOSURFACTANTS

2.1. The most researched glycolipid biosurfactants

The most well known biosurfactants are glycolipids, which are composed of carbohydrates linked by means of ether or ester bonds with either long-chain aliphatic acids or hydroxyaliphatic acids. Sophorolipids, trehalose lipids, mannosylerythritol lipids (MELs) and RLs are the most widely researched glycolipids.[2,12]

Sophorolipids are extracellularly produced by several yeast species, such as *Candida bombicola*, *C. apicola*, *Rhodotorula bogoriensis*, *Wickerhaminella domercqiae* and *C. batistae*.[13] These compounds contain two β-1,2 linked glucose units, with acetylated 6- and 6′-hydroxyl groups, connected to the lipid part by a glycosidic linkage.[14] Generally, sophorolipids occur as a mixture of macrolact-

ones and the free acid form of at least six to nine different sophorolipid congeners.[12] Applications of sophorolipids were reported in the biomedical field, in the synthesis of metal-bound nanoparticles in cosmetics and in the formulation of pharmaco-dermatological products.[13,15] Furthermore, these biosurfactants show potential for some recovery processes.[1]

Trehalose lipids are glycolipids containing trehalose as the sugar moiety, which is the basic component of the cell wall glycolipids in *Mycobacteria* and *Corynebacteria*.[16] Depending on the producing organism, trehalose lipids show differences in the size and structure of mycolic acid, the number of carbon atoms and the degree of unsaturation. The most reported trehalose lipid is trehalose 6,6′--dimycolate,[17] but different trehalose-containing glycolipids are known to be produced by several other microorganisms, such as *Arthrobacter*, *Nocardia*, *Rhodococcus* and *Gordonia*. Trehalose lipids have been applied in bioremediation to enhance the bioavailability of hydrocarbons. Recently, the increasing interest in trehalose lipids is related to their functions in cell membrane interaction and their potential as antitumor therapeutic agents.[17]

The mannosylerythritol glycolipids are reported as metabolites of yeasts strains, which belong to the genera *Pseudozyma* sp. and *Ustilago* sp., produced on soybean oil or *n*-alkane.[17] MELs are a mixture of partially acylated derivatives of 4-*O*-*β*-D-mannopyranosyl-D-erythritol, containing C_2, C_{12}, C_{14}, $C_{14:1}$, C_{16}, $C_{16:1}$, C_{18} and $C_{18:1}$ fatty acids as the hydrophobic groups.[18] Interesting applications of MELs have been reported in the biomedical field as antimicrobial, antitumor and immunomodulating molecules, in the biotechnological field for gene and drug delivery, and in cosmetic applications as skin moisturizers.[15,17,18]

RLs are glycosides, produced mainly by *P. aeruginosa* and by the genus *Burkholderia*, composed of one (for mono-rhamnolipids) or two (for di-rhamnolipids) rhamnose sugar moieties linked to one or two *β*-hydroxy fatty acid chains. RLs in which one or two molecules of rhamnose are linked to one or two molecules of *β*-hydroxydecanoic acid (Fig. 1) are the best-studied biosurfactant compounds. While the OH group of one of the acids is involved in a glycosidic linkage with the reducing end of the rhamnose disaccharide, the OH group of the second acid is involved in ester formation. RLs are the principal glycolipid produced by *P. aeruginosa*.[12] The major field of application of RLs is in bioremediation processes.[1,10,19] Additionally, these molecules, due to their physicochemical and biological properties, have great potential for applications in the biomedicine, pharmacology, cosmetics, food and agricultural industries.[12,15,17,19] RLs have also been used in the preparation of nanoparticles and microemulsions.[17]

2.2. Rhamnolipids

2.2.1. Discovery

The discovery of RLs dates back to 1946, when Bergström *et al.*[20,21] reported an oily glycolipid, which was named piolipic acid, composed of L-rhamnose and *β*-hydroxydecanoic acid,[22,23] produced by *P. pyocyaneus* (today *P. aeruginosa*), when cultivated on glucose. Further characterization of the structure by Jarvis and Johnson[23] showed that the isolated compound was composed of two *β*-hydroxydecanoic acids (connected *via* an ester bond) linked through a glycosidic bond to two rhamnose moieties. Additionally, Edwards and Hayashi,[24] using periodate oxidation and methylation, reported that the linkage between the two rhamnose moieties is an *α*-1,2-glycosidic linkage. Based on this, they chemically described the RL as 2-*O*-*α*-L-rhamnopyranosyl-*α*-L-rhamnopyranosyl-*β*-hydroxydecanoyl-*β*-hydroxydecanoate (di-RL structure). This was the first glycolipid discovered containing a link between a sugar and a hydroxylated fatty acid residue.[25] From 1972 to 2000, various RL structures produced by *P. aeruginosa* strains growing on different carbon sources (*n*-paraffins, glycerol, *n*-alkanes, glucose, *etc*.), were isolated and reported. The isolated RLs included all types of RL homologues (RL1, RL2, RL3 and RL4), and their number increased with the progress of analytical methods.[8]

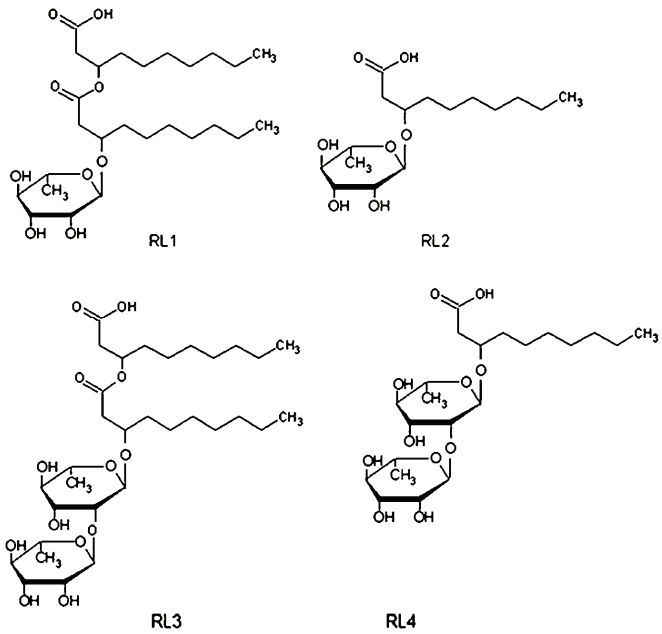

Fig. 1. Structure of rhamnolipid biosurfactants: mono-rhamno-di-lipidic structure (RL1), mono-rhamno-mono-lipidic structure (RL2), di-rhamno-di-lipidic structure (RL3) and di-rhamno-mono-lipidic structure (RL4).

Furthermore, some studies reported the complex composition of bacterially produced RL mixtures, including, for example, an RL mixture composed of 67 % di-rhamno-di-lipid, 22 % mono-rhamno-di-lipid, 9 % di-rhamno-mono-lipid, and less than 3 % of mono-rhamno-mono-lipid.[26] The development of sensitive, high throughput analytical techniques has led to the further discovery of a wide diversity of RL congeners and homologues (about 60) produced in different concentrations by various *Pseudomonas* species and other bacteria.[8]

2.2.2. *Pseudomonas* spp. as producers of rhamnolipids

For more than 60 years, much work has been undertaken to stimulate the development and establishment of RL production by *P. aeruginosa*.[5] When comparing the production of RLs by different *Pseudomonas* species, Onbasli and Aslim[27] found that *P. luteola* and *P. putida* gave higher RL yields than *P. aeruginosa*, *P. fluorescens* and *P. stutzerri*, but the composition and distribution of the RL homologues were not described for these strains, while the production of RLs by *P. chlororaphis* had lower yield than *P. aeruginosa*.[28] Furthermore, reports about RLs obtained from *Pseudomonas* species other than *aeruginosa* lack information about their surface properties, which is the most important indicator of surfactant quality.

