

## PRACTICALLY VALUABLE PROPERTIES OF THE SURFACTANT SYNTHESIZED BY *RHODOCOCCLUS* GENUS ACTINOBACTERIA

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Currently, microbial surfactants are the objects of intense research because of their surface-active and emulsifying properties, high antimicrobial, anti-adhesive activity, and ability to destroy biofilms. The review provides current literature data on the properties of surfactants synthesized by *Rhodococcus* genus actinobacteria, determining their practical significance. The researchers' interest in the surfactants of *Rhodococcus* bacteria is primarily due to their key role in the destruction of xenobiotics (aliphatic, heterocyclic and polycyclic aromatic hydrocarbons). Information on the antimicrobial and anti-adhesive activity of surfactants of *Rhodococcus* genus bacteria remains scarce at present, while the immunomodulatory properties of these products of microbial synthesis are studied more actively than for other microbial surfactants known in the world. The data of our experimental studies on the practically valuable properties of surfactants synthesized by *Rhodococcus erythropolis* IMV Ac-5017 are presented. Unlike surfactants of other representatives of *Rhodococcus* genus bacteria, surfactants of IMV As-5017 strain are multifunctional preparations. Because in addition to the high efficiency of the destruction of oil pollution, including complex with heavy metals, surfactants are characterized by high antimicrobial and anti-adhesive activity, including the ability to destroy biofilms.

**Keywords:** *Rhodococcus*, surfactants, destruction of xenobiotics, antimicrobial and anti-adhesive activity, destruction of biofilms, immunomodulatory properties.

The extensive metabolic capabilities of *Rhodococcus* genus bacteria enhance interest of researchers [1]. The metabolic capabilities provide considerable potential for these actinobacteria as destructors of xenobiotics [2–4] and their ability to synthesize number of practically valuable compounds like surfactants [5–7], antibiotics [8, 9], enzymes [10–12], pigments, siderophores, etc. [13]. Among these metabolites, greater emphasis is being placed on the surfactants. They have a wide range of physicochemical (ability to reduce surface and interfacial tension, emulsification of various substrates, metal deposition) and biological (antimicrobial, anti-adhesive, immunomodulatory, cytotoxic activity) properties [14–17]. The mentioned properties allow us to consider biosurfactants as target products of a multifunctional purpose, promising for use

in various industries, agriculture, medicine, and environmental technology.

In 2012, we published the review [18], which summarized the available literature data on the potential of actinobacteria related to the genus *Rhodococcus* as destructors of aromatic, heterocyclic and aliphatic xenobiotic compounds, as well as producers of surfactants, antibiotics, exopolysaccharides, enzymes, and prospects for their practical application.

The purpose of this review is to analyze and summarize current literature data, as well as own experimental studies of the physicochemical and biological properties of surfactants synthesized by *Rhodococcus* genus bacteria that appeared after the publication of the review [18] or were not mentioned in it.

Notably, in 2017 was published a review of Elsayed et al. [13] on the biologically active substances synthesized by *Rhodococcus*. However, it does not contain information on the surfactants of these actinobacteria.

### **The role of surfactants in the destruction of xenobiotics**

The first publications on the prospects for the practical use of surfactants synthesized by *Rhodococcus* genus bacteria are from the early 80's of the twentieth century (cit. according to [19]). These works have established the involvement of surfactants in the decomposition of oil and other hydrocarbons.

In the works published during 2013–2019, the authors note that the key role in the processes of xenobiotic degradation with the participation of *Rhodococcus* genus actinobacteria belongs to the synthesized surfactants [20–33].

Notably, today, researchers use two approaches to evaluate the role of microbial surfactants in the decomposition of xenobiotics. The first approach involves the study of the ability to synthesize surfactants during the cultivation of *Rhodococcus* on media containing toxic compounds as a source of carbon and energy. In these studies, the ability to synthesize surfactants was evaluated by the index of surface tension [20, 21, 25–28, 32], the emulsification index [21, 25, 28, 29, 32], oil spreading test [21, 26], the chemical composition of metabolites, which was determined by thin-layer chromatography [21, 24, 27, 32], gas chromatography-mass spectrometry [23], or determination of product concentration after extraction with organic solvents [30]. The second approach involves *in vitro* research and consists of xenobiotic-contaminated systems model using (water, soil), in which surfactant-containing culture fluid and solutions isolated surfactants were used as preparations for purification [21, 24, 30].

### **Xenobiotics as substrates for growth and synthesis of surfactants of *Rhodococcus* genus bacteria**

**Crude oil.** Notably, that in the current literature, there is not much information on the synthesis of surfactants by *Rhodococcus* genus bacteria growing on crude oil. The authors [20, 21, 31] used seawater or model (high NaCl content) water to prepare nutrient media to bring laboratory conditions closer to real ones since the problem of seawater purification from spilled oil is still relevant.

Thus, a group of scientists from the Netherlands [31] found that in the case of co-cultivation of surfactant-producing *Pseudomonas putida* F1 and *R. qingshengii* TUHH–12 strains in seawater for 45 days at a temperature of 20° C, the degree of oil degradation (initial concentration in medium was 5 g/L) was 60%. The authors of this paper noted that a temperature of 20° C is lower than the optimum for the growth of both strains. However, performed studies allowed to evaluate the potential effectiveness of these microorganisms using as oil destructors under adverse conditions.

