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BIOSURFACTANTS: STRUCTURE, FUNCTIONS AND PRODUCTIONS

Ye. B. YANVAROV, V. V. HAVRYLIAK

Department of Technology of Biologically Active Substances,
Pharmacy and Biotechnology,
Lviv Polytechnic National University, Ukraine

E-mail: yehor.b.yanvarov@lpnu.ua

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Surfactants are widely used in many areas of our life. However, synthetic surfactants have a serious negative impact on the environment. They do not decompose well and can accumulate in ecosystems. Microbial biosurfactants can be an alternative to synthetic surfactants. They are characterized by a diverse structure, stable at critical temperatures, pH and can be obtained from various renewable raw materials.

Aim. Analysis and generalization of the available information on the main characteristics and features of the synthesis of surface-active substances of microbial origin.

Results. The article describes the structure of the most important groups of biosurfactants of microbial origin, such as rhamnolipids, trehalolipids, and sophorolipids. The main producers of biosurfactants, as well as the areas of their application were characterized. Information about the main ways of their biosynthesis was discussed. Special attention in the review was paid to factors that are essential for the cultivation of microorganisms — the main producers of biosurfactants.

Key words: biosurfactants; rhamnolipids; trehalolipids; sophorolipids; biosynthesis; cultivation.

Surfactants belong to an important class of chemical compounds found in many everyday products. They are included in cleaning agents, detergents, cosmetics, herbicides, pesticides, etc. In other words, surfactants are actively used in agriculture, food, pharmaceutical, textile, paper, oil industries [1, 2].

Surfactants are mainly amphiphilic organic compounds that dissolve in organic solvents, non-polar fats and polar media. They accumulate on the surfaces between liquid phases with different polarity (air-water, oil-water), thus reducing surface and interphacial tension [3].

Most of the surfactants used today are synthesized chemically from petrochemical resources, and they decompose only partially, which has a very bad effect on the environment [1].

This became an impetus for scientists to direct their research towards more environmentally friendly surfactants, such as microbial surfactants (biosurfactants). They are known as biosurfactants [4], which are obtained by microbial synthesis, have similar properties and are much more environmentally friendly than chemically obtained surfactants. Rising consumer concerns about the environment, as well as increased environmental protection legislation, led to the development of microbial surfactants as an alternative to existing synthetic ones [5].

Biosurfactants have gained wide popularity because they are an ecological alternative to synthetic compounds with surface activity and have a number of significant advantages: they are characterized by a diverse structure, less toxic, highly active and stable at critical temperatures, pH and salinity. They can also be produced from renewable raw materials by means of a wide range of microorganisms. But their greatest advantage is that they are environmentally friendly chemicals, as they are biodegradable [6].

Microbial surfactants have various functional properties, which include: foaming,

cleaning, wetting, emulsifying, dispersion, corrosion inhibition, surface activity, etc. [7—9]. Such properties make it possible to effectively use them in the following ways: in biotechnology for bioremediation, namely cleaning oil spills in a natural way; as components in cleaning agents and detergents; as emulsion stabilizing agents in the cosmetic, pharmaceutical and food industries [10].

Surface-active compounds of microbial origin

The classification of biosurfactants is based on the microbial source and their chemical components. They can also be divided into low-molecular weight (glycolipids, lipopeptides, phospholipids) and high-molecular weight surfactants (polymeric and solid) [10].

The vast majority of currently known biosurfactants belong to glycolipids. These are carbohydrates combined with long-chain aliphatic or hydroxyaliphatic acids. The most common among the group of glycolipids are rhamnolipids, trehalolipids, and sophorolipids.

Rhamnolipids have one or two molecules of rhamnose bound to one or two of β-hydroxy-decanoic acid molecules (Fig. 1). They belong to the best studied glycolipids. The synthesis of rhamnose found in glycolipids was first described for *Pseudomonas aeruginosa* by Jarvis and Johnson [11].