3. PROPERTIES OF RHAMNOLIPID MIXTURES FROM *P. aeruginosa*

3.1. Diversity

Interest in RLs arises from two contrasting facts. On the one hand, RLs show relatively high surface activities, and are produced in relatively high yields after fermentation processes, which are relatively well understood. On the other hand, they are considered as virulence factors involved in the processes of *P. aeruginosa* pathogenesis, resulting in an investigation of RL biosynthesis in order to control their production and effects.[29]

P. aeruginosa produces various extracellular mono- and di-rhamnolipids. Two types of RLs (Fig. 1), termed RL1 and RL3, consisting of one or two L-rhamnose units and two units of ß-hydroxydecanoic acid, are the principal RLs. There are also RL2 and RL4 RLs, which consist of one or two L-rhamnose units and one unit of ß-hydroxydecanoic acid.[30]

3.2. Composition

Naturally produced RLs always appear as mixtures of different RL congeners, as was observed when produced by various strains of *P. aeruginosa*.[31–38] The complexity of the composition of RL mixtures was found to depend on various factors such as: origin of the bacterial strain, type of carbon substrate,[39–41] culture conditions,[42] age of the culture,[34] the *P. aeruginosa* strain itself,[34,38] as well as the method of RL isolation and purification.[43,44]

Some RL congeners are predominant in all RL-producing *P. aeruginosa* strains and are classified as the major RL structures, while others, produced only sometimes or with low abundance, are the minor RL structures. Both the major and the minor RL congeners contribute to the complete profile of RLs. In all studies of RL mixtures produced by *P. aeruginosa* strains, it was shown that, aside from their varying composition, these mixtures contain mono-RL and di--RL structures. In the lipid part, chain lengths from C_8 to C_{12} and Rha–C_{10}–C_{10} and Rha–Rha–C_{10}–C_{10} were the predominant congeners.[34,38,45,46] The ratio of total di- to mono-RL fractions for RL produced by *P. aeruginosa* is reported to range from 1 to 5.[10,38,47]

Despite the diversity of the reported RL structures, there are relatively few studies which both quantitatively and qualitatively analyzed RLs.[7,38,43,47–49] The composition of RL mixtures produced is important, since this defines the physicochemical properties of the products, which further impacts on their potential application.

3.3. Physicochemical properties

RL mixtures show physicochemical properties that are different from single RL congeners. Hence, the most abundant structure will have the biggest impact on the characteristics and dictate the behavior of the total RL mixture.

RLs can reduce the surface tension of water from 72 to 30 mN m^{-1} and the interfacial tension of water/oil to values < 1 mN m^{-1}.[33] RLs are weak acids due to the carboxylic acid moieties and are known to undergo aggregation in solution.[33] At concentrations above the critical micelle concentration (CMC), RLs form micelles, vesicles or lamella, depending on the solution properties.[50] The CMC values for RLs depend on the chemical composition of the congeners present in each RL mixture (the ratio and composition of the homologues, the presence of unsaturated bonds, the branching and length of the alkyl chain, or the size of the hydrophilic head group), and on their chemical environment, and was reported to range from 5 to 200 mg L^{-1}.[38,47,48,51] Furthermore, in a comparative study, a statistically significant increase in the CMC values was observed on lowering the ratio of total mono-/di-rhamnolipid and ratio of Rha–C_{10}–C_{10}/Rha--Rha–C_{10}–C_{10} ($P \leq 0.05$), indicating that mono-rhamnolipids start to form micelles at lower concentrations than di-rhamnolipids.[38]

RLs were shown to have higher solubilization capacity, which is expressed as the molar solubility ratio (*MSR*) for non-aqueous phase liquids (NAPL), as well as for solid-phase organics, such as polycyclic aromatic hydrocarbons (PAH) and hydrocarbon mixture than some of the commonly used synthetic surfactants (Tween, Triton and alkylbenzyl sulfonate).[3]

RLs are also an example of a readily degraded agent, as determined by the OECD 301 Ready Biodegradability Test.[52] Furthermore, invertebrate toxicity

tests performed in accordance with OECD 202 showed RL to have very low toxicity.[3] RLs also have high affinity for a variety of metals, including cadmium, copper, lanthanum, lead and zinc.[3]

4. METHODS FOR THE CHARACTERIZATION OF RHAMNOLIPID MIXTURES

4.1. Identification and quantification

The quest for a cost-efficient production of RL biosurfactant and alternatives, plus more efficient, RL-producing strains of bacteria is associated not only with economic aspects of their production, but also with new demands in the analysis of RL mixtures.[53] A large variety of analytical methods have been employed to identify and quantify the different RL species and RL production. These methods range from indirect analysis based on the physical properties of RLs (determination of surface tension and hemolytic test),[29,54] colorimetric measurement (cetyltrimethylammonium bromide agar test, anthrone method and orcinol test)[54–56] to spectroscopic analysis of sample structure by infrared and nuclear magnetic resonance spectroscopy and a sophisticated analysis of composition by mass spectrometry (MS).[34,43,46,57,58]

One current method for RL identification and quantification is high performance liquid chromatography (HPLC) analysis coupled with electrospray ionization mass spectrometry (ESI-MS).[33,34,43,53,57] Furthermore, fragmentation of the pseudomolecular ion using tandem MS analysis of the parent ion is often performed in order to provide additional structural information, such as discrimination of isomeric pairs with subtle structural variations.[43] Matrix assisted laser desorption ionization-time of flight MS (MALDI-TOF MS) approaches were recently developed for high-throughput screening of naturally occurring mixtures of RLs from *Pseudomonas* spp.[58]

4.2. Tensioactive properties

Methods based on the tensioactive properties of RL biosurfactant are typical for all surface-active compounds and involve the calculation of the value of the hydrophilic–lipophilic balance (*HLB*), the drop collapsing test, the oil spreading test, determination of the surface activity and the *CMC* values,[9,29] as well as the determination of the interfacial tension and the wetting properties.[59]

Determination of the *HLB* value, which varies between 0 and 20, enables the prediction of the behavior of surface-active compounds: 0–3 antifoaming agents, 4–6 W/O (water/oil) emulsifiers, 7–9 wetting agents, 8–18 O/W (oil/water) emulsifiers, 13–15 typical detergents and 10–18 solubilizers/hydrotropes. The *HLB* value can only be used as a preliminary guide, and further analysis by more exact and precise analytical techniques is required. According to Griffin,[60] the *HLB* can be calculated as $HLB = 20\times(MW_{HP}/MW_{SA})$, where MW_{HP} is the molecular

weight of the hydrophilic part and MW_{SA} is the molecular weight of the whole surface-active agent.[9]

The drop-collapsing test and oil-spreading test are methods for rapid screening of RL-producing bacterial strains.[29] Both tests are based on a similar approach, where the responses depend on the presence of RLs in culture supernatant. In the case of the drop-collapsing test, a sample of supernatant is applied to a polystyrene plate containing shallow wells covered with oil,[61] and spreads over the oil only if the sample of culture supernatant contains RLs. In the oil--spreading test, a drop of bacterial supernatant is added on top of an oil/water interface,[62] where the presence of RLs causes the oil to be repelled, resulting in the formation of a clearing zone, the diameter of which can be correlated with the activity of the tensioactive compounds in the supernatant.[29]

A more precise method for determining the surfactant properties of RLs in bacterial culture supernatants or of isolated RL biosurfactants is the direct measurement of surface activity and the determination of the *CMC* (specific to each surfactant), which is performed by measurement of the surface tension after sequential dilution of the solution.[29] However, the determination of the *CMC* suffers the drawbacks that it is time-consuming and not applicable for high--throughput screening.[29] Additionally, the results of these methods, as well as of all the previous indirect tests based on surface tension, could be affected by the potential presence of other tensioactive compounds.

One method to quantify solid–liquid adsorption is to measure the interfacial tension, which can be performed by tensiometry or by contact angle goniometry.[59,63] Surface tension and contact angle measurements as a function of surfactant concentration are directly related to the difference in the adsorption of surfactants on solid–vapor (S–V) and solid–liquid (S–L) interfaces. Contact angle measurements also aid the elucidation of the nature of interactions between a surfactant molecule and a solid surface.[64] Özdemir and Malayoglu[64] investigated the wetting behavior of a mixture of mono- and di-rhamnolipid and SDS (sodium dodecyl sulfate) molecules on glass, PET (polyethylene terephthalate) and gold surfaces by measuring the advancing contact angle, and elucidated the preferences of surfactant molecules adsorbed onto SL (solid–liquid)–SV (solid–vapor) and L–V (liquid–vapor) interfaces.

