doi: https://doi.org/10.15407/microbiolj83.03.092

BIOTECHNOLOGICAL POTENTIAL OF THE *ACINETOBACTER* GENUS BACTERIA

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Until recently, there were rare scientific reports on the biotechnological potential of non-pathogenic bacteria of the Acinetobacter genus. Although the first reports about the practically valuable properties of these bacteria date back to the 70s and 80s of the twentieth century and concerned the synthesis of the emulsan bioemulsifier. In the last decade, interest in representatives of the Acinetobacter genus as objects of biotechnology has significantly increased. The review presents current literature data on the synthesis by bacteria of this genus of high-molecular emulsifiers, low-molecular biosurfactants of glyco- and aminolipid nature, enzymes (lipase, agarase, chondroitinase), phytohormones, as well as their ability to solubilize phosphates and decompose various xenobiotics (aliphatic and aromatic hydrocarbons, pesticides, insecticides). Prospects for practical application of Acinetobacter bacteria and the metabolites synthesized by them in environmental technologies, agriculture, various industries and medicine are discussed. The data of own experimental studies on the synthesis and biological activity (antimicrobial, anti-adhesive, ability to destroy biofilms) of biosurfactants synthesized by A. calcoaceticus IMV B-7241 strain and their role in the degradation of oil pollutants, including complex ones with heavy metals, are presented. The ability of A. calcoaceticus IMV B-7241 to the simultaneous synthesis of phytohormones (auxins, cytokinins, gibberellins) and biosurfactants with antimicrobial activity against phytopathogenic bacteria allows us to consider this strain as promising for practical use in crop production to increase crop yields.

Keywords: Acinetobacter genus, biosurfactants, bioemulsifiers, enzymes, phytohormones, degradation of xenobiotics.

Among the bacteria that produce practically important metabolites, there are peculiar "record holders" that are capable of synthesizing a wide range of biologically active agents. They include representatives of the generitypes *Bacillus* and *Paenibacillus* [1–3], *Streptomyces* [4, 5], *Pseudomonas* [6, 7], and *Rhodococcus* [8, 9].

In the last decade, more and more information has emerged about the bacteria of the genus *Acinetobacter*. If in 1986 only two species of this genus were described (*Acinetobacter calcoaceticus* and *Acinetobacter lwoffii* [10]), then currently they are about 78 [www.bacterio.net]. Interest in representatives of the genus *Acinetobacter* is due to the following reasons. First, these bacteria are found in various natural places of existence (soil, fresh water, oceans, sediments, areas contaminated with xenobiotics) [10, 11]. Secondly, universal catabolic pathways and broad substrate specificity of metabolic systems ensure their active participation in the cycle of matters in nature [10]. Thirdly, *Acinetobacter baumannii* strains are characterized by multiple antibiotic resistance and cause healthcare-associated infections with heavy mortality [11–13]. Fourthly, nonpathogenic representatives of the genus *Acinetobacter* are capable of synthesizing practically valuable compounds: bioemulsifiers [14–16], small-molecule biosurfactants (surface-active agent) [17, 18], enzymes [19, 20], gibberellins [21, 22]. Fifthly, these bacteria are promising for use in environmental technologies due to their ability to effectively destroy various xenobiotics [23–25].

The purpose of this review is to analyze and summarize literature data as well as own experimental studies on the use of *Acinetobacter*

genus bacteria as potential biological agents in biotechnological processes.

Synthesis of practically important metabolites by *Acinetobacter* genus bacteria

The first practically valuable final product produced by *Acinetobacter* genus bacteria was emulsan. In the late 70s of the twentieth century, information appeared in the literature about the isolation of acidic etherified by fatty acids of a high-molecular exopolysaccharide synthesized by *A. calcoaceticus (Arthrobacter)* RAG-1 [26–28]. The polysaccharide was called emulsan due to its ability to emulsify hydrophobic substrates containing aliphatic, aromatic and cyclic components.

Biosurfactants. Reports on the ability of representatives of this genus to synthesize low-molecular biosurfactants date back to 2009 [29–31].

Subsequent reports of new strains of the genus *Acinetobacter* capable of biosurfactant synthesis appeared in 2012–2013 [17, 32, 33].

During 2014–2020, about a dozen papers were published that reported on the synthesis of biosurfactants by representatives of the genus *Acinetobacter* on hydrophobic (petroleum, diesel fuel, oil) [18, 34–42] and hydrophilic (glucose, saccharose, glycerol, ethanol, tryptone) [38, 43–45] substrates. In 2019, brief information about some producers of biosurfactants was provided in the review [46].