Trehalose lipids have only a few structural types (Fig. 2). The disaccharide trehalose, bound at the C-6 and C-6′ positions to mycolic acids, is characteristic of most *Nocardia*, *Mycobacterium*, and *Corynebacterium* species. The trehalose lipids of these organisms differ in the structure of mycolic acid, size, number of carbon atoms, and degree of unsaturation [11].

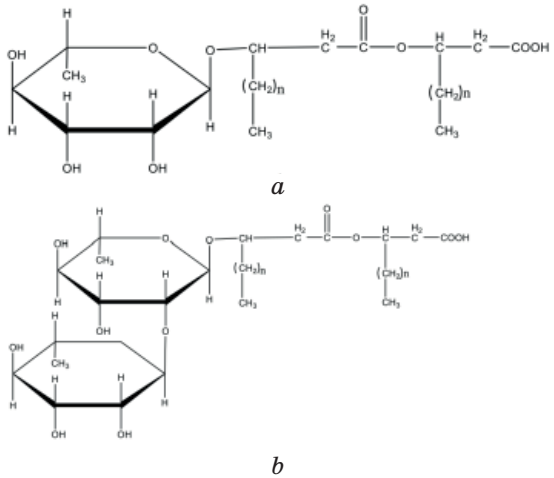

Fig. 1. **Structural formulas of rhamnolipids:** *a* — monorhamnolipid; *b* —diramnolipid [12]

Sophorolipids are mainly produced by yeasts such as *Torulopsis bombicola*, *Torulopsis petrophilum*. The dimeric carbohydrate of sophorose binds to a long-chain hydroxy fatty acid (Fig. 3). Sophorolipids are a mixture of six to nine different hydrophobic sophorosides [11].

Table 1 presents generalized information about the main producers of biosurfactants and their main areas of application.

Rhamnolipids

Rhamnolipids consist of a β-hydroxy fatty acid bound by the carboxyl end to a rhamnose sugar molecule. They are most often produced by *Pseudomonas aeruginosa*. Rhamnolipids can be divided into mono- and dirhamnolipids.

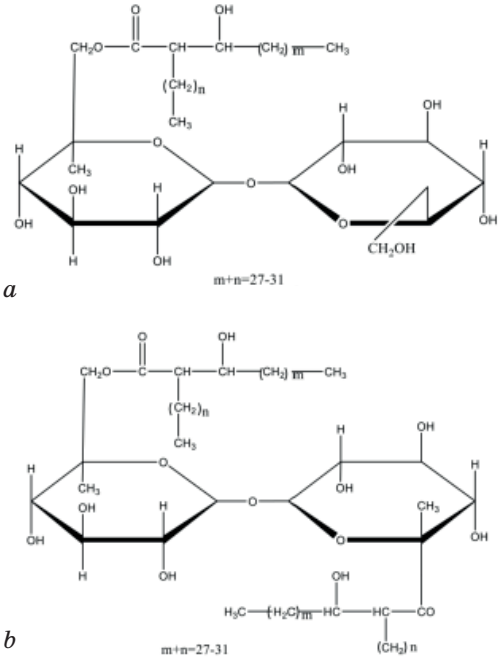

Fig. 2. **Structural formulas of Trehalose Lipids:** *a* — Trehalose monomycolates; *b* — trehalose dimycolates [12]

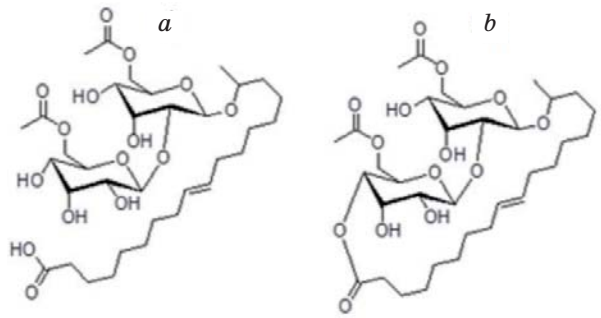

Fig. 3. **Structural formulas of sophorolipids:** *a* — acidic; *b* — lactone [13]