5. PRODUCTION OF RHAMNOLIPIDS FROM *P. aeruginosa*

5.1. Bottlenecks for production

The high cost of production, isolation and purification of RLs, and the low yield has determined that, despite 60 years of research in the area of RL production, the economic feasibility of these glycolipids is still pending.[65] Often, the amount and type of a raw material can contribute considerably to the production cost, as raw materials account for 10–30 % of the total production cost in most

biotechnological processes.[12] Solutions resulting in more economic production include the use of cheap and renewable resources, optimized and efficient bioprocesses, over-production by mutant or recombinant strains,[12,66] as well as the application of response surface methodology (RSM) as a modern concept for optimization of biotechnological processes.[67]

The major problems for large-scale applications of RLs are safety and yield issues related to their main producers - strains of *P. aeruginosa*. Despite the fact that in the UK, *P. aeruginosa* spp. are classified as type II opportunistic pathogens that are not highly infective, large-scale fermentation production would require compliance to special procedures by employees involved in the production.[68] However, commercial-scale production is already being undertaken in the USA, with no reported problems.[68]

Problems related to the pathogenic status of *P. aeruginosa* could be solved by two strategies: heterologous production of RLs in non-pathogenic bacteria and identification of potential new non-pathogenic RL producers, for which the RL yields are acceptable.[29,68] The heterologous production of RLs would bring about two major advantages as compared to their production by *P. aeruginosa*. The first is the increased safety when handling large amounts of culture broths, while the second is the possibility of constitutive RL production, in contrast to the very tightly regulated production in *P. aeruginosa*.[29] Although several attempts to obtain heterologous production of *Pseudomonas* RLs have been reported, none of them produced RLs at levels comparable with those of the best *P. aeruginosa* strains.[29] In terms of the commercial production of RLs, there is still vast potential for genetic optimization.[29] Other species and genera of bacteria were also found to be RL producers.[8] Most RL-producing species belong to the closely related genera *Pseudomonas* and *Burkholderia*.[47] The most prominent nonpathogenic RL producers from the genus *Pseudomonas* are *P. chlororaphis*, *P. alcaligenes* and *P. putida*, and from the genus *Burkholderia*, they are *B. glumae*, *B. plantarii*, *B. pseudomallei* and *B. thailandensis*. These species from the genus *Burkholderia* primarily produce Rha–Rha–C_{14}–C_{14}, but a number of other congeners, including mono-RLs, mostly Rha–C_{14}–C_{14}, were recently detected in cultures of *B. pseudomallei* and *B. thailandensis*.[47] Additionally, in the mentioned study, the RL mixture was further characterized and the results, such as ratio of di-RL to mono-RL, the major RL congeners and genetic background of RL biosynthesis, were compared to RL produced by *P. aeruginosa*. On the other hand, one non-pathogenic RL producer, *P. chlororaphis*, synthesizes exclusively mono-RL congeners, primarily Rha–C_{10}–C_{12} and Rha–C_{10}–$C_{12:1}$, under static conditions of fermentation, with a yield of 1 g L^{-1}.[28] RLs were also detected in cultures of many other genera and species of widely different taxonomical origins.[29] However, for most of these strains, structural determination of the putative RLs was not accomplished and, sometimes, the actual identification of the

producing strain was not firmly confirmed.[29] Due to this, their biotechnological potential is currently unknown.

Other major obstacles in the production of RL are related to the yields, the substrates needed to produce them, and the downstream processing required. At present, strains of *P. aeruginosa* produce 10–20 g L^{-1} of RL in the laboratory (although there have been some reports of hyperproducer strains), which is low in comparison to the yields obtained for other glycolipid biosurfactants, MEL and sophorolipids (100 g L^{-1}).[68] One advantage of RLs, and glycolipid biosurfactants generally, is the possibility for production from a range of renewable substrates, such as industrial waste. Additionally, complex regulation of RL biosynthesis in *P. aeruginosa* (environment conditions, nutrition factors and quorum sensing mechanism), which is not yet fully understood, prevents the development of hyperproducing strains, either by mutagenesis and selection or by genetic manipulation. Failure to achieve high yields may eventually preclude rhamnolipids from use in many possible applications.[68]

5.2. Commercial production

Rhamnolipids from *P. aeruginosa* are officially produced by only a few companies in the USA.[68] Small quantities of RL from the strain *P. aeruginosa* NY3 are produced by AGAE Technologies (www.agaetech.com). This company produces highly purified rhamnolipid as a mixture, which can be a regular or liquid product, or separate mono- and di-RL fractions (90 or 95 % purity). The Jeneil Biotech Company, which is generally a food additive producer, and Rhamnolipid Companies, Inc. (www.rhamnolipid.com), a specialist producer of these biosurfactants, both manufacture RL on a larger scale. Jeneil Biotech RL products range from the crudest preparation comprising fermentation broth with approximately (2 % RLs) to partially purified products (99 % RLs).[68] Rhamnolipid Companies, Inc. offers raw 99 % rhamnolipid in 1–25 % aqueous solutions and 1–25 % solutions with stabilizers, refined RL in 0.1 to 7 % in silicone, mineral oil, alcohol or other carriers, and refined powder mono- and di-RL.

5.3. Substrates for improved production

RLs are considered to be secondary metabolites and as such, their production coincides with the onset of the bacterial stationary phase.[3] Production of RLs on the cellular level, as well as under conditions for laboratory or industrial production, are not fully mastered, even though the biosynthesis of RLs and their regulation are partially explained.[10,69,70] Traditional engineering by random and targeted genetic alteration, process design, and recombinant strategies have not yet proven successful.[65] For enhanced process development, there is an urgent need for in-depth information concerning the regulation of RL production during

bioreactor cultivation of RL-producing bacteria, in order to design knowledge-based genetic and process engineering strategies.[65]

Reported data indicate that the production of RLs is possible from simple carbon sources, such as glycerol and glucose, or complex carbon sources (olive oil, sunflower oil) or wastes (crude whey, distillery waste, molasses, corn steep liquor, sunflower oil mill effluent, olive oil mill effluent (OOME), frying oil, soap stock) and minerals ($NaNO_3$, NH_4Cl) or combined nitrogen source ($NaNO_3$ and yeast extract or NH_4Cl and peptone).[10,29] This indicates that economically viable levels of biosurfactant production could be achieved using renewable resources for the carbon source, with special emphasis on the importance of the utilization of industrial by-products and agricultural wastes as cost-effective alternative substrates for microbial growth and biosurfactant production.[38,41,66,68–75]

The study of Dubey and Juwarkar[71] is an example of RL production from renewable sources, in which *P. aeruginosa* BS2 was cultivated on whey for 48 h, with an RL yield of 0.92 g L^{-1}. In other studies, Mercade *et al.*[74] cultivated *Pseudomonas* sp. JAMM in a medium with OOME (100 g L^{-1}) and $NaNO_3$ (2.5 g L^{-1}), which resulted in an RL yield of 14 g kg^{-1} OOME after 150 h of cultivation. Haba *et al.*,[75] using sunflower and olive frying oil as carbon sources and $NaNO_3$ as the nitrogen source, studied the production of biosurfactant by several *P. aeruginosa* strains. Most of the examined *P. aeruginosa* strains showed good growth on both sources and one of them produced a yield of 2.7 g L^{-1} of RL. In another study, Haba *et al.*[34] found *P. aeruginosa* AT10 produced an RL yield of 16.5 g L^{-1} on soybean residual fatty acids. Benincasa *et al.*[39] tested *P. aeruginosa* LBI for RL production by batch fermentation on soap stock as a carbon source; the maximal production of biosurfactant was 15.9 g L^{-1}. Rikalovic *et al.*[38] tested the effect of different carbon sources on RL production by several *P. aeruginosa* isolates. The best RL yield of 1.3 g L^{-1} was obtained using sunflower frying oil as a carbon source and peptone and NH_4Cl as nitrogen sources. In the study of Aparna *et al.*,[45] *Pseudomonas* sp. 2B produced 4.97 g L^{-1} of RL when cultivated on molasses, peptone and NH_4Cl.

6. APPLICATIONS OF RHAMNOLIPIDS

The main properties of biosurfactants, *i.e.*, detergency, foaming, emulsifying, demulsifying, solubilizing, wetting, thickening, metal sequestering, vesicle forming and phase dispersion properties, among others, can be exhibited by RLs.[5,76] All of these properties are associated with the amphiphilic character of the RL molecules, and confer upon them the ability to accumulate between fluid phases, thus reducing surface and interfacial tensions.[5] Environmental uses of RLs are currently considered as the major field for potential application of RLs,[3,46,77–80] but an increasing number of patents indicates the successful appli-

cation of RLs in the food industry, agriculture, the cosmetics industry, pharmacology, and in nanotechnology.[9] The applications of rhamnolipids, which will be further discussed, are summarized in Table I.