It should be noted that in many papers [36, 40, 43–45], the authors did not determine the concentration of biosurfactants, and the ability to synthesize these metabolites was evaluated by reducing the surface tension and/or emulsifying index of the cell-free culture fluid. This is due to the fact that the main research of these works' authors is aimed at the potential use of biosurfactants in environmental technologies for the destruction of xenobiotics, and the formation of biosurfactants is considered as an additional property of microbes capable to destruct hydrophobic toxic compounds.

It is worth mentioning that the concentration of biosurfactants synthesized by representatives of

Table 1

Biosurfactants of *Acinetobacter* genus bacteria

Strain	Carbon source, concentration	BS concen-tration, g/L	Chemical nature of biosurfactants	References
Hydrophobic substrates				
A. baumannii MKS2	Crude oil, 1 %	0.12	Glycolipids	[39]
Acinetobacter sp. D3-2	Crude oil, 0.5 %	0.52	Lipopeptides	[35]
A. bouvetii BP18	Crude oil, 3%, Diesel fuel, 2 %	0.17	–	[37]
A. pittii ABC	Hexadecane, 1 %	0.57	Lipopeptides	[41]
A. junii BD	Refined soybean oil, 80 g/L	4.0	Rhamnolipids	[18]
A. calcoaceticus IMV B-7241	Waste oil after frying meat, 4 %	8.5	Complex of glyco-, amino- and neutral lipids	[47]
A. calcoaceticus IMV B-7241	Waste oil after frying farm potatoes, 6 %	7.9	Complex of glyco-, amino- and neutral lipids	[47]
Hydrophilic substrates				
A. calcoaceticus K-4 (IMV B-7241)	Ethanol, 2 %	3.6*	Glyco- and amino-lipid complex	[30]
A. calcoaceticus IMV B-7241	Waste of biodiesel production, 7 %	5.0	Glyco- and amino-lipid complex	[48]
A. calcoaceticus NRRL B-59190	Purified glycerol, 1%	2.0	Rhamnolipids	[31]
A. calcoaceticus NRRL B-59191	Purified glycerol, 1%	2.2	Rhamnolipids	[31]
A. calcoaceticus B-59190	Sodium citrate, 20 g/L	2.0	Rhamnolipids	[34]
A. baumannii MN3	Saccharose, 4 %	4.68	Lipopeptides	[34]

Legend: BS – biosurfactants, * – conditional biosurfactant concentration, "–" – no data.

the genus *Acinetobacter* on hydrocarbon substrates (petroleum, hexadecane) established in the papers [35, 37, 39, 41] was low and amounted to only 0.12–0.57 g/L. Parthipan et al. [38] found that the concentration of synthesized biosurfactants reached 4.68 g/L when *A. baumannii* MN3 growing on an optimized medium (saccharose concentration 4 %, nitrogen source – urea).

In 2018, we published the paper [47], in which we reported on the ability of *A. calcoaceticus* IMV B-7241 strain to synthesize biosurfactants on waste (overcooked) sunflower oil of various qualities.

It should be noted that to date, there is no information in the literature on the synthesis of biosurfactants by *Acinetobacter* genus bacteria on waste oil. There is also no information on the use of biodiesel production waste for the production of biosurfactants by representatives of this genus. Our studies [48] showed that during the cultivation of *A. calcoaceticus* IMV B-7241 on this industrial waste (7 %), the biosurfactant concentration was 5 g/L.

Summarized information on the synthesis of biosurfactants by *Acinetobacter* bacteria is given in Table 1.

It should be noted that Table 1 includes only information on the producer for which the concentration of synthesized biosurfactants in g/L is established. The data shown in Table 1 indicate that strain *A. calcoaceticus* IMV B-7241 is more efficient producer of biosurfactants than described in the literature. Thus, this strain synthesizes biosurfactants in sufficiently high concentrations (5.0–8.5 g/L) on various hydrophilic and hydrophobic substrates, including industrial waste.

Until recently, there was no information in the literature about the biological activity of biosurfactants of *Acinetobacter* genus bacteria. Only in 2020, information appeared about the antimicrobial, antineoplastic activity and ability to destroy bacteria films of biosurfactants *A. junii* B6 [42] and *A. indicus* M6 [45].