Table 1. **Producers of biosurfactants and their areas of application**

No.	Surfactants	Strain/producer	Area of application	Reference
1	Rhamnolipids	*Pseudomonas aeruginosa*	Oil production	[14]
2	Rhamnolipids	*Pseudomonas aeruginosa*, *Bacillus subtilis SPB1*	Detergents (stain removal)	[15, 16]
3	Rhamnolipids	*Lysinibacillus sphaericus IITR51*	Antimicrobial effect (ability to dissolve hydrophobic pesticides)	[17]
4	Sophorolipids	*Starmerella bacillaris*	Winemaking	[18]
5	Trehalose Lipids	*Rhodococcus fascians BD8*	Antimicrobial and antiadhesive activity against pathogenic bacteria	[19]
6	Sophorolipids	*Candida bombicola*	Antiviral activity	[20]
7	Rhamnolipids	*Pseudomonas aeruginosa S2*	Bioremediation of areas contaminated with oil products	[21]
8	Rhamnolipids	*Pseudomonas aeruginosa BS20*	Bioremediation of areas contaminated with hydrocarbons	[21]
9	Rhamnolipids	*Pseudoxanthomonas sp. PNK-04*, *Pseudomonas alcaligenes*	Ecological application	[21]
10	Rhamnolipids	*Pseudomonas cepacia CCT6659*	Bioremediation of the marine and soil environment	[21]
11	Sophorolipids	*Candida lipolytica UCP0988*	Removal of oil and motor oil from sand	[21]
12	Trehalose Lipids	*Rhodococcus erythropolis 3C-9*	Liquidation of oil spills	[21]
13	Trehalose Lipids	*Rhodococcus erythropolis B7g*	Bioremediation	[22]

The main producers of rhamnolipids are *Pseudomonas* bacteria: *P. chlororaphis, P. fluorescens, P. plantarii, P. putida*. Among the bacteria known to us, there are those that synthesize mono- and di-rhamnolipids, and there are those that produce only monorhamnolipids [23].

The synthesis of rhamnolipids is best studied for *Pseudomonas* strains [24]. Literature data indicate that different carbon sources can be the main precursors for the biosynthesis of rhamnolipids, which is why different PA strains produce rhamnolipid variants [25]. The biosynthesis of rhamnolipids includes three main steps that lead to the formation of dTDP-1-rhamnose and β-hydroxy fatty acid [26]. At the 1st stage, β-hydroxydecanoyl is transferred by the RhlA enzyme to coenzyme A, resulting in the formation of β-hydroxydecanoyl-CoA, an intermediate product in *de novo* fatty acid synthesis [27]. RhlA ensures the formation of the lipid part of rhamnolipid — β-hydroxydecanoyl-β-hydroxydecanoate, using the type II fatty acid synthase pathway. The precursor, dTDP-1-rhamnose, which provides the glycosyl part of rhamnolipids, can be synthesized by gluconeogenesis or Entner–Doudoroff pathway while the algC and rmlBDAC operon genes control the dTDP-l-rhamnose biosynthesis [24]. The two precursors, dTDPl-rhamnose and β-hydroxy fatty acids, are condensed into monorhamnolipids and di-rhamnolipids by the enzymes of rhamnosyltransferase I (RhlB) and rhamnosyltransferase II (RhlC), respectively. Rhamnosyltransferase I (RhlB) and rhamnosyltransferase II (RhlC) are encoded by the rhlB and rhlC genes, respectively, while the lasRI and rhlRI genes are responsible for activating the expression of the rhlAB and rhlC genes [28].

Over the past decades, a significant amount of research has been carried out on optimizing the ways to obtain rhamnolipids using biotechnology, which allowed to considerably expand the scope of their application in various industries. This was also facilitated by their biodegradability and biocompatibility, as well as the availability of raw materials for synthesis [23].

In total, five main areas of rhamnolipid applications can be distinguished, which satisfy a wide range of industrial needs:

Cleaning agents and detergents: Rhamnolipids are natural emulsifiers, which has led to their widespread use in detergents, soaps, shampoos, laundry detergents, etc. [29].

Pharmaceuticals: rhamnolipids are low toxic, and demonstrate antimicrobial activity against *Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus*, and *Listeria monocytogenes* microbes [30].