It was shown in [21] that *R. soli* 102-Na5 strain isolated from oil-contaminated Taean beach sediment (Korea) during growth on a medium with crude oil (1%, v/v) containing 30 g/l NaCl, metabolized 85% of the substrate for 14 days at 28 °C. Under these cultivation conditions, the surface tension index was 36.7 mN/m.

Researchers from China [20] found that *Rhodococcus* sp. JZX–01 strain, isolated from oil-contaminated soils, assimilated 65.3% of oil (initial concentration in the medium was 50 g/l) for 9 days at 30° C, pH 7.2. The concentration of sodium chloride in the medium was 0.5 g/l. The authors note that even in the case of increasing of NaCl content to 30 g/l, the degree of oil degradation was 50.4%. The conclusion about the ability of JZX–01 strain to synthesize surfactants was made on the basis of the surface tension index ( $\sigma = 31.3$  mN/m).

The degree of oil destruction (initial concentration in the medium was 0.5%) after 120 h of *R. erythropolis* IMB B-7012 cultivation was 68.9% [32]. The authors investigated the ability to synthesize surfactants during strain growth on hexadecane. It was found that in this case, the surface tension of the cell-free culture fluid was reduced to 50 mN/m, and the index of emulsification of hexadecane was 51%. In [32], the chemical composition of surfactants was established, but their concentration was not given.

Pi et al. [33] found that *R. erythropolis* M-25 can metabolize 70.7% of oil (initial concentration in the medium was 3%) for 30 days. The authors note that this strain forms a surfactant, but this paper does not provide any indicators of their synthesis.

**Aromatic/polycyclic hydrocarbons.** The authors of [25, 27, 34] found that the cultivation of some *Rhodococcus* genus bacteria on media with aromatic polycyclic hydrocarbons is often accompanied by the synthesis of surfactants that facilitate the consumption of these xenobiotics by microorganisms. Typically, during mentioned

studies, the ability to synthesize surfactants was determined by the surface tension index.

Thus, in [27] it was shown that during cultivation of *Rhodococcus* sp. NJ2 on a medium with 200 mg/l fluoranthene (polycyclic hydrocarbon), the surface tension of the culture fluid decreased to 28 mN/m. On the 10<sup>th</sup> day of cultivation, the degree of xenobiotic destruction was 74%.

A group of scientists from Bulgaria [25] established the ability of free and immobilized *R. wratislawiensis* BN38 cells to assimilate monosubstrates and mixture of substrates (phenol, hexadecane) with simultaneous surfactant synthesis. The substrate was added in cycles fractionally by 500 mg/l of each hydrocarbon. The cycle was considered completed after the complete destruction of phenol and hexadecane, after which the substrates were reintroduced into the medium. It was found that free cells completely assimilated a mixture of hydrocarbons (8 g/l of phenol and hexadecane were completely assimilated after 16 cycles). In the case of cells' immobilization in hydroxypropyl cellulose/poly (N-isopropylacrylamide) cryogels, the destruction of 20 g/l of hydrocarbons was observed for 40 cycles. During cultivation, the surface tension index decreased to 32 mN/m.

Kundu et al. [34] showed that *R. pydinovorans* NT2 decomposed 98% of 400 mg/l of 4-nitrotoluene for 120 hours. The assimilation of xenobiotics was accompanied by the synthesis of surfactants, although its concentration was low, only 45 mg/l. Later, the same group of authors found that NT2 strain was able to grow on a medium containing 2,4 dinitrotoluene [35] and 2,6-dinitrotoluene [36] as carbon source; however, the degree of decomposition of these xenobiotics was low. Therefore, in subsequent studies [37] Kundu et al. determined the cultivation conditions of *R. pydinovorans* NT2, which would provide intensification of the destruction of 2,4- and 2,6-dinitrotoluene. It was found that with the presence in the culture medium of 0.11 g/L of magnesium sulfate and maintaining the temperature at 37.5 °C the degree of destruction of both xenobiotics (initial concentration was 470-474 mg/l) after 72 h was 97.55%. It should be noted that before the optimization of cultivation conditions, after 108 h the decomposition rate of 2,4 dinitrotoluene did not exceed 67%, and of 2,6-dinitrotoluene – 27% [37].

Our studies [38] have shown that *R. erythropolis* IMV Ac-5017 synthesizes surfactants during

cultivation on aromatic hydrocarbons. Under the conditions of IMV Ac-5017 strain growth on a medium with phenol and toluene (0.5%), the conditional concentration of surfactants was 3.3 and 1.3, respectively. Higher concentrations of phenol and toluene were toxic to *R. erythropolis* IMV Ac-5017. Benzene and naphthalene in low concentrations (0.3%) inhibited the biosynthesis of surfactants (the conditional concentration of surfactants did not exceed 0.6).

**Other hydrocarbons.** The ability to synthesize surfactants by bacteria of the *Rhodococcus* genus during growth on diesel fuel [22, 39] and kerosene [26] has been reported.

Thus, in [22] it was found that during the growth of free and immobilized in Na-alginate cells of *Rhodococcus* sp MK1 on a medium with diesel fuel (1%), the degree of its destruction after 7 days was 70 and 45%, respectively. The authors note that the lower efficiency of substrate degradation by immobilized cells is reasoned by the synthesized cell-bound surfactants, and immobilization complicates their effect on the emulsification and availability of diesel fuel for cells. The researchers determined qualitative composition (fatty and mycolic acids) of the synthesized surfactants only. The data about their concentration were not given.