Thus, it is reported in the paper [45] that complete inhibition of the growth of methicillin-resistant biological strains was observed in the presence of biosurfactants *A. indicus* M6 in concentration (mg/mL): *Escherichia coli, Pseudomonas aeruginosa* – 20; *Streptococcus epidermis* – 50; *Klebsiella pneumoniae* and *Candida albicans* – 100. Treatment of biofilms for 7 days with a solution of biosurfactants (500 mg/mL) was accompanied by their destruction by 80–82 %. In the presence of biosurfactants of strain *A. indicus* M6 at a concentration of 200 mg/mL showed a significant decrease in lung cancer cells (A549) due to inhibition of their proliferation in the G1 phase.

Minimum inhibitory concentrations (MIC) of biosurfactants *A. junii* B6 against *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6051, *Micrococcus luteus* ATCC 4698, *P. aeruginosa* ATCC 27853, *K. pneumonia* ATCC 13883, *E. coli* ATCC 25922, *Salmonella typhi* ATCC 6539, *C. albicans* ATCC 10231 and *Candida utilis* ATCC 9950 were 5 μg/mL [49]. In the presence of biosurfactants at concentrations of 1250 and 2500 μg/mL, the degree of destruction of biofilms reached (%): *Proteus mirabilis* − 10 and 30; *S. aureus* – 30 and 50; *P. aeruginosa* − 30 and 70, respectively. The IC_{50} index used to evaluate the cytotoxic effect of biosurfactants on U87, KB, and HUVEC cancerous cells was (mg/mL): 7.8±0.4, 2.4±0.5, and 5.7±0.1, respectively [42].

Our first studies of the antimicrobial and anti-adhesive activity of biosurfactants synthesized by *A. calcoaceticus* IMV B-7241 on various substrates (ethanol, hexadecane, refined glycerol) date back to 2014–2016 [49–51]. In 2017 [52], it was established that biosurfactants of IMV B-7241 strain are effective decomposers of bacterial and yeast biofilms. More recent studies [53, 54] have shown that biosurfactants synthesized by *A. calcoaceticus* IMV B-7241 on industrial waste (waste oil of various quality and biodiesel production waste) is also characterized by high antimicrobial, anti-adhesive activity and the ability to biofilm destruction.

MIC of *A. calcoaceticus* IMV B-7241 biosurfactants in relation to some test-cultures, the adhesion of bacterial and yeast cells on the abiotic surface treated with biosurfactant, as well as the effect of biosurfactants on biofilm destruction is shown in Table 2.

These data indicate that the biosurfactants of IMV B-7241 strain exhibit high anti-adhesive activity (adhesion 22–47 %) and effectively destroyed biofilms (41–83 %) at a lower concentration (2.5−320 μg/mL) than synthesized by *A. junii* B6 (1250 and 2500 μg/mL) [42] and by *A. indicus* M6 (500 μg/mL) [45].

So, despite the fact that the biosurfactants of *Acinetobacter* genus bacteria have been the target of scientific research for only ten years, currently some of them are competitive in the market of these microbial synthesis products. Firstly, they can be obtained in sufficiently high concentrations (up to 7–8 g/L) on cheap and available in large amounts industrial waste; secondly, they are promising not

Table 2

Biological activity of *Acinetobacter calcoaceticus* IMV B-7241 biosurfactants

Substrate	Test culture	MIC, μg/mL	Adhesion on polyvinyl chloride *, %	Destruction of biofilm**, %
Ethanol	*B. subtilis* БТ-2	9	33	69 (80)
	E. coli ІЕМ-1	20	22	49 (320)
	C. albicans Д-6	9	27	N.d.
Purified glycerol	*B. subtilis* БТ-2	9	25	87 (320)
	E. coli ІЕМ-1	34	33	41 (320)
	C. albicans Д-6	68	25	42 (320)
Hexadecane	*B. subtilis* БТ-2	27	33	57 (320)
	E. coli ІЕМ-1	11	45	45 (320)
	C. albicans Д-6	54	47	42 (320)
Waste of biodiesel production	*B. subtilis* БТ-2	0.96	N.d.	N.d.
	E. coli ІЕМ-1	3.8	N.d.	N.d.
	C. albicans Д-6	15.2	86	N.d.
Waste oil after frying farm potatoes	*B. subtilis* БТ-2	28.8	34	63 (233)
	E. coli ІЕМ-1	0.9	N.d.	83 (29)
	C. albicans Д-6	57.6	44	N.d.

Legend: N.d. – not determined; "*" – surfactant concentration of 2.5–5.0 μg/mL; "**" – in parentheses indicates the concentration of biosurfactants, μg/mL.

only for the degradation of xenobiotics, but due to their antimicrobial, anti-adhesive, antineoplastic properties – for potential use in the pharmaceutical industry and medicine.