Cosmetics: rhamnolipids, as an active ingredient, can be effectively used for skin treatment, in particular burns, wound healing, as well as for the elimination of wrinkles [31].

Agriculture: rhamnolipids are used to improve soil quality and recultivation. Rhamnolipids are also currently being researched as biopesticides, and their ability to destroy plant pathogens is being studied, which will facilitate the absorption of fertilizers and nutrients through plant roots [32].

Bioremediation and removal of oil spills: rhamnolipids, due to their good emulsifying properties, are able to effectively remove oil spills from contaminated soil [21].

However, despite all the advantages, rhamnolipids are still not able to completely displace surfactants of chemical origin from the market. This is due to the peculiarities of the rhamnolipid synthesis process on an industrial scale and production costs [23]. It is known that the biosurfactant cultivation process accounts for 70–80% of all production costs [23].

TREHALOSE LIPIDS

Trehalose lipids are good emulsifiers. They are mainly used to eliminate oil spills. Trehalose lipids are characterized by high bioactivity, as they easily penetrate membranes, demonstrate hemolytic activity, and affect cell differentiation [33–38]. They also have antimicrobial properties and play a significant role in the treatment of infectious diseases [39]. Bacteria of the genus *Rhodococcus* can synthesize both exo- and endogenous trehalose lipids.

It should be noted that fundamental studies of the metabolic pathways involved in the synthesis of trehalose lipids are still insufficient. Schematically, this process can be described as follows: carbohydrate and fatty acid components of trehalose lipids are synthesized independently and subsequently esterified. Mycolate is formed as a result of the Claisen-type condensation of two fatty acids, while the synthesis of the remaining carbohydrate component, trehalose-6-phosphate, is catalyzed by trehalose-6-phosphate synthetase, which binds two d-glucopyranose units at the C1 and C1′ positions. The precursors are UDP-glucose and glucose-6-phosphate [40]. A detailed pathway for the biosynthesis of all mycolic acids in *M. tuberculosis* and trehalose-dicorynomycolates was proposed by Takayama et al. [41].

Trehalose lipids obtained with *Rhodococcus* are structurally diverse: trehalose monomycolates, dimycolates, and trimycolates; various nonionic acylated derivatives of trehalose and anionic trehalose tetraethers and succinoyl trehalose lipids. Considering such a variety of currently known and studied trehalose lipids, it is likely that new strains of *Rhodococcus* obtained from other environments will be able to discover trehalose lipids with new interesting properties [42].

Trehalose lipids are widely used in many areas: soil bioremediation, food industry, and agriculture [39].

SOPHOROLIPIDS

Sophorolipids are glycolipids structurally composed of the disaccharide sophorose (2′-O-β-D-glucopyranosyl-1-β-D-glucose) linked by a β-glycosidic bond to a long chain of fatty acids [43, 44]. They are synthesized in the form of a mixture of chemical structures in which the carbon end of the fatty acid can be free or esterified at the C4′, C6′ or C6» position to form the lactone form (Fig. 3) [13]. These structures can also differ from each other in terms of the carbon amount, hydrogenation, unsaturation and acetylation. In general, acidic sophorolipids are more soluble and have better foaming ability, but lactone ones have better antimicrobial properties and surface tension [45, 46].

Candida apicola and *Candida bombicola*, described in the 1970s, were the first microorganisms used for the biosynthesis of sophorolipids. *C. bombicola (Starmerella bombicola)* is the main microorganism used for the production of sophorolipids [47].