Petrikov et al. [39] showed that under conditions of growth on hydrocarbon substrates, including diesel fuel, *Rhodococcus* sp. S67 formed surface-active trehalose tetraester in a low concentration (310 mg/l). The authors did not consider *Rhodococcus* sp. S67 as a promising surfactant producer. The reason is that it is an effective xenobiotic destructor, which is part of the MicroBak biological product for cleaning the environment from oil [40], and the ability to synthesize surfactants increases the degree of destruction of hydrocarbon contaminants. It should be noted that in 2018, a work [41] was published in which it was reported that *Rhodococcus* sp. S67 is the first representative of the *Rhodococcus* genus which synthesizes surfactants at 10 °C on solid-state hexadecane (2%). Even though the amount of surfactants formed under such conditions is low (0.15 g/l), the obtained results indicate the possibility of S67 strain using to eliminate oil pollution in cold climate regions with.

Thus, Chen et al. [23] found that *Rhodococcus* sp. LH can grow in a medium with 1% diesel fuel at low temperatures (15° C). One of the mechanisms involved in the decomposition of this substrate is the synthesis of surfactants, in particular, fatty

acids, the presence of which was established by the gas chromatography-mass spectrometry method.

During the cultivation of *Rhodococcus* sp. LF-13 and *Rhodococcus* sp. LF-22 marine strains on a medium with 1% kerosene observed a decrease in the surface tension of cell-free culture fluid to 31.8 and 29.7 mN/m, respectively [26]. However, the researchers have found that resting cells are also capable of forming surfactants, and therefore, the biosynthesis of surfactants is not associated with the growth of LF-13 and LF-22 strains.

In [24] it was shown that after 14 days of *Rhodococcus* sp. Y2-2 psychrotolerant strain cultivation on a mixture of gasoline, diesel, and kerosene (500 mg/l of each hydrocarbon) at a temperature of 10° C and pH 7 the degree of destruction was 84%. It was found by using the method of thin-layer chromatography that Y2-2 strain synthesizes a complex of surface-active glyco- and aminolipids.

Therefore, the data on the formation of surfactants by *Rhodococcus* genus bacteria on the toxic hydrocarbons indicate the feasibility of their use in the composition of preparations for xenobiotics destruction.

#### ***In vitro* destruction of xenobiotics**

In [21, 24, 30], the role of surfactants in the destruction of xenobiotics in soil was determined on the corresponding model systems.

Thus, Korean scientists have found that 1 g of soil contaminated with oil (200 mg/kg) was added to the surfactant-containing culture fluid (10 ml) obtained after *Rhodococcus soli* 102-Na5 growing in oil medium (10 g/l), and the degree of degradation of oil in such conditions was 60% after 7 days at 28° C [21].

As cells in the culture fluid of the oil-oxidizing bacteria were deactivated by the addition of 200 mg/l sodium azide, the authors concluded that the key role in the degradation of soil oil belongs to surfactants. Since the cells of the oil-oxidizing bacteria were deactivated in the culture fluid by the addition of 200 mg/l sodium azide, the authors concluded that surfactants play a key role in the degradation of oil in the soil. *R. soli* 102-Na5 ability to synthesize surfactant was determined by the oil spreading test method and the surface tension index ( $\sigma = 36.7$  mN/m). Thin-layer chromatography method has shown that the chemical nature of the surfactant of 102-Na5 strain is a complex of mono- and dirhamnolipids [21].

It was shown in [24] that the surfactants of *Rhodococcus* sp. Y2-2 psychrotolerant strain intensify the destruction of toluene, benzene, and xylene in the soil at low temperatures. Hydrocarbons were added to the soil at a concentration of 4000 mg/kg, then a culture fluid (biomass concentration was 25 g/kg of soil) was added. After 14 days at 10 °C, the degree of destruction was 44%. The authors noted that the degree of destruction of hydrocarbons increased to 60% due to the additional introduction of chemical surfactants (in particular, sulfonates) into the soil and to 80% when both chemical surfactants and glucose or ethanol were added as a source of additional carbon.

Ivshina et al. [30] found that in the presence of *R. ruber* IEGM 231 surfactants activates the decomposition process in the soil of both a mixture of polycyclic aromatic hydrocarbons (PAHs) and a mixture of PAHs with polycyclic aromatic sulfur heterocycles. To obtain preparations of surfactant, the IEGM 231 strain was grown on hexadecane, followed by ultrasonic treatment of the culture fluid to release cell-bound surfactants and the surfactants were extracted with methyl tert-butyl ether. The concentration of polycyclic aromatic hydrocarbons was 0.63 g/kg of dry soil, and a mixture of PAHs with polycyclic aromatic sulfur heterocycles was 1 g/kg of dry soil. In the presence of surfactants at a concentration of 1.4 g/l after 48 h at room temperature, the degree of destruction was:

- mixtures of polycyclic aromatic hydrocarbons (%): naphthalene – 95, acenaphthene – 35, phenanthrene – 25, anthracene – 30;

- mixtures of PAHs with polycyclic aromatic sulfur heterocycles (%): naphthalene – 75, acenaphthene – 35, phenanthrene – 25, anthracene – 30, dibenzothiophene – 85, 4-methyldibenzothiophene – 80.

It is known that environmental pollution is often mixed. Thus, soil and water contaminated with oil products may also contain heavy metals [42].