TABLE I. Fields for rhamnolipid applications

Field of application	Specific processes / purposes
Bioremediation	Organic flushing agents (PAH, NAPL, hydrocarbon mixtures Metal flushing agents (heavy metals Cu, Zn, Pb, Ni, Cr) Biodegradation of organics (petroleum and petroleum derivatives) Biodegradation of organics in metal–organic co-contaminated systems
Food industry	Multipurpose ingredients (decrease surface and interfacial tension, formation and stabilization of emulsions, improvement of stability, texture, volume and conservation of products) Source of L-rhamnose Surface conditioning
Cosmetic and pharmacy industries	Health care formulations Drug delivery systems Skin care products
Biomedicine	Antimicrobial and antiviral activity Cellular immunosuppression Wound healing, treatment prevention of gum disease and periodontal regeneration of ulcer Inhibition of growth of human breast cancer cell lines
Agronomy	Control of zoosporic plant pathogens by affecting motility, causes lysis, inhibition of spore germination and mycelium growth
Formulation of cleaners and wetting agents	Replacements for the synthetic compounds in liquids and powders cleaning formulations Superior wetting abilities compared to synthetic surfactants on different types of surfaces
Bio- and nanotechnologies	Synthesis of nanoparticles (metal nanoparticles) Drug delivery systems Formulation of microemulsions

6.1. Bioremediation

RLs have been studied and shown to have potential in bioremediation of organics, as organic flushing agents, as metal flushing agents, and in the biodegradation of organics in metal–organic co-contaminated systems.[3,10]

6.2. Biodegradation and uptake of hydrocarbons

Numerous studies confirmed that biosurfactants, especially RLs, could affect the biodegradation of hydrocarbons, both aliphatic and aromatic.[81] Furthermore, it was shown that the addition of RLs to pure cultures of *P. aeruginosa* enhanced the biodegradation of hexadecane, octadecane, *n*-paraffins and phenanthrene,[82–84]

as well as degradation in soil systems in the presence of hexadecane, tetradecane, pristane, creosote or hydrocarbon mixtures.[3] Additionally, besides the role of RLs in the biodegradation of hydrocarbons, reports showed that RLs facilitate the uptake of hydrocarbons by *P. aeruginosa*.[46,77,85,86]

Although some studies reported positive effects on the biodegradation of petroleum hydrocarbons in presence of RL biosurfactant, a lack of influence or even a negative effect of biosurfactant supplementation was observed just as frequently.[87] Some reports indicated that the potential reason for inhibition of degradation is that RLs are favored as the carbon source for bacterial metabolism.[52] Recently, it was observed that the presence of RLs, or other surfactant compounds, may induce changes in the microbial community, which in turn corresponded to differences in the degradation patterns.[87] Some earlier reports suggested mechanisms of hydrocarbon biodegradation facilitated by RL, and assumed that RLs, due to their physicochemical properties, increased the hydrocarbon solubility and bioavailability or that RLs interacted with the bacterial cell, making the cell surface more hydrophobic and easily accessible to hydrophobic substrates.[52] On the other hand, some recently published studies proposed three mechanisms of interaction between microorganisms and hydrocarbons: access to water-solubilized hydrocarbons, direct contact of cells with large oil drops and contact with pseudo-solubilized or emulsified oil,[88] as well as combinations of these interactions.[87] However, regardless of whether the biodegradation process is enhanced or inhibited, the effects are bacterial strain-specific in the sense of strain characteristics and response to environmental conditions.[83] Although much work was realized by many groups to explain the role of RLs, and biosurfactants generally, in the degradation of water-immiscible substrate, their significance and exact purpose in this process still remain unclear.

6.3. Flushing agents for organic pollution

Biodegradation of NAPL and soil-phase organics, such as PAH, is often a slow and non-feasible process.[3] The addition of surfactants to a flushing solution could enhance the flushing efficiency, either by mobilization or by an increase in the solubilization of these compounds.[3] Thus, to be effective, a surfactant must have good solubilization capacity and/or be able to reduce interfacial tension. RLs were shown to have an *MSR* for the model NAPL, hexadecane that was 20 times greater than the *MSR* for hexadecane alkyl benzyl sulfonate.[3] In studies examining the use of RLs for the removal of residual hexadecane from soil, it was shown that RL (20 % removal) was more effective than either SDS (negligible removal) or Tween 80 (6 % removal). Additionally, it was shown the optimal removal of NAPL compounds (60 %) could be achieved by altering the pH and ionic strength, thereby maximizing the reduction of the surface tension.[3] Similar results were obtained for RL solubilization of solid phase materials. For

example, the *MSR* of rhamnolipid–octadecane was ten and five times higher than the *MSR*s for Triton-X-114–octadecane and for Corexit 0600–octadecane, respectively.[3] Moreover, the *MSR* of rhamnolipid-phenanthrene was 1.7 to 2.8 times higher than for 13 different synthetic surfactants that were tested.[3] Furthermore, in a comparison of the removal of a hydrocarbon mixture (undecane, pentadecane, hexadecane, octadecane, pristane, naphthalene, phenanthrene and pyrene) from soil, RLs were more effective than Triton X-100 or Tween 60 for all hydrocarbon components.[3] Finally, RL-enhanced removal of phenanthrene, pyrene and polychlorinated biphenyls and a variety of PAH from soil were reported.[3]

6.4. Bioremediation of heavy metals

Juwarkar *et al.*[79] conducted column experiments to evaluate the potential of environmentally compatible RL biosurfactants produced by *P. aeruginosa* BS2, to remove Cd and Pb from artificially contaminated soil. Results showed that dirhamnolipid removed not only the leachable or available fraction of Cd and Pb, but also the bound metals, whereas tap water removed only the mobile fraction.[79] Washing contaminated soil with tap water revealed that only ≈ 2.7 % of Cd and 9.8 % of Pb in contaminated soil were in freely available or weakly bound forms and were able to be removed, whereas washing with RL had removed 92 % of Cd and 88 % of Pb after 36 h of leaching.[79] Wang and Mulligan[80] evaluated the feasibility of using RL foam to remove Cd and Ni from a sandy soil. Application of RL foam increased the efficiency and enabled the removal of 73.2 % and 68.1 % of Cd and Ni, respectively, whereas the RL solution alone flushed 61.7 % and 51 % of Cd and Ni, respectively.[80] Mulligan *et al.*[89] designed batch washing experiments to evaluate the feasibility of using biosurfactants to remove heavy metals from sediments. Thus, surfactant from *Bacillus subtilis*, RLs from *P. aeruginosa*, and sophorolipid from *Torulopsis bombicola* were evaluated on a sediment containing 110 mg kg^{-1} of Cu and 3300 mg kg^{-1} of Zn. A single washing with 0.5 % RL removed 65 % of the copper and 18 % of the zinc, whereas 4 % sophorolipid removed 25 % of Cu and 60 % of Zn.[89] Avramovic *et al.*[90] studied the chromium(VI) tolerance of *P. aeruginosa* NCAIM (P) B001380 and showed that the strain was chromium tolerant and had potential for application in heavy metal bioremediation.

6.5. Bioremediation of co-contaminated sites

Sandrin *et al.*[91] studied the effectiveness of RL biosurfactants in the remediation of a Cd and naphthalene co-contaminated site. They observed that reduced cadmium toxicity as a result of the addition of *P. aeruginosa* RL led to enhanced naphthalene biodegradation by *Burkholderia* sp. NCBI U37342.[91] These authors suggested that reduction of metal toxicity by RL might involve a

combination of RL complexation with cadmium and RL interaction with the cell surface to alter Cd uptake, resulting in enhanced rates of bioremediation. In another co-contaminant study, it was observed that the inhibition of phenanthrene mineralization in the presence of Cd was reduced by the pulsed addition of RL.[52] Dahrazma and Mulligan[78] reported higher rates of Cu and Ni removal from sediments by adding 1 % NaOH to a solution of RL. Efficient removal of Zn and Cu from co-contaminated soil with a 12.6 % oil content using RLs was also demonstrated.[92]

6.6. Food industry

Some properties of RLs, such as emulsion formulation and stabilization, as well as anti-adhesive and antimicrobial activity, make them interesting for the food industry as multipurpose ingredients.[4] Apart from their role as surface active agents, there are reports that RLs could have several other functions in food.[12,93] Some examples are an improvement of dough stability, texture, volume and conservation of bakery products, obtained by the addition of RL surfactants,[94] while some other authors suggested the use of RLs for improvement of properties of butter cream, croissants and frozen confectionery products.[93]

Finally, RLs could serve a source of L-rhamnose, a compound used commercially in the production of high quality flavor compounds. L-Rhamnose is a methyl pentose natural sugar, classified as one of the rarer sugars, and is found in several animal, plant and bacterial polysaccharides, as well in RLs. This compound was already successfully obtained by hydrolyzing RL surfactants produced by *P. aeruginosa*.[95] L-Rhamnose is a sugar that the Food and Drug Administration (FDA) has approved as a food additive and hence, it has found use in the flavor industry as a precursor for the production of 2,5-dimethyl-4-hydroxy--3(2*H*)-furanone, the high-quality flavor aroma furaneol (trademark of Firmenich SA, Geneva),[12] which resembles strawberry and raspberry. It is also the starting raw material in the reaction flavors developed during the preparation of various foods, such as bread, grilled meats, *etc*. Thus, there is a great deal of interest in obtaining commercial quantities of rhamnolipids to provide a source of L-rhamnose, which already has the above mentioned applications in the food industry.