The biosynthesis of sophorolipids is best described for *Candida bombicola* yeast [48]. The biosynthesis of sophorolipids occurs in the nitrogen-limited stationary phase. The biosynthesis process begins with the hydroxylation of a fatty acid present in the environment. This fatty acid can be in the form of alkanes, alcohol, aldehydes, triglycerides

or fatty acid esters, if these are not present in the environment, the fatty acid is formed by *de novo* synthesis from acetyl-CoA. It should be noted that at the low glucose concentration in the environment, these hydrophobic carbon sources are metabolized through β-oxidation and are used to maintain cell viability instead of sophorolipid synthesis [49]. The process of activation of fatty acids occurs by hydroxylation of the terminal carbon atom with the participation of the CYP52M1 enzyme. Characteristically, the CYP52M1 enzyme is expressed exclusively in the stationary phase. This expression can be enhanced by the DAP1 protein, which stabilizes and regulates the CYP450 protein and is involved in the metabolism of lipids and sterols [50]. In the next two steps, two glucose molecules bind to the activated fatty acid. The first glucose molecule in the C1 position binds to the hydroxyl group of the fatty acid under the action of glucosyl transferase I (UgtA1). UDP-glucose (glucose uridine diphosphate) is the donor of the glycosyl group in this reaction. At the next stage, the glucose molecule binds to the first glucose molecule at the C2′ position with the participation of glucosyltransferase II (UgtA2). Both UgtA1 and UgtA2 enzymes are expressed at a high level at the beginning of the stationary phase. It is interesting that the glucose contained in the environment is not included in the structure of sophorolipids directly, but is metabolized by glycolysis, while sophorolipid glucose is formed as a result of gluconeogenesis [51]. Sophorolipids in non-acetylated acidic form are transformed by acetylation or lactonization under the action of acetyltransferase or lactonesterase, respectively [50].

It is known that sophorolipids can improve the activity of pesticides and herbicides, and are also able to increase agricultural crop yields [13].

The results of some studies have revealed the antimicrobial activity of sophorolipids *in vitro*. They can be used as adjuvants to other antimicrobial agents against certain pathogens by inhibiting the growth and destroying the biofilm. Sophorolipids at concentrations of 5% by volume inhibited the growth of gram-negative *Cupriavidus necator ATCC 17699* and gram-positive *Bacillus subtilis BBK006* [52].

In addition, sophorolipids are able to dissolve and emulsify hydrophobic contaminants (diesel, motor oil, grease, kerosene and crude oil), remove heavy metals (mercury, zinc, lead, chromium, cadmium, iron and copper), as well as pesticides in the aqueous phase [53–55].

THE INFLUENCE OF VARIOUS FACTORS ON THE RATE OF CULTIVATION OF BIOSURFACTANTS

Many factors influence the cultivation process of biosurfactant-producing micro-organisms. The key ones are: carbon source, nitrogen source, temperature, pH, agitation rate, and fermentation time. The type of bioreactor also affects the biosurfactant cultivation process.

Carbohydrates, oils and fats are used as a source of carbon in the nutrient medium [4]. They can be both water-soluble (glycerol, glucose, sucrose) and insoluble (vegetable oils, crude oils). The most common source of carbon is glucose. Tomar and Srinikethan (2016) investigated biosurfactant synthesis during the cultivation of *Pseudomonas aeruginosa MTCC 7815* in a medium containing glucose, glycerol, fructose, and starch. The maximum emulsification index E_{24} = 76.77% and the minimum surface tension (34.53 mN/m) were characteristic of biosurfactants grown in an environment in which glucose acted as the main carbon source [56].

Santa Anna et al. (2002) demonstrated that yeast extract, meat extract, NH_4NO_3, $NaNO_3$ and urea can be a source of nitrogen for *Bacillus sp.* and *Pseudomonas sp.* It was investigated that during the cultivation of *Pseudomonas aeruginosa*, in a mineral salt medium, using sodium nitrate instead of other nitrogen sources, the yield of rhamnolipid was 3.16 g/l [57].

Hippolyte et al. (2018) studied that the temperature increase stimulates the biosurfactant production from *Lactobacillus paracasei N2* and does not have a negative effect on surface tension reduction. The maximum yield and activity of biosurfactants were observed in the temperature range of 33–34 °C and molasses concentration of 5.49–6.35% (w/v). At this temperature range, peptone and molasses are effectively used by the *Lactobacillus paracasei N2* strain with the maximum yield of biosurfactant, which is characterized by high antimicrobial and surface activity. Beyond this temperature range, the authors observed a decrease in the yield and weakening of the biosurfactant activity produced by *Lactobacillus paracasei N2*. An increase in temperature could lead to the cell growth inhibition and the weakened biosurfactant synthesis [58]. Agarry et al. (2015) investigated that rhamnolipids grown using *Bacillus* and *Pseudomonas* strains have the highest activity at the temperature of 30 °C [59].