Our studies [43, 44] showed that in the presence of surfactants synthesized by *R. erythropolis* IMV Ac-5017 on traditional substrates and industrial waste, the degree of decomposition of oil in water (3 g/l) and soil (20 g/kg), including in the presence of heavy toxic metals (0.01–0.5 mmol Cu<sup>2+</sup>, Cd<sup>2+</sup>, Pb<sup>2+</sup>) amounted to 70–95% after 20–30 days. The activating effect of Cu<sup>2+</sup> on the decomposition of oil pollution complex with Cd<sup>2+</sup> and Pb<sup>2+</sup> was established: after processing with the surfactant of *R. erythropolis* IMV As-5017, the destruction of oil



in water and soil was 85–95%, and after removal of copper cations it decreased to 45–70%. We suggest that the intensification of oil decomposition in the presence of copper cations may be due to their stimulating effect on the activity of alkane hydroxylases of both the surfactant producing strain and the natural (autochthonous) oil-oxidizing microbiota.

Notably, that there is information in the literature on the prospects of microbial surfactants using for the environment purification from heavy metals, however, it mainly concerns rhamnolipid and lipopeptides [45–47]. We have not been able to find publications on the role of surfactants synthesized by *Rhodococcus* bacteria in the neutralization of both heavy toxic metals and the destruction of oil pollution complex with heavy metals.

#### **The stability of surfactants**

One of the areas of research on the possibility of microbial surfactants using in environmental technologies is the determination of the stability of these microbial products properties under conditions as close as possible to natural ones.

It was established in [28] that solutions of trehalose lipids synthesized by *Rhodococcus* sp. PML026 on sunflower oil (2%, v/v), at a concentration of 250 mg/l, formed emulsions with sunflower oil, which remained stable under the adverse conditions (high temperature, various pH values, and concentrations of sodium chloride). Thus, in the temperature range from 26 to 50° C the emulsification index was 44–42%, at pH values from 2 to 12 – 34–46%, at a NaCl concentration from 5 to 25 – 36–38%. Regardless of the temperature and concentration of sodium chloride, the surface tension of trehaloselipid solutions remained virtually unchanged and was at the level of 27–31 mN/m, and only increased to 50 mN/m when the pH increased to 12.

Stancu [29] showed that the surfactants synthesized by *R. erythropolis* IBBPo1 on alkanes (cyclohexane, n-hexane, n-decane) or aromatic hydrocarbons (toluene, styrene, ethylbenzene) emulsified crude oil with high efficiency (emulsification index was 100 %). The synthesized surfactants turned out to be extremely heat-resistant: their properties remained stable at a temperature from 30 to 100° C.

It was found in [48] that rhamnolipids synthesized by *R. fascians* SDRB-G7 on olive oil were characterized by high stability at pH from 2 to 12 and concentration of NaCl from 2 to 20%. The emulsification index of the surfactant-containing

supernatant (surfactant concentration was 2.44 g/l) was (%): for all the studied concentrations of sodium chloride 87–93, at pH 2–4 – 65–71, and in the pH range from 6 to 12 – 50–52.

Table 1 summarizes the data on the decomposition of hydrocarbon nature xenobiotics with the participation of *Rhodococcus* genus actinobacteria, that synthesize surfactants. In most part of the works, the ability to destroy xenobiotics is established during the cultivation of *Rhodococcus* on the corresponding hydrocarbons, which is not entirely correct, since the environmental conditions differ significantly from the laboratory ones. In a review [44], we noted that biological methods of environmental purification are based on the direct introduction of oil-oxidizing microorganisms (bioaugmentation) or the use of various substances that stimulate the natural (autochthonous) microbiota (biostimulation), including microbial surfactants. The role of microbial surfactants is in the solubilization of hydrocarbons, increasing their availability for the natural oil-oxidizing microbiota, which plays a key role in the processes of destruction. Thus, the ability to assimilate hydrocarbons as a carbon source during cultivation under laboratory conditions and even synthesize surfactants at the same time rather indicates the possibility of using such strains of the *Rhodococcus* genus to create preparations for bio-augmentation. As for biostimulation using microbial surfactants, the corresponding conclusion can be made only after conducting studies on model systems, as described in the literature [21, 44, 30] and our works [43, 44].

#### **Antimicrobial and anti-adhesive activity**

Even though the ability of representatives of the *Rhodococcus* genus to synthesize glycolipid surfactants was established in the 70's and 80's of the twentieth century [49, 50], to date, the most part of studies focus on the study of these surfactants as destroyers of xenobiotics for use in environmental technologies (see Table 1). At present, there is little information in the literature on the biological properties of *Rhodococcus* surfactants, in particular, their antimicrobial [51–53], anti-adhesive [51, 54] activity, their ability to destroy biofilms [51].

**Antimicrobial action.** In [51] was shown that surfactants synthesized by *R. fascians* BD8 on n-hexadecane showed antimicrobial activity against resistant pathogens. Thus, in the presence of a sufficiently high concentration of surfactants (500 µg/ml) growth inhibition was