6.7. Surface conditioning

Bacterial biofilms present on surfaces in the food industry are potential sources of contamination, which may lead to food spoilage and disease transmission, and thus, controlling the adherence of microorganisms to food contact surfaces is an essential step in providing safe and quality products to consumers.[96] The promising results from studies of the disruption of *Bordetella bronchiseptica* biofilm by RL[97] and reduction of adhesion of *Streptococcus salivarius* and *C. tropicalis* by RL[98] suggested a potential application of RLs for

surface conditioning in the food industry. Moreover, studies by Meylheuc *et al.*[99,100] showed inhibition of the adhesion of the pathogen *Listeria monocytogenes* to two types of surfaces typically used in the food industry using biosurfactant obtained from *P. fluorescens*, while Dagbert *et al.*[101] showed that the surfactant produced by *P. fluorescens* also has good potential as a corrosion inhibitor.

It is important to note that RLs, as products obtained from *P. aeruginosa*, which are considered to be opportunistic pathogens, still face some difficulties (particularly the long process required by regulations for the approval required by governmental agencies) related to application in food industry as food ingredients or integration of these biosurfactants in industrial processes on any large scale level.[4] This obstacle could, in the future, be prevented by using RL produced by, as already mentioned, nonpathogenic bacterial species, such as *P. chlororophasis*.

6.8. Cosmetic and pharmacy industries

Cosmetic surfactants perform detergency, wetting, emulsifying, solubilizing, dispersing and foaming effects.[15] Biodegradability, low toxicity and ecological acceptability, which, at the same time, are the benefits of a naturally derived surfactant that promises cosmetic safety are, therefore, in high demand. In particular, application of RLs in the field of cosmetics and pharmaceuticals as emulsifiers, penetrating agents and drug delivery systems is an emerging area of research.[102,103]

RLs are used in health care products in several different formulations,[15] for example, in insect repellents, antacids, acne pads, anti-dandruff products, contact lens solutions, deodorants, nail care products and toothpastes.[3,104] These formulations require surfactants with high surface activity and, in particular, emulsifying activities,[105] which is the essence of the texture consistency of these products.[48] Furthermore, requirements for the biological activities for cosmetics should expand the application of RLs, and a delivery system has been achieved, not only for emulsions but also for liposomes.[106] Patents for cosmetics containing RLs have been granted for anti-wrinkle and anti-aging products,[107] which were launched in several dosage forms as commercial skin care cosmetics[108] because of their skin compatibility and extremely low skin irritation.[48]

6.9. Biomedicine

Early on, the wide-ranging antimicrobial properties of RLs were noted. Interestingly, they were shown to be active against a large variety of bacteria, including both Gram-negative and Gram-positive species.[8] In several studies, the antimicrobial properties of mixtures of RL congeners produced by three different strains of *P. aeruginosa* were investigated.[31,33,34] The various RL combinations displayed antimicrobial activity against nearly all the tested Gram-positive spe-

cies, including *Staphylococcus*, *Mycobacterium*, and *Bacillus*, and significant activity against a number of Gram-negative species, with *Serratia marcescens*, *Enterobacter aerogenes*, and *Klebsiella pneumoniae* being especially sensitive.[29]

Rhamnolipids were also shown to affect cellular immunosuppression[109] and wound healing, treatment and prevention of gum disease and periodontal regeneration,[110] and to display differential effects on human keratinocytes and fibroblast cultures.[111] Moreover, Piljac *et al.*[112] reported the successful treatment of a decubitus ulcer with an ointment containing 0.1 % of a di-rhamnolipid.

Thanomsub *et al.*[113] tested the cytotoxic activity of a crude RL extract, Rha––Rha–C_{10}–C_{10} and Rha–Rha–C_{10}–C_{12}, produced by *P. aeruginosa* B189 isolated from a milk factory, against herpes simplex virus, insect and cancer cell lines. Rha–Rha–C_{10}–C_{10} exhibited significant inhibition of growth of human breast cancer cell lines (MCF-7), with minimum inhibitory concentration (*MIC*) of 6.25 μg mL^{-1}, Rha–Rha–C_{10}–C_{12} had an *MIC* of 50 μg mL^{-1} against insect cell line C6/36, while the crude RL extract showed no cytotoxic activity.[113] The potential mechanism of activity, regarding the structure of the biosurfactant, is a toxicological effect on the cell membrane permeability. Furthermore, Rha–Rha––C_{10}–C_{10} and Rha–Rha–C_{10}–C_{12} had no inhibition effect on the normal cell line (Vero cell) at concentrations up to 50 μg mL^{-1}. This confirmed the specific toxicity of these compounds to the cell lines used. However, the inhibitory mechanisms against these cell lines are as yet unknown and are under investigation.[113]

6.10. Agronomy

RLs also showed the ability to control certain zoosporic plant pathogens, including *Phytophthora cryptogea* and *Pythium* spp.[114–116] Purified mono- and di-RL, in concentrations ranging from 5 to 30 mg L^{-1}, caused cessation of motility and lysis of the entire zoosporic population in less than 1 min.[3] This observation led to the development of a RL-containing biofungicide formulation, used to prevent crop contamination by pathogenic fungi.[5] This product is considered to be non-mutagenic and of low acute toxicity to mammals. It was approved by the FDA for direct use on vegetables, legumes and fruit crops.[4] Dorey *et al.*[117] reported the role of RLs in triggering defense responses and protection against the fungus *Botrytis cinerea* in grapevines.[117] The authors showed that RLs inhibited spore germination and mycelium growth, thus efficiently protecting grapevines against the fungus by inducing the plant defense system. A product based on an aqueous RL solution (0.01 %) was claimed to act as a novel agent for stimulating the natural defense reactions of plants against pathogenic fungi.[5,117]

6.11. Formulation of cleaners and wetting agents

One of the major commercial domestic applications of biosurfactants is in the field of cleaning and laundry products. The interfacial chemistry created by

adsorbed surface-active molecules, of either biological or chemical origin, dominates the end-use properties of materials in many different applications.[59] The properties and efficacy of detergent formulations are critically dependent on the interfacial activity of various surfactants, which are present in their composition.[64] At present, the liquids and powders generally contain alkyl sulfonates, such as linear alkylbenzene sulfonates, but the glycolipid biosurfactants and among them rhamnolipids, produced by *P. aeruginosa*, are possible candidates to be used for the, at least, partial replacements of these synthetic compounds.[68] One of the major challenges in the use of RLs is the fact that they are produced as a mixture of different congeners, which affects physicochemical properties and behavior, as mentioned previously.

Bafghi and Fazaelipoor[118] investigated RLs in the formulation of a washing powder. The results showed that the biosurfactant was effective in removal of oil from the samples. The formulation presented in this study was also compared with some commercial powders for the removal of edible oil, chocolate and albumen stains. The results showed that the RL-inclusive formulation was comparable to the commercial powders in terms of stain removal. Biodegradability tests performed on pure RL and the RL-inclusive formulation confirmed the good biodegradability of this biosurfactant.

Özdemir and Malayoglu[64] investigated the wetting behavior of a mixture of mono- and di-rhamnolipid (in 1:1 ratio of mono:di-rhamnolipid) on glass, PET and gold surfaces by measuring the advancing contact angle, and elucidated the preferences of the surfactant molecules adsorbed onto SL–SV and L–V interfaces, with SDS as the reference surfactant. The study showed that at low concentrations of RL and reference surfactant, the contact angle varied in a certain range depending on the character of the surfactant interactions with the surface.[64] Moreover, on hydrophobic surfaces, the adhesion tension had a specific dynamic, depending on surfactant concentration, while on hydrophilic surfaces a steady decrease in adhesion tension was observed with both surfactant solutions.[64]

Costa *et al.*[59] also studied the wetting behavior of RLs produced by *P. aeruginosa* LBI grown on a waste oil substrate, and the chemical surfactant SDS, on glass, PET, poly(vinyl chloride) (PVC), poly(ε-caprolactone) (PCL) and a polymer blend (PVC–PCL) by measuring the contact angle of sessile drops. The comparison of the wetting profiles showed dynamic changes in the contact angle at low SDS and RL concentrations – the contact angle increased and when the concentration of the surfactant increased further, the contact angle decreased.[59] The results showed that RLs produced by *P. aeruginosa* LBI exhibited superior wetting abilities compared to SDS. This is the first work that evaluated the wetting properties of RLs on polymer blends.

6.12. Bio- and nano-technology

Biosurfactants have been increasingly attracting attention in the field of nanotechnology as a "green" alternative for high-performance nanomaterials.[17] During the last decade, unique properties of biosurfactants, including versatile self-assembling and biochemical properties, which do not usually occur among chemically derived surfactants, were studied and analyzed.[119–121] RLs, alone or in combination with other glycolipid biosurfactants, have potential roles as systems for drug delivery, synthesis of nanoparticles and formulation of microemulsions.