The operating temperature and pH ranges must be selected very carefully, as the temperature change must not cause changes in the biosurfactant composition. The optimal temperature of the culture medium for the maximum biosurfactant yield depends on the physiology of the producing organism. Guerra-Santos et al. (1984) investigated that for the synthesis of *Pseudomonas sp.* rhamnolipids, the most optimal pH range is within 6.0–6.5, and with an increase in pH to 7.0, a sharp decrease occurs [60]. For microorganisms of the *Pseudomonas aureofaciens NB-1* strain, it was determined that the amount of surfactant is 54% higher when extracted with ethyl acetate and isopropanol (2:1) and is 3.71 g/l at pH 11 than at pH 3 [61].

Akbari, E., Rasekh, B., Maal, K.B. et al. (2021) determined the optimal agitation rate during the cultivation of *Kocuria rosea ABR6*. The experiments were carried out at the speeds of 80 rpm, 100 rpm, 120 rpm, and 140 rpm, while the remaining parameters stayed unchanged. It turned out that the optimal agitation rate for biosurfactant synthesis using *Kocuria rosea ABR6* is 120 rpm [62].

The duration of fermentation should be determined taking into account the possible formation of secondary metabolites, which can interfere with the formation of emulsion and adsorption of biosurfactant molecules. The incubation period plays a very important role in the synthesis of biosurfactants, since biomolecules are synthesized at different time intervals. For example, the maximum biosurfactant yield during the fermentation of *Acinetobacter sp.* was reached after 168 h in the medium supplemented with olive oil, while the optimal biosurfactant yield was recorded for *Aeribacillus pallidus YM-1* after only 10 h when grown in the medium containing glucose [63, 64].

Therefore, biosurfactants (rhamnolipids, trehalolipids, and sophorolipids) have numerical advantages over synthetic surface active substances. However, their production remains quite expensive. There are several ways to optimize their biosynthesis, in particular, improvement of producer strains, and selection of optimal cultivation parameters for each specific producer. All cultivation parameters that significantly affect the yield of biosurfactants must be selected for each producer experimentally or by modeling the process. The correct selection of parameters will enable to significantly optimize the biosurfactant synthesis process and reduce the cost of production.

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Conflict of Interest

Authors declare that there is no conflict of interest.

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БІОСУРФАКТАНТИ: СТРУКТУРА, ФУНКЦІЇ, ВИРОБНИЦТВО

ЯНВАРЬОВ Є. Б., ГАВРИЛЯК В. В.

Національний університет «Львівська політехніка»,
Львів, Україна

E-mail: yehor.b.yanvarov@lpnu.ua

Поверхнево активні речовини використовують у багатьох сферах. Проте синтетичні ПАР чинять серйозний негативний вплив на довкілля, оскільки погано розкладаються і можуть накопичуватися в екосистемах. Мікробні біосурфактанти можуть бути альтернативою синтетичним поверхнево активним речовинам. Вони характеризуються різноманітною структурою, стабільні при критичних температурах та pH. Їх можна отримувати з різноманітної відновлюваної сировини.

Мета: аналіз та узагальнення наявної інформації про основні характеристики та особливості синтезу поверхнево активних речовин мікробного походження.

Результати. У статті детально описано структуру таких найважливіших груп біосурфактантів мікробного походження, як рамноліпіди, трегалозоліпіди та софороліпіди, охарактеризовано їхні основні продуценти, а також сфери застосування. Обговорено відомості про основні шляхи синтезу біосурфактантів. Особливу увагу в огляді приділено факторам, які мають суттєве значення для культивування мікроорганізмів — основних продуцентів біосурфактантів.

Ключові слова: біосурфактанти; рамноліпіди; трегалозоліпіди; софороліпіди; біосинтез; культивування.