Table 1

Destruction of hydrocarbons by surfactant synthesized by *Rhodococcus* genus bacteria

| Determination method | Strain                            | Compound that degrades                                      | Initial concentration of hydrocarbon | The degree of destruction, % | Exposition, days | Source |
|----------------------|-----------------------------------|-------------------------------------------------------------|--------------------------------------|------------------------------|------------------|--------|
| During cultivation   | <i>Rhodococcus</i> sp. JZX-01     | Oil                                                         | 50 g/l                               | 65,3                         | 9                | [20]   |
|                      | <i>R. soli</i> 102-Na5            | Oil                                                         | 1%, v/v                              | 85                           | 14               | [21]   |
|                      | <i>R. erythropolis</i> IMB B-7012 | Oil                                                         | 0,5%, v/v                            | 68,9                         | 5                | [32]   |
|                      | <i>R. erythropolis</i> M-25       | Oil                                                         | 3%, v/v                              | 70,7                         | 30               | [32]   |
|                      | <i>R. wratislawiensis</i> BN38    | A mixture of phenol and hexadecane (fractional application) | 8 g/l of each hydrocarbon            | 100                          | 16 cycles        | [25]   |
|                      | <i>Rhodococcus</i> sp. MK1        | Diesel fuel                                                 | 10 g/l                               | 70                           | 7                | [22]   |
|                      | <i>Rhodococcus</i> sp. Y2-2       | A mixture of hydrocarbons (gasoline, diesel, and kerosene)  | 500 mg/l of each hydrocarbon         | 84                           | 14               | [24]   |
|                      | <i>Rhodococcus</i> sp. NI2        | Fluoranthene                                                | 200 mg/l                             | 74                           | 10               | [27]   |
|                      | <i>R. pydinovorans</i> NT2        | 4-nitrotoluene                                              | 400 mg/l                             | 98                           | 120 h            | [34]   |
|                      | <i>R. pydinovorans</i> NT2        | 2,4-dinitrotoluene                                          | 474 mg/l                             | 97,55                        | 72 h             | [37]   |
| Model system         | <i>R. pydinovorans</i> NT2        | 2,6-dinitrotoluene                                          | 470 mg/l                             | 97,55                        | 72 h             | [37]   |
|                      | <i>Rhodococcus</i> sp. Y2-2       | A mixture of toluene, benzene, xylene                       | 4000 mg/kg of soil                   | 44                           | 14               | [24]   |
|                      | <i>R. soli</i> 102-Na5            | Oil                                                         | 200 mg/kg of soil                    | 60                           | 7                | [21]   |
|                      | <i>R. ruber</i> IEGM 231          | A mixture of PAHs                                           | 0.63 g/kg of dry soil                | Naphthalene – 95*            | 48 h             | [30]   |
|                      |                                   | A mixture of polycyclic aromatic sulfur heterocycles + PAHs | 1 g/kg of dry soil                   | Naphthalene – 75*            |                  |        |

Legends: \* – the degree of destruction of one compound from the mixture is given, detailed

observed for *Escherichia coli* ATCC information in the text. 10536 – 25, *Enterococcus hirae* ATCC 10541 – 32, *Proteus mirabilis* ATCC 21100 – 27, *Staphylococcus epidermidis* KCTC 1917 – 14, *Candida albicans* ATCC 10231 – 30. Surfactant solutions showed the highest antimicrobial activity against *Vibrio harveyi* ATCC 14126 and *Proteus vulgaris* ATCC 27973 – growth inhibition by 89 and 95%, respectively. In the case of a decrease in the concentration of surfactant solutions of *R. fascians* BD8, their antimicrobial effect decreased, and at a concentration of 35 µg/ml growth inhibition of the studied test cultures did not exceed an average of 2–3% [51].

Surfactant glycolipids synthesized by *R. erythropolis* (strain not shown in the paper) on a medium with 1 mmol eicosan caused an antimicrobial effect against bacteria and fungi [52]. The growth inhibition zones of *Pseudomonas aeruginosa* after 300 min exposure was 4 mm, *E. coli* was 3.5 mm after 420 min, *Aspergillus niger* – 3 mm after 240 min, and *A. flavus* – 3 mm after 360 min exposure.

The review [53] reported that trehalose lipids (TL-1 and TL-2) did not show antimicrobial activity against gram-negative bacteria and yeast, however, TL-2 at a concentration of 300 mg/l, inhibited the germination of *Glomerella cingulata* conidia, causing a fungistatic action.

Our studies [55–57] showed that surfactants synthesized by *R. erythropolis* IMV Ac-5017 on a wide range of carbon substrates (ethanol, purified glycerin, waste from biodiesel production, refined and spent sunflower oil) were characterized by high antimicrobial activity against bacteria and yeast. In [55] showed that after treatment for 2 h with surfactants (0.15–0.4 mg/ml), cell survival ( $10^5$ – $10^7$  in ml) of phytopathogenic bacteria of the *Pseudomonas* and *Xanthomonas* genus was 0–40%. Minimum inhibitory concentrations of surfactants of *R. erythropolis* IMV Ac-5017 against bacteria (*E. coli* IEM-1, *Bacillus subtilis* BT-2, *Pseudomonas* sp. MI-2) and yeast (*C. albicans* D-6, *C. utilis* BVS-65, *C. tropicalis* PE-2) ranged from 2 to 500 µg/ml [56, 57].

**Anti-adhesive activity.** It was found in [51] that trehalose lipids of *R. fascians* BD8 at a concentration of 500 µg/ml decreased the adhesion on polystyrene of bacterial test cultures (*P. mirabilis* ATCC 21100, *E. coli* ATCC 10536, *E. hirae* ATCC 10541, *S. epidermidis* KCTC 1917) by 40–70%, and *C. albicans* ATCC 10231 by 90–95%.