Bioemulsifiers. The producer of the high-molecular bioemulsifier emulsan was initially identified as a representative of the genus *Arthrobacter* RAG-1 [26–28], but later assigned to the genus *Acinetobacter*, which at that time included only one species – *A. calcoaceticus* [55]. In the international American typical culture collection, the strain is registered as *A. lwoffii.* In 1999, the emulsan-producing strain was renamed and assigned to type *A. venetianus* [56]. In the second half of the 80s of the twentieth century, there was information on *A. calcoaceticus* BD4 strain, capable of emulsan synthesis [57] and *A. calcoaceticus* A2 as a producer of biodispersan [58, 59]. In the 90s of the twentieth century, the bioemulsifier alasan synthesized by *A. radio-resistens* KA53 was described [60].

A brief description of emulsan, alasan, and biodispersan is given in Table 3.

In 2008 [62], it was found that apoemulsan RAG-1 (protein-free emulsan) is a complex consisting of a high-molecular exopolysaccharide and an R-type lipopolysaccharide. The exopolysaccharide has a molecular weight of 200–250 kDa and is able to form stable emulsions of the "oil in water" type.

The highest concentration of emulsan and dispersan (4–5 g/L) was achieved under cultivation of *A. venetianus* RAG-1 and *A. calcoaceticus* A2 on medium with ethanol [58, 61].

Information on the practical use of emulsan and alasan, the main ones being environmental cleaning from xenobiotics, oil transportation and cleaning of tankers from oil, is provided in the reviews [46, 61, 63, 64]. Due to the ability of biodispersan to change the surface properties of limestone and increase its dispersion in aqueous solutions, it is promising for use in the production of paper, paint and ceramics [46, 59].

Over the past ten years, the literature has described new emulsifiers synthesized by representatives of the genus *Acinetobacter* [14–16, 65, 66] on hydrocarbon [14, 15], oil-containing [16, 66], hydrophilic (ethanol, tryptone) [65, 66] substrates, as well as on mixtures of glycerol and hexadecane [66]. By their chemical nature, bioemulsifiers are glycoproteins [14, 16, 65], lipopolysaccharides [66], and polysaccharides [15].

Table 4 shows summarized information on emulsifiers studied during 2010–2019 years. These data indicate that the most active producers are *A. beijerinckii* ZRS, *Acinetobacter* sp. Ab9-ES and *Acinetobacter* sp. Ab33-ES strains, which synthesize from 4 to 5 g/L of the final product with high emulsifying activity.

So, bioemulsifiers of *Acinetobacter* genus bacteria, which were first described more than 40 years ago, and still remain the target of scientific research. This is primarily due to the fact that, unlike

other products of microbial synthesis synthesized by a wide range of bacteria, these emulsifiers are a "kind of business card" of *Acinetobacter* genus representatives.

Enzymes. Over the past 10 years, a number of papers have been published that have established the ability of *Acinetobacter* genus bacteria to synthesize various hydrolytic enzymes: lipases (EC 3.1.1.3) [20, 67–74], agarases (EC 3.2.1.81) [75, 76], chitinases (EC 3.2.1.14), gelatinases (EC 3.4.24) [70], including cold-active ones [67, 68, 70], as well as chondroitinases (EC 4.2.2.4) [19].

Most of the research is devoted to the synthesis of lipases, which are promising for use in various areas of the chemical industry (detergents, chemical synthesis). It should be noted that the first reports on the synthesis of these enzymes by representatives of the genus *Acinetobacter* date back to the 80–90s of the twentieth century, and in 2004 a review was published [77], which summarized the relevant information available at that time. In our review, we provide information on the lipases of *Acinetobacter* genus bacteria, which appeared in the last decade (Table 5).

Agarase enzymes can be used for processing seaweed biomass and their further application in the production of biofuels [75, 76].

Zhu et al. [19] found that *Acinetobacter* sp. C26 strain synthesizes a unique enzyme, chondroitinase, which is promising in the treatment of spinal cord traumas. The papers [78–81] state that spinal cord traumas are a destructive condition that has no effective treatment at the moment. This condition occurs due to the inability of central nervous system neurons to regain after trauma. This is partly due to the presence of chondroitin sulfate molecules in the environment of the damaged spinal cord, which block the recovery process. The enzyme chondroitinase split these molecules, thereby promoting nerve regeneration and functional recovery. Chondroitinase can also promote neuroprotection of injured neurons [78, 79].