6.12.1. Drug delivery systems

In 1988, RL liposomes were patented as drug delivery systems, useful as microcapsules for drugs, proteins, nucleic acids, dyes and other compounds, as biomimetic models for biological membranes and as sensors for detecting pH variations.[17] These novel liposomes were described as safe and biologically decomposable, with suitable affinity for biological organisms, stable and with long service and shelf life.[17] Recently, in a study of Sharma *et al.*,[122] RLs and sophorolipids were mixed with lecithins to prepare biocompatible microemulsions in which the phase behavior was unaffected by changes in temperature and electrolyte concentration, making them desirable for cosmetic and drug delivery applications.[17]

6.12.2. Synthesis of nanoparticles

Another interesting aspect of the applications of RL is the synthesis of metal nanoparticles as an alternative (a more ecological technology) to traditional methods of production.[121] There are several reports with RL applications in this field. Kumar *et al.*[123] synthesized silver nanoparticles using purified RLs from *P. aeruginosa* BS-161R, which showed a broad spectrum of antimicrobial activity. Xie *et al.*[124] successfully synthesized silver nanoparticles in RL reverse micelles. Palanisamy and Raichur[125] demonstrated a simple and eco-friendly method for synthesis of spherical nickel oxide nanoparticles by a microemulsion technique using RLs as an alternative surfactant.

In two recent studies, RL biosurfactants were used as capping agents for the synthesis of ZnS nanoparticles.[126,127] Narayanan *et al.*[126] demonstrated a novel method for the synthesis of ZnS nanoparticles in aqueous medium and showed that RL biosurfactant has potential as an effective capping agent for the synthesis of uniform nanoparticles. Hazra *et al.*[127] reported a facile eco-friendly procedure for biosynthesis of RL capped ZnS nanoparticles, their structural characterization, biocompatibility, cytotoxicity assessment and their applicability as a nanophotocatalyst for the degradation of a textile azo dye. The results obtained explained the importance of environmentally friendly RLs as an effective and

inexpensive capping and stabilizing agent for the development of stable and biocompatible ZnS nanoparticles as nanophotocatalysts in the textile industry and for wastewater and effluent treatment.[127]

6.12.3. Microemulsions

Xie *et al.*[128] showed that RLs have potential for application in the formulation of microemulsions with medium-chain alcohols as co-surfactants. Furthermore, the same authors observed that the phase behavior and microstructure of these microemulsions were related to the conformational changes of the RL molecules at the oil/water interface.[128,129] In another study, RLs were successfully used as the surfactant to synthesize spherical nickel oxide nanoparticles by a microemulsion technique.[125]

Nguyen and Sabatini[130] focused their research on developing alcohol-free biosurfactant-based microemulsions. RL-based mixtures were found to have doubled the solubilization parameter as compared to sodium bis(2-ethylhexyl) sulfosuccinate/sodium dihexyl sulfosuccinate/sodium mono- and di-methylnaphthalene sulfonate at the same total molar concentration.[130] Additionally, these authors developed a phase diagram for surfactant mixtures containing methyl ester ethoxylate, RL and oleyl alcohol with limonene oil, which could be used as a guideline for selecting a surfactant system and surfactant ratio to formulate microemulsions with a given oil. The RL biosurfactant used by Nguyen and Sabatini[130] was the least hydrophobic type (fatty acid tails of C_8 chain length), since its tail length was the shortest within the typical range (the tail length usually varies from C_8 to C_{14}). Potential application of longer tail RL would make the surfactant system more hydrophobic and, as a result, either the optimum formulation at lower salinity for the studied oils would be achieved, or it would be easier to formulate microemulsions with oils that are more hydrophobic than the studied oils.[130] In a recent report, Nguyen *et al.*[121] formulated and evaluated microemulsions of lecithin/RL/sophorolipid biosurfactants with a range of oils.

7. CONCLUDING REMARKS AND FUTURE PERSPECTIVE

Next to the already established biosurfactants alkyl polyglycosides and sophorolipids, RLs shows the highest economic potential among all the currently investigated microbial biosurfactants.[9] The reasons for this are their environmental friendly properties (biodegradability, low aquatic toxicity, production from renewable resources or industrial wastes), as well as additional benefits of their physicochemical characteristics and biological origin. In spite of the several drawbacks discussed above, strains of *P. aeruginosa* are the most promising candidates for RL production, because they can metabolize a variety of carbon sources, including renewable sources, with good yields of RL surfactants and their RL mixtures contain all types of RL structures. In comparison to other gly-

colipid biosurfactants, RLs have a broader range of applications, from environmental to industrial spectra. The increasing commercial importance of RL is suggested by the number of companies that are official manufacturers of RL biosurfactant produced by *P. aeruginosa*, and the great number of related patents. However, in the near future, it seems that the most likely progress in the application of RLs will be in the field of bioremediation, biodegradation of hydrocarbons and removal of heavy metals. In addition to the research focused on commercial application of RLs in bioremediation, further investigation and understanding of the mechanisms of action of these compounds in the environment appears to be of importance.

Acknowledgment. This work was supported by Project III 43004, funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

ИЗВОД

РАМНОЛИПИДНИ БИОСУРФАКТАНТ ИЗ *Pseudomonas aeruginosa* – ОД ОТКРИЋА ДО ПРИМЕНЕ У САВРЕМЕНОЈ ТЕХНОЛОГИЈИ

МИЛЕНА Г. РИКАЛОВИЋ[1], МИРОСЛАВ М. ВРВИЋ[1] и ИВАНКА М. КАРАЦИЋ[1]

[1]*Хемијски факултет, Универзитет у Београду, Студентски трг 12–16, Београд и* [2]*Медицински факултет, Институт за хемију у медицини, Универзитет у Београду, Вишеградска 26, Београд*

Рамнолипиди су, највероватније, следећа генерација биосурфактаната која ће доминирати на тржишту сурфактаната. Налазе се одмах иза алкил-полигликозида, који су већ прихваћени на тржишту, и софоролипида, који се за сада налазе у неколико формулација за чишћење. Ипак, највећи број нових публикација и патената везаних за гликолипидне биосурфактанте се односи на рамнолипиде. Главни продуценти рамнолипида су *Pseudomonas aeruginosa.* Ови биосурфактанти су смеше различитих рамнолипидних структура, које показују физичко-хемијске особине различите од појединачних структура, при чему најзаступљеније рамнолипидно једињење има највећи утицај на укупне карактеристике смеше. Особине рамнолипида, као што су биоразградивост, ниска токсичност, продукција из обновљивих извора, антимикробна (посебно антифунгална) активност, заједно их чине потенцијално погодним за широку комерцијалну примену. До сада је главна примена рамнолипида била у области биоремедијације, а растући број патената везаних за примену рамнолипида у козметичкој, агро- и прехрамбеној индустрији, формулацијама за чишћење и нано-технологији указују да је њихова будућност имплементација у овим областима.

(Примљено 27. јуна, ревидирано 19. септембра, прихваћено 20. септембра 2014)

REFERENCES

1. M. Pacwa-Płociniczak, M. G. A. Płaza, Z. Piotrowska-Seget, S. S. Cameotra, *Int. J. Mol. Sci.* **12** (2011) 633
2. J. D. Desai, I. M. Bant, *Microbiol. Mol. Biol. Rev.* **61** (1997) 47
3. R. M. Maier, G. Soberon-Chávez, *Appl. Microbiol. Biotechnol.* **54** (2000) 625
4. M. Nitschke, S. G. V. A. O. Costa, *Trends Food Sci. Tech.* **18** (2007) 252
5. M. Nitschke, S. G. V. A. O. Costa, J. Contiero, *Process Biochem.* **46** (2011) 621
6. J. D. van Hamme, A. Singh, O. P. Ward, *Biotechnol. Adv*. **24** (2006) 604