After treating the polystyrene surface with surfactant solutions of *R. ruber* IEGM 231 at a concentration of 10–100 mg/ml, inhibition of cell adhesion of gram-positive (*Arthrobacter simplex* IEGM 667, *B. subtilis* ATCC 6613, *Brevibacterium linens* IEGM 1830, *Corynebacterium glutamicum* IEGM 1861, *Micrococcus luteus* IEGM 401) and gram-negative (*E. coli* K-12 and *P. fluorescens* NCIMB 904) bacteria was observed up to 76% [54].

In [58] we found that anti-adhesive activity of surfactants from *R. erythropolis* IMV Ac-5017, synthesized on refined sunflower oil, depended on the degree of purification of surfactants (supernatant, solution of purified SA), the concentration of SA, type of abiotic surface (glass, plastic, ceramics, steel, polyvinyl chloride) and test cultures. The cell adhesion of the majority studied bacteria (*E. coli* IEM-1, *B. subtilis* BT-2, *P. vulgaris* BT-1, *S. aureus* BMC-1, *P. aeruginosa* P-55, *Enterobacter cloacae* AC-22, *Erwinia aroidaeae* B-433) after treatment of abiotic surfaces with the supernatant of the culture fluid with surfactants of 0.06–0.25 mg/ml averaged 20–45%, *C. albicans* D-6 yeast – 30–75% and was lower than in case of using solutions of purified surfactants of a similar concentration. The higher anti-adhesive activity of the supernatant in comparison with solutions of purified surfactants indicates the possibility of eliminating the expensive stage of isolation and purification upon receipt of preparations with anti-adhesive properties. Later [59], it was shown that both supernatants and surfactant solutions of *R. erythropolis* IMV Ac-5017 (0.03–0.12 mg/ml) effectively (by 60–80%) reduced the adhesion of *E. coli* IEM-1 and *C. albicans* D-6 on a silicone base and acrylic denture material.

In [56], we found that surfactants synthesized during the cultivation of *R. erythropolis* IMV Ac-5017 on ethanol exhibit high anti-adhesive activity at lower concentrations (0.005 mg/ml) than surfactants obtained after culturing the strain on sunflower oil [58–59].

**Ability to destroy biofilms.** Currently, there are only a few reports in the literature on the effect of surfactants of *Rhodococcus* genus actinobacteria on biofilms. Thus, in [51] it was found that trehalose lipids of *R. fascians* BD8 at a concentration of 250 µg/ml effectively destroyed biofilms of *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *E. hirae* ATCC 10542, *C. albicans* SC5314 on various surfaces (polystyrene, glass, silicone). Notably, the

authors evaluated the effect of microbial surfactants on biofilms only qualitatively using confocal laser scanning microscopy; therefore, it is not possible to compare the effectiveness of their action with other surfactants.

Our studies [56] showed that surfactants synthesized by *R. erythropolis* IMV Ac-5017 on ethanol at significantly lower concentrations (16–64 µg/ml) destroyed 30–70% of biofilms of *E. coli* IEM-1, *B. subtilis* BT-2 and yeast of the *Candida* genus on polystyrene.

Therefore, in the modern literature, information on the antimicrobial and anti-adhesive activity of surfactants from *Rhodococcus* genus actinobacteria is significantly less than the corresponding information on rhamnolipids [53, 60, 61], aminolipids (*Bacillus*) [53, 62, 63] and sophorolipids [61, 64]. In our point of view, this is because environmental protection technologies (xenobiotic destruction) have been traditional spheres of the potential practical application of *Rhodococcus* surfactants for many years. Also, the concentration of surfactants synthesized by *Rhodococcus* is lower than other glycolipids, and the cost of the substrates (hydrocarbons) used for their synthesis remains high. These factors significantly increase the cost of the target product. If the use of surfactants in the form of a culture fluid is possible in environmental technologies, the use of surfactants as antimicrobial and anti-adhesive agents requires additional purification, which consequently increases their cost.

### Immunomodulatory and cytotoxic action

In addition to antimicrobial and anti-adhesive activity, surfactants synthesized by the *Rhodococcus* genus bacteria also have immunomodulatory properties [65–75]. The first review, which summarized information on the immunomodulatory properties of trehalose dimycolate, dates back to 2001 [65]. Later, in 2015, a review was published in which analysis of scientific work on the immunomodulatory properties of surfactants synthesized specifically by *Rhodococcus* was carried out [66].

**Granuloma formation.** The molecular mechanisms underlying the formation of granulomas after the introduction of surfactants to the body, in particular, trehalose dimycolates (TDM), are not yet fully understood. There is an assumption that a complex biochemical cascade of reactions is formed. Perhaps TDM directly affect macrophages,

causing their migration and stimulating procoagulant activity through the induction of chemokine and proinflammatory cytokine synthesis [66].

In [67], histological changes in the internal organs of ICR mice were investigated under the action of glycolipids of various compositions (glucose mycolates (GM), TDM, manose mycolates (MM), fructose mycolates (FM), synthesized by *Rhodococcus ruber* M1. GM and TDM proved to be the most effective, since on the 14<sup>th</sup> day after their intravenous administration (500 µg in the form of w/o/w emulsion) there was an increase in 1.5 and 3.5 times in liver and spleen indices, respectively, and on the 7<sup>th</sup> day – an increase in lung index almost in 4 times. After conducting histological studies of the cells of these organs, the authors showed that their mass increase is explained by an increase in the number of granulomas in the cells.

Matsuhara et al. [68] showed that GM and TDM of *R. ruber* M1 stimulate the formation of granulomas in spleen, liver and lung cells of ICR mice at a lower concentration of 300 µg in the form of a w/o/w emulsion. *In vitro* studies demonstrated the ability of GM and TDM to stimulate migration of peritoneal macrophages in 2.5 and 3 times, respectively, and also lead to 1.6 times higher level of interleukin-1 (IL-1) synthesis.