It should be noted that the first reports of microbial synthesis of chondroitinase by *Bacteroides stercoris* HJ-15 strain appeared in 2002 [82]. This strain synthesized type ASII chondroitinase, whose activity in the supernatant was 0.42 U/mL. The *Sphingomonas paucimobilis* strain (the strain number is not given) was somewhat more productive (the strain number is not given): the activity of ABC-type chondroitinase reached 0.94 U/mL [83]. The recombinant strain *Pichia pastoris* X33 synthesized chondroitinase with the activity of 2.72 U/mL [84], which is several times lower compared to that of *Acinetobacter* sp. C26 (8.5 U/mL) [19].

The summarized data on the synthesis of enzymes by representatives of the *Acinetobacter* genus are given in Table. 5. It should be noted that while *Acinetobacter* sp. XMZ-26 lipase producer is characterized by the highest synthesizing capacity compared to other bacteria of this genus, it is significantly inferior to the promising producer of *Aspergillus tamarii* JGIF06, which synthesizes lipase on a coconut oil medium with an activity of 25,000 U/mL [71]. At the same time, the *Acinetobacter* sp. AG LSL-1 strain, as an agarase producer (activity 3 U/mL) is more promising than *Alteromonas* sp. C-1 (activity 2.3 U/mL), which was discovered more than 15 years ago and was considered one of the most active producers of this enzyme (cited for [75]). The *Acinetobacter* sp.C26 strain is a more active producer of the unique chondroitinase enzyme (activity 8.5 U/mL) compared to the recombinant *Pichia pastoris* X33 strain (2.72 U/mL) [84].

Phytohormones. Bacteria of the *Acinetobacter* genus are common in the soil, some of them are associated with plants, have a positive effect on their growth and development, including due to the ability to synthesize phytohormones [21, 22, 85–90].

The first reports on the synthesis of phytohormones by representatives of the *Acinetobacter* genus appeared about ten years ago [21, 22, 85]. Thus, a group of scientists from Korea [21] found that strain *A. calcoaceticus* SE370 formed ten different gibberellins, including biologically active GK_1, GK_3, and GK_4 (0.45–6.25 ng/100 mL of culture fluid). Gulati et al. [22] showed that the phosphate-immobilizing strain *A. rhizosphaerae* BIHB 723 synthesized indoleacetic acid (IAA) in the amount of 15.6 ng/mL. In the paper [85] was found that two strains of *Acinetobacter* sp. PUCM1007 and *A. baumannii* PUCM1029 produced indoleacetic acid in a sufficient high concentration (10–13 μg/mL). We assume that the increased synthesis of IAA by these strains is due to the presence of a precursor of tryptophan auxins biosynthesis (0.3 mm) in the culture medium.

Later, in 2014 Zhao et al. [86] showed that *A. calcoaceticus* D10, when cultivated in a medium with saccharose and tryptophan (100 mg/L) formed 22.77 mg/L of indoleacetic acid. In 2018 [87], there was a report on the synthesis of IAA (2.8 μg/mL) by the *Acinetobacter* sp. UQ202 strain

Table 3

Chemical composition and properties of emulsan, alasan and dispersan

Emulsifier	Producer	Chemical composition	Monosaccharide composition of the carbohydrate part	Basic properties	References
Emulsan	*Acinetobacter venetianus* RAG-1	Polysaccharide, protein, fatty acids	D-galactosamine, L-galacturonic acid, diamino-2-deoxy-N-acetylglucosamine	An effective emulsifier in low concentrations (0.01-0.001 %), does not emulsify pure aliphatic, aromatic, or cyclic hydrocarbons; however, their mixtures are emulsified effectively. Maximum emulsifying activity is achieved in the presence of divalent cations (2-10 mm Mg^{2+}) and PH 5.0–7.5	[26–28]
Emulsan	*Acinetobacter calcoaceticus* BD4	Polysaccharide, protein	Rhamnose, mannose, glucose, glucuronic acid in a ratio of 4:1:1:1	The polysaccharide and protein themselves do not exhibit emulsifying properties. The combination of protein and polysaccharide fractions is accompanied by the restoration of emulsifying ability	[57, 61]
Alasan	*Acinetobacter radioresistens* KA53	Polysaccharide, protein, alanine	Glucosamine, galactosamine, alanine, galactose and glucose	Reduces the interfacial tension from 69 to 41 mN/m, increases the solubility and rate of biological decomposition of polyaromatic hydrocarbons. The viscosity of solutions at 30-50 °C increases by 2.6 times without changes in emulsifying activity	[60, 61]
Biodispersan	*Acinetobacter calcoaceticus* A2	Polysaccharide, protein	Glucosamine, galactosamine, glucose, galactose, glucuronic acid, N-acetyl galactose-aminouronic acid	The active ingredient is a polysaccharide that acts on powdered calcium carbonate and changes its surface properties, which is accompanied by increased dispersion in water, contributes to the formation of cracks in limestone during its grinding	[58, 59]