7. H. Górna, Ł. Lawniczak, A. Zgoła-Grześkowiak, E. Kaczorek, *Bioresour. Technol.* **10** (2011) 3028
8. A. M. Abdel-Mawgoud, F. Lépine, E. Déziel, *Appl. Microbiol. Biotechnol.* **86** (2010) 1323
9. M. M. Müller, J. H. Kügler, M. Henkel, M. Gerlitzki, B. Hörmanna, M. Pöhnlein, C. Syldatk, R. Hausmann, *J. Biotechnol*. **162** (2012) 366
10. M. G. Rikalovic, M. M. Vrvic, I. M. Karadzic, in *Bioremediation: Challenges, Future and Prospectives*, J. B. Velázquez-Fernández, S. Muñiz-Hernández, Eds. Nova Science Publishers, New York, 2014, p. 299
11. P. Singh, S. S. Cameotra, *Trends Biotechnol*. **22** (2007) 142
12. K. Muthusamy, S. Gopalakrishnan, T. K. Ravi, P. Sivachidambaram, *Curr. Sci*. **94** (2008) 736
13. I. N. A. van Bogaert, W. Soetaert, in *Microbiology Monographs 20 – Biosurfactants: From Genes to Applications*, G. Soberón-Chávez, A. Steinbüchel, Eds., Springer, Berlin, 2011, p. 179
14. E. Rosenberg, E. Z. Ron, *Appl. Microbiol. Biotechnol*. **52** (1999) 154
15. N. Lourith, M. Kanlayavattanakul, *Int. J. Cosmetic Sci.* **31** (2009) 255
16. A. Franzetti, I. Gandolfi, G. Bestetti, T. J. P. Smyth, I. M. Banat, *Eur. J. Lipid Sci. Technol*. **112** (2010) 617
17. L. Fracchia, M. Cavallo, M. G. Martinotti, I. M. Banat, in *Biomedical Science, Engineering and Technology*, D. N. Ghista, Ed., InTech, Rijeka, 2012, p. 326
18. J. Arutchelvi, M. Doble, in *Microbiology Monographs 20 – Biosurfactants: From Genes to Applications*, G. Soberón-Chávez, A. Steinbüchel, Eds., Springer, Berlin, Germany, 2011, p. 145
19. I. M. Banat, A. Franzetti, I. Gandolfi, G. Bestetti, M. G. Martinotti, L. Fracchia, T. J. Smyth, R. Marchant, *Appl. Microbiol. Biotechnol.* **187** (2010) 427
20. S. Bergström, H. Theorell, H. Davide, *Arkiv Chem. Mineral Geol. 23A* **13** (1946) 1
21. S. Bergström, H. Theorell, H. Davide, *Arch. Biochem. Biophys*. **10** (1946) 165
22. G. Hauser, M. L. Karnovsky, *J. Bacteriol*. **68** (1954) 645
23. F. G. Jarvis, M. J. Johnson, *J. Am. Chem. Soc*. **71** (1949) 4124
24. J. R. Edwards, J. A. Hayashi, *Arch. Biochem. Biophys*. **111** (1965) 415
25. N. Shaw, *Microbiol. Mol. Biol. Rev*. **34** (1970) 365
26. S. Arino, R. Marchal, J. P. Vandecasteele, *Appl. Microbiol. Biotechnol.* **45** (1996) 162
27. D. Onbasli, B. Aslim, *J. Environ. Biol*. **30** (2009) 161
28. N. W. Gunther, A. Nunez, W. Fett, D. K. Y. Solaiman, *Appl. Environ. Microbiol*. **71** (2005) 2288
29. A. M. Abdel-Mawgoud, R. Hausmann, F. Lépine, M. M. Műller, E. Déziel, in *Microbiology Monographs 20 – Biosurfactants: From Genes to Applications*, G. Soberón-Chávez, A. Steinbüchel, Eds., Springer, Berlin, 2011, p. 13
30. A. Tahzibi, F. Kamal, M. M. Assadi, *Iran. Biomed. J.* **8** (2004) 25
31. A. Abalos, A. Pinazo, M. R. Infante, M. Casals, F. Garcia, A. Manresa, *Langmuir* **17** (2001) 1367
32. A. M. Abdel-Mawgoud, M. M. Aboulwafa, N. A. H. Hassouna, *Appl. Biochem. Biotechnol*. **157** (2009) 329
33. M. Benincasa, A. Abalos, I. Oliveira, A. Manresa, *Antonie Van Leeuwenhoek* **85** (2004) 1
34. E. Haba, A. Abalos, O. Jauregui, M. J. Espuny, A. Manresa, *J. Surfactants Deterg*. **6** (2003) 155
35. J. C. Mata-Sandoval, J. Karns, A. Torrents, *J. Chromatogr*. **864** (1999) 211

36. O. Pornsunthorntawee, P. Wongpanit, S. Chavadej, M. Abe, R. Rujiravanit, *Bioresour. Technol.* **99** (2008) 1589
37. M. Rikalovic, G. Gojgic-Cvijovic, M. M. Vrvic, I. Karadzic, *J. Serb. Chem. Soc.* **77** (2012) 27
38. M. G. Rikalovic, A. M. Abdel-Mawgoud, E. Déziel, G. Dj. Gojgic-Cvijovic, Z. Nestorovic, M. M. Vrvic, I. M. Karadzic, *J. Surfactants Deterg.* **16** (2013) 673
39. M. Benincasa, J. Contiero, M. A. Manresa, I. O. Moraes, *J. Food Eng.* **54** (2002) 283
40. M. Benincasa, F. R. Accorsini, *Bioresour. Technol*. **99** (2008) 3843
41. K. S. M. Rahman, T. J. Rahman, S. McClean, R. Marchant, I. M. Banat, *Biotechol. Prog.* **18** (2002) 1277
42. S. G. V. A. O. Costa, M. Nitschke, R. Haddad, M. N. Eberlin, J. Contiero, *Process Biochem*. **41** (2006) 483
43. E. Déziel, F. Lépine, D. Dennie, D. Boismenu, O. A. Mamer, R. Villemur, *Biochim. Biophys. Acta* **1440** (1999) 244
44. M. Nitschke, S. G. Costa, J. Contiero, *Appl. Biochem. Biotechnol.* **160** (2010) 2066
45. A. Aparna, G. Srinkethan, H. Smitha, *Colloids Surfaces, B* **95** (2012) 23
46. M. Nie, X. Yin, C. Ren, Y. Wang, F. Xu, Q. She, *Biotechnol. Adv*. **28** (2010) 635
47. D. Dubeau, E. Déziel, D. Woods, F. Lépine, *BMC Microbiol.* **9** (2009) 263
48. E. Haba, A. Pinazo, O. Jauregui, M. J. Espuny, M. R. Infante, A. Manersa, *J. Surfact. Deterg.* **6** (2003) 155
49. M. M. Müller, B. Hörmann, M. Kugel, C. Syldatk, R. Hausmann, *Appl. Microbiol. Biotechnol*. **89** (2011) 585
50. A. Lebron-Paler, J. E. Pemberton, B. Becker, W. H. Otto, C. K. Larive, R. M. Maier, *Anal. Chem*. **78** (2006) 7649
51. M. Nitschke, S. G. V. A. O. Costa, J. Contiero, *Biotechnol. Prog*. **21** (2005) 1593
52. P. Maslin, R. M. Maier, *Biorem. J.* **4** (2000) 295
53. M. Heyd, A. Kohnert, T.-H. Tan, M. Nusser, F. Kirschhöfer, G. Brenner-Weiss, M. Franzreb, S. Berensmeier, *Anal. Bioanal. Chem.* **391** (2008) 1579
54. I. Siegmund, F. Wagner, *Biotechnol. Tech*. **5** (1991) 265
55. E. V. Chandrasekaran, J. N. BeMiller, in *Methods in Carbohydrate Chemistry*, R. L. Whistler, Ed., Academic Press, New York, 1980, p. 89
56. J. E. Hodge, B. T. Hofreiter, in *Methods in Carbohydrate Chemistry*, R. L. Whistler, M. L. Wolfrom, Eds., Academic Press, New York, 1962, p. 380
57. E. Déziel, F. Lépine, S. Milot, R. Villemur, *Biochim. Biophys. Acta* **1485** (2000) 145
58. N. P. J. Price, K. J. Ray, K. Vermillion, T. M. Kuo, *Carbohydr. Res.* **344** (2009) 204
59. S. G. V. A. O. Costa, S. R. de Souza, M. Nitschke, S. M. M. Franchetti, M. Jafelicci., R. B. Lovaglio, J. Contiero, *J. Surfactants Deterg*. **12** (2009) 125
60. W. Griffin, *J. Soc. Cosmet. Chem*. **5** (1954) 235
61. D. K. Jain, D. L. Collins-Thompson, H. Lee, J. T. Trevors, *J. Microbiol. Methods* **13** (1991) 271
62. M. Morikawa, Y. Hirata, T. Imanaka, *Biochim. Biophys. Acta* **1488** (2000) 211
63. E. A. Vogler, *Langmuir* **8** (1992) 2005
64. G. Özdemir, U. Malayoglu, *Colloids Surfaces, B* **39** (2004) 1
65. M. M. Müller, R. Hausmann, *Appl. Microbiol. Biotechnol*. **91** (2011) 251
66. S. Maneerat, *J. Sci. Technol.* **27** (2005) 675
67. S. Mukherjee, P. Das, R. Sen, *Trends Biotechnol.* **24** (2006) 509
68. R. Marchant, I. M. Banat, *Trends Biotechnol*. **30** (2012) 558