The ability of TDM to form granulomas *in vivo* was confirmed by Ueda et al. [69]. TDM of *R. terrae* led to an increase in the index of lungs and spleen by 3 and 3.4 times, and TDM of *Rhodococcus* sp. 4306 – by 2.6 and 3 times, respectively. In this case, TMM, GM, MM, FM of *Rhodococcus* sp. 4306 did not affect the indicated indexes [69].

**Cytokine-inducing activity.** Since 2007, studies of immunomodulatory activity of *R. ruber* IEGM 231 surfactants have begun [70]. It was established that surfactants of *Rhodococcus* increase the synthesis of several cytokines (Table 2): IL-12, IL-18, IL-1β, IL-1β, IL-6 and tumor necrosis factor (TNF-α) [15, 71–74]. Trehalose mono-, di-, and tri- mycolates synthesized by *R. ruber* IEGM 231 affect the production of TNF-α and interleukins IL-1β and IL-6 when used as an emulsion treated with ultrasound (23 kHz 10 sec) [71].

Dutch scientists have shown that Rubratin drug, the active substance of which is a fragment of *R. ruber* cell wall, affects the production of cytokines. Rubratin stimulates IL-1β, IL-2, IL-6 and TNF-α synthesis in patients with bladder cancer. However, this drug led to T-cell activation in only 2 out of 5 patients [75].



Table 2

## The influence of surfactants on the synthesis of cytokines

| Strain                      | Effective surfactant concentration | Production of cytokines, pg/ml                 | Model                                                | Source |
|-----------------------------|------------------------------------|------------------------------------------------|------------------------------------------------------|--------|
| In vivo                     |                                    |                                                |                                                      |        |
| <i>R. ruber</i> IEGM 231    | 50 mg/kg                           | IL-1β – 72,98<br>TNFα – 71,2                   | White outbred mice                                   | [15]   |
| <i>R. aurantiacus</i> 80005 | 300 µg per mice                    | Intravenously<br>IFN – 5 IU/ml                 | ICR mice                                             | [72]   |
|                             |                                    | intraperitoneally IFN – 2,5<br>IU/ml           |                                                      |        |
| In vitro                    |                                    |                                                |                                                      |        |
| <i>R. ruber</i> IEGM 231    | 1 µg/ml                            | IL-1β – 41<br>TNFα – 180<br>IL-6 – 600         | Human peripheral<br>blood monocytes                  | [71]   |
|                             | 100 µg/ml                          | IL-1β – 68<br>IL-8 – 458<br>TNFα – 95          | Neutrophils of human<br>peripheral blood             | [73]   |
|                             | 0.1 µg/ml                          | IL-12 (p70) – 9.1<br>IL-18 – 7.8               | Mononuclear fraction<br>of human peripheral<br>blood | [74]   |
|                             | 100 µg/ml                          | IL-10 – 520                                    |                                                      |        |
|                             | 1 µg/ml<br>10 µg/ml<br>100 µg/ml   | IL-12 (p70) – 11<br>IL-18 – 2.2<br>IL-10 – 500 | Monocyte fraction<br>of human peripheral<br>blood    |        |

**Antitumor effect.** Investigation of possible antitumor effect of trehalose lipids began since the 80s. The effect of GM, TDM, MM and FM from *R. ruber* M1 on cancer cells was investigated in [76]. On the *in vivo* sarcoma model, FM and GM use resulted in a decrease in tumor mass by 32 and 57%, respectively. At the same time, MM stimulated tumor growth. The most effective were TDM as tumor weight decreased by 92%. Notably, the studied surfactants were administered intravenously to ICR mice at a dose of 30  $\mu$ g/10 g of animal weight in an amount of 10 injections, but a single administration of TDM at a dose of 300  $\mu$ g/10 g of animal weight stimulated tumor growth.

In [77] showed that trehalose lipids synthesized by *Rhodococcus* sp. TB-42 at a concentration close to the critical concentration of mycelium-releasing (3–4  $\mu$ M) exhibit antiproliferative activity *in vitro* on a model of human promyelocytic leukemia using the HL-60 cell line.

**Reactive oxygen species (ROS) activity.** One of the indicators that are used to assess the effect of compounds on the immune system is the production of ROS, which is the first line of defense against various infections. But it is difficult to say whether ROS increase has a positive effect on the immune response. In [73, 74] showed that ROS production

depended on the concentration of glycolipids in the working solution, the type of cells (neutrophils, monocytes, or the mononuclear fraction of blood cells) and the duration of incubation.

Surfactants synthesized by *R. ruber* IEGM 231 induced ROS release by leukocytes and neutrophils of human peripheral blood [74]. The production of ROS and IL-8 by peripheral blood neutrophils was shown by Baeva et al. [73]. No changes were observed in the synthesis of IL-1 $\beta$  and TNF- $\alpha$  in the presence of surfactants.

#### Other properties of *Rhodococcus* surfactants, promising for use in medicine

The possibility of using of surfactants synthesized by *Rhodococcus* sp. to protect proteins from denaturation due to high temperatures is shown in [78]. The authors created a surfactant-protein system using trehalose lipid preparations at a concentration close to the concentration of critical micelle formation. At the same time, surfactants did not affect the structure of the protein molecule and protected it from the effects of high temperatures (up to 70° C).