69. D. H. Dusane, S. S. Zinjarde, S. Smita, V. P. Venugopalan, R. J. C. McLean, M. M. Weber, P. K. S. M. Rahman, *Biotechnol. Genet. Eng. Rev.* **27** (2010) 159
70. G. Soberon-Chavez, F. Lepine, E. Déziel, *Appl. Microbiol. Biotechnol*. **68** (2005) 718
71. K. Dubey K, A. Juwarkar, *World J. Microbiol. Biotechnol*. **17** (2001) 61
72. A. B. Moldes, A. M. Torrado, M. T. Barral, J. M. Domianguez, *J. Agric. Food Chem.* **55** (2007) 4481
73. Z. A. Raza, A. Rehman, M. S. Khan, Z. M. Khalid, *Biodegradation* **18** (2007) 115
74. M. E. Mercade, M. A. Manresa, M. Robert, M. J. Espuny, C. De Andres, J. Guinea, *Bioresour. Technol.* **43** (1993) 1
75. E. Haba, M. J. Espuny, M. Busquets, A. Manresa, *J. Appl. Microbiol*. **88** (2000) 379
76. P. D. Mukherjee, R. Sen, *Trends Biotechnol*. **24** (2006) 509
77. S. S. Cameotra, P. Singh, *Int. Biodeter. Biodegr*. **62** (2008) 274
78. B. Dahrazma, C. N. Mulligan, *Chemosphere* **69** (2007) 705
79. A. A. Juwarkar, K. V. Dubey, A. Nair, S. K. Singh, *Indian J. Microbiol*. **48** (2008) 142
80. S. Wang, C. N. Mulligan, *Water Air Soil Pollut*. **157** (2004) 315
81. R. M. Miller, in *Bioremediation: science and applications*, H. D. Skipper, R. F. Turco, Eds., Soil Science Society of America, Madison, WI, 1995, p. 322
82. G. S. Shreve, S. Inguva, S. Gunnam, *Mol. Mar. Biol. Biotechnol*. **4** (1995) 331
83. Y. M. Zhang, R. M. Miller, *Appl. Environ. Microbiol.* **60** (1994) 2101
84. Y. M. Zhang, R. M. Miller, *Appl. Environ. Microbiol.* **61** (1995) 2247
85. R. Beal, W. B. Betts, *J. Appl. Microbiol*. **89** (2000) 158
86. W. H. Noordman, D. B. Janssen, *Appl. Environ. Microbiol*. **68** (2002) 4502
87. Ł. Chrzanowski, Ł. Ławniczak, K. Czaczyk, *World J. Microbiol. Biotechnol*. **28** (2012) 401
88. A. Franzetti, I. Gandolfi, G. Bestetti, T. J. Smyth, I. M. Banat, *Eur. J. Lipid Sci. Tech.* **112** (2010) 617
89. C. N. Mulligan, R. N. Yong, B. F. Gibbs, *J. Hazard. Mat*. **85** (2001) 111
90. N. S. Avramovic, S. D. Nikolic-Mandic, I. M. Karadzic, *J. Serb. Chem. Soc.* **78** (2013) 639
91. R. T. Sandrin, M. C. Andrea, R. M. Maier, *Appl. Environ. Microbiol.* **66** (2000) 4585
92. C. N. Mulligan, C. N. Yong, B. F. Gibbs, *Environ. Prog.* **18** (1999) 31
93. N. Kosaric, *Food Technol. Biotechnol.* **39** (2001) 295
94. I. P. H. Van Haesendonck, E. C. A. Vanzeveren, (International application patent PCT), W.O. 2004/040984 (2004)
95. R. J. Linhardt, R. Bakhit, L. Daniels, F. Mayerl, W. Pickenhagen, *Biotechnol. Bioeng*. **33** (1989) 365
96. S. K. Hood, E. A. Zottola, *Food Control* **6** (1995) 9
97. Y. Irie, G. A. O'Toole, M. H. Yuk, *FEMS Microbiol. Lett.* **250** (2005) 237
98. L. R. Rodrigues, I. M. Banat, H. C. Mei, J. A. Teixeira, R. Oliveira, *J. Appl. Microbiol.* **100** (2006) 470
99. T. Meylheuc, C. J. van Oss, M. N. Bellon-Fontaine, *J. Appl. Microbiol*. **91** (2001) 822
100. T. Meylheuc, C. J. van Oss, M.N. Bellon-Fontaine, *Int. J. Food Microbiol*. **109** (2006) 71
101. C. Dagbert, T. Meylheuc, M. N. Bellon-Fontaine, *Electrochim. Acta* **51** (2006) 5221
102. A. Ortiz, J. A. Teruel, M. J. Espuny, A. Marqués, A. Manresa, F. J. Aranda, *Int. J. Pharm*. **325** (2006) 99
103. G. Piljac, V. Piljac, (U.S. Patent), 5,455,232 (1995)
104. V. Kleckner, N. Kosaric, in *Biosurfactants: Production, Properties, Applications*, N. Kosaric, Ed., Marcel Dekker, New York, 1993, p. 373

105. E. Vasileva-Tonkova, D. Galabova, E. Karpenko, A. Shulga, *Lett. Appl. Microbiol.* **33** (2001) 280
106. Y. Ishigami, Y. Gama, H. Nagahora, T. Hongu, M. Yamaguchi, (U.S. Patent), 4,902,512 (1988)
107. T. Piljac, G. Piljac, (Europan Patent Office), E.P. 1, 889,623 A2 (1999)
108. K. Desanto, (Aurora Advanced Beauty Labs St. Petersburg, FL), Word Patent 2008/013899, (2008)
109. G. Piljac, V. Piljac, (U.S. Patent) 5,466,675 (1995b)
110. T. Stipcevic, T. Piljac, J. Piljac, T. Dujmic, D. Piljac, (PCT Patent Application) WO 01/10447 A1 (2001)
111. T. Stipcevic, T. Piljac, R. R. Isseroff, *J. Dermatol. Sci.* **40** (2005) 141
112. A. Piljac, T. Stipcevic, J. Piljac-Zegarac, G. Piljac, *J. Cutan. Med. Surg.* **12** (2008) 142
113. B. Thanomsub, W. Pumeechockchai, A. Limtrakul, P. Arunrattiyakorn, W. Petchleelaha, T. Nitoda, H. Kanzaki, *Bioresour. Technol.* **98** (2007) 1149
114. K. De Jonghe, I. De Dobbelaere, R. Sarrazyn, M. Höfte, *Plant Pathol.* **54** (2005) 219
115. M. Perneel, L. D'hondt, K. De Maeyer, A. Adiobo, K. Rabaey, M. Höfte, *Environ. Microbiol.* **10** (2008) 778
116. A. L. Varnier, L. Sanchez, P. Vatsa, L. Boudesocque, A. Garcia-Brugger, F. Rabenoelina, A. Sorokin, J. H. Renault, S. Kauffmann, A. Plugin, C. Clément, F. Baillieul, S. Dorey, *Plant Cell Environ.* **32** (2009) 178
117. S. Dorey, F. Baillieul, C. Clement, A. Varnier, M. Terganier, (International Patent), WO 2007/045767 (2007)
118. M. K. Bafghi, M. H. Fazaelipoor, M. Hassan, *J. Surfactants Deterg.* **15** (2012) 679
119. D. Kitamoto, T. Morita, T. Fukuoka, M. Konishi, T. Imura, *Curr. Opin. Colloid Interface Sci.* **14** (2009) 315
120. D. Kitamoto, K. Toma, M. Hato, in *Handbook of nanostructured biomaterials and their applications in nanobiotechnology*, Vol. 1, H. Singh Nalwa, Ed., American Science Publishers, Valencia , CA, USA, 2005, p. 239
121. T. T. L. Nguyen, A. Edelen, B. Neighbors, D. A. Sabatini, *J. Colloid Interface Sci.* **348** (2010) 498
122. V. K. Sharma, R. A. Yngard, Y. Lin, *Adv. Colloid Interface Sci.* **145** (2009) 83
123. C. G. Kumar, S. K. Mamidyala, B. Das, B. Sridhar, G. S. Devi, M. S. Karuna, *J. Microbiol. Biotechnol.* **20** (2010) 1061
124. Y. Xie, R. Ye, H. Liu, *Colloids Surfaces, A* **279** (2006) 175
125. P. Palanisamy, A. M. Raichur, *Mater. Sci. Eng. C* **29** (2009) 199
126. J. Narayanan, R. Ramji, H. Sahu, P. Gautam, *IET Nanobiotechnol.* **4** (2010) 29
127. C. Hazra, D. Kundu, A. Chaudhari, T. Jana, *J. Chem. Technol. Biotechnol.* **88** (2013) 1039
128. Y. Xie, Y. Li, R. Ye, *J. Dispers. Sci. Technol.* **26** (2005) 455
129. Y. W. Xie, R. Q. Ye, H. L. Liu, *Colloids Surfaces, A* **292** (2007). 189
130. T. T. Nguyen, D. A. Sabatini, *J. Surfactants Deterg.* **12** (2009) 109.