There are reports on the study of the effect of trehalose lipids on the activity of enzymes. Thus, in the study published by Zagaroza et al. [79] showed

that trehalose lipids synthesized by *R. erythropolis* 51T7 interact with the porcine pancreatic phospholipase A2 and inhibit its catalytic activity. The ability of trehalose lipids to incorporate into phospholipid membranes of eukaryotic cells was also revealed, which is typical for the mechanism of antimicrobial action of surfactants [80]. In this case, human red blood cells swell, followed by hemolysis in concentrations of surfactants significantly lower than the critical concentration of mycelium. The authors indicate that surfactants cause hemolysis of human erythrocytes by the colloid osmotic mechanism, most likely through the formation of so-called “pores” enriched with surfactants in the erythrocyte membrane.

Therefore, trehalose mycolates synthesized by representatives of *Rhodococcus* genus are promising compounds with immunomodulatory activity. They stimulate the synthesis of several cytokines (interferons, interleukins, tumor necrosis factor), which play an important role in humoral immune response. Further studies on the mechanism of microbial surfactants effect on immune cells will allow to develop new therapies for cancer and immune-dependent diseases treatment.

Thus, an analysis of modern literature data on the properties of surfactants of *Rhodococcus* genus actinobacteria, which determine their practical significance, showed that as in the previous 20–30 years, the main studies are aimed at the potential use of these products of microbial synthesis in environmental technologies for destruction of xenobiotics (aliphatic, heterocyclic and polycyclic aromatic hydrocarbons).

The literature data concerning the antimicrobial and anti-adhesive activity of glycolipid surfactants synthesized by *Rhodococcus*, as well as their ability to destroy biofilms, are scarce, although these properties are studied quite actively for other microbial surfactants [5, 17, 57, 60–64]. However, there is much more information in the literature on the immunomodulatory properties of surfactants from *Rhodococcus* genus actinobacteria [65–80], in contrast to the world-famous surfactants like lipopeptides [81, 82], or rhamnolipids [83, 84].

In our opinion, the predominant orientation of researchers on the use of *Rhodococcus* surfactants in environmental technologies results from the fact that up to now, expensive hydrocarbon substrates are mainly used for their production, and the surfactant-synthesizing ability of *Rhodococcus* genus bacteria

remains lower than producers of rhamno- and sophorolipids. These factors significantly reduce the effectiveness of technologies for producing *Rhodococcus* surfactants, although in recent years there have been reports of the creation of surfactants during the cultivation of *Rhodococcus* on the so-called “non-traditional” substrates for these bacteria: oil-containing and hydrophilic (including waste from biodiesel production) as well as mixed ones. These data were summarized in our review [7]. In the same review [7], we outlined the benefits of *R. erythropolis* IMV Ac-5017 strain we are studying, which synthesizes high concentrations of extracellular surfactants on a wide range of substrates, including toxic industrial wastes. Also, the surfactants of IMV Ac-5017 strain are multifunctional preparations, because, in addition to the high efficiency of destruction of oil pollution, including complex with heavy metals, they are characterized by high antimicrobial and anti-adhesive activity, which is inherent in the supernatant of culture fluid, which allows the exclusion of costly stage of separation and purification of surfactants from technological process. Finally, in addition to extracellular surfactants, the strain synthesizes phytohormones of the auxin, cytokinin and gibberellin nature [85, 86], which makes it promising for the creation of waste-free integrated biotechnology for obtaining a complex microbial preparation with various biological properties.

## ПРАКТИЧНО ЦІННІ ВЛАСТИВОСТІ ПОВЕРХНЕВО-АКТИВНИХ РЕЧОВИН, СИНТЕЗОВАНИХ АКТИНОБАКТЕРІЯМИ РОДУ *RHODOCOCUS*

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### Резюме

Нині мікробні поверхнево-активні речовини (ПАР), завдяки їх поверхнево-активним та емульгувальним властивостям, високій антимікробній, антиадгезивній активності та здатності руйнувати біоплівки, є об'єктами інтенсивних досліджень.

В огляді наведені сучасні дані літератури про властивості ПАР, синтезованих актинобактеріями роду *Rhodococcus*, що визначають їх практичну цінність. Інтерес дослідників до поверхнево-активних речовин родококів зумовлений насамперед їх ключовою роллю у деструкції ксенобіотиків (аліфатичних, гетероциклічних та поліциклічних ароматичних вуглеводнів). Нечисельними на теперішній час залишаються відомості про антимікробну та антиадгезивну активність ПАР представників роду *Rhodococcus*, тоді як імуномодулюючі властивості цих продуктів мікробного синтезу досліджуються активніше, ніж інших відомих у світі мікробних ПАР. Наведено дані власних експериментальних досліджень щодо практично

цінних властивостей ПАР, синтезованих штамом *Rhodococcus erythropolis* ІМВ Ас-5017. На відміну від поверхнево-активних речовин інших родококів, ПАР штаму ІМВ Ас-5017 є препаратами мультифункціонального призначення, оскільки крім високої ефективності деструкції нафтових забруднень, у тому числі й комплексних з важкими металами, ПАР характеризуються високою антимікробною та антиадгезивною активністю, у тому числі й здатністю до деструкції біоплівки.

*Ключові слова:* родококи, поверхнево-активні речовини, деструкція ксенобіотиків, антимікробна та антиадгезивна активність, руйнування біоплівки, імуномодулюючі властивості.

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