Table 4

Other bioemulsifiers synthesized by representatives of the *Acinetobacter* genus

Strain	Carbon source	Chemical composition	Molecular weight, kDa	Concentration, g/L	Emulsification index E_{24}, %	Areas of practical application	References
A. beijerinckii ZRS	Hexadecane, 2.73%	Glycolipo-protein	40	5.16	83	Bioremediation	[14]
A. bouvetii UAM25	Hexadecane, 7.6 g/L	Polysaccha-ride	1010	0.15	12.5	Bioremediation	[15]
A. bouvetii UAM25	Ethanol, 10.58 g/L	Lipohetero-polysaccha-ride	3999 (F1) 352 (F2)	0.145	8 U/mL	Bioremediation	[66]
A. bouvetii UAM25	Glycerol, 8 g/L + Hexadecane, 2.81 g/L	Lipohetero-polysaccha-ride	4320 (F1) 485 (F2)	0.151	9.9 U/mL	Bioremediation	[66]
A. bouvetii UAM25	Fried oil, 7.11 g/L	Lipohetero-polysaccha-ride	3279 (F1) 551 (F2)	0.225	18.5 U/mL	Bioremediation	[66]
A. lwoffii TA 38	Tryptone, 10 g/L	Glycoprotein	–	1.9	140 U/mL	Bioremediation	[65]
Acinetobacter sp. Ab9-ES	Refined olive oil, 2 %	Glycoprotein	–	4.52	83.8	Bioremediation	[16]
Acinetobacter sp. Ab33-ES	Refined olive oil, 2 %	Glycoprotein	–	4.31	80.8	Bioremediation	[16]

Legend: "– " – no data provided, F1 – fraction 1, F2 – fraction 2.

Table 5

Enzymes of the *Acinetobacter* genus bacteria

Strain	Carbon source	Enzyme	Activity*, U/mL	Scope	References
Acinetobacter sp. C26	Chondroitin sulfate, 0,6 %	Chondroitinase	8.23	Therapy for spinal cord injuries	[19]
Acinetobacter sp. EH28	Tributyrin, 1 %	Lipase	3.8	Synthesis of ethyl caprylate	[20]
Acinetobacter sp. AU07	Refined castor oil, 2 %	Lipase	14.5	Chemical industry	[69]
A. johnsonii LP28	Refined olive oil, 20 g/L	Lipase	3.09	Chemical industry	[67]
Acinetobacter sp. XMZ-26	Refined olive oil, 1 %	Lipase	584.85	Chemical industry	[68]
Acinetobacter sp. UBT1	Castor seedcake, 2%	Lipase	292.29	Chemical industry	[74]
A. junii PS12B	Glucose, agar, 0.5%	Agarase	0.3	Biofuel production	[76]
Acinetobacter sp. AG LSL-1	Sucrose 3%, agar, 0.3%	Agarase	3.01	Biofuel production	[75]

Legend: "–" – data not given; "*" – activity in the supernatant.

under cultivation in a medium with tryptone and tryptophan (800 μg/mL).

Our studies [89, 90] have shown that *A. calcoaceticus* IMV B-7241 strain, when cultivated on various substrates, including industrial waste (technical glycerol, biodiesel production waste), synthesizes phytohormones of auxin, cytokinin and gibberellin nature. During cultivation on ethanol and technical glycerol, the concentration of phytohormones (μg/l) was: auxins − 104.2 and 122.0; cytokinins – 3.5 and 363.9 [90]; biologically active gibberellin GK_4 − 6.88 and 7.36 [90].

In subsequent studies [91], we established the possibility of a significant increase of auxin synthesis when tryptophan (100–300 mg/L) is introduced into the medium cultivation of *A. calcoaceticus* IMV B-7241. Thus, in the presence of a precursor of auxin biosynthesis in a medium with biodiesel production waste, the concentration of phytohormones reached 1405–4851 μg/L.

It should be noted that the ability of *A. calcoaceticus* IMV B-7241 strain to the simultaneous synthesis of phytohormones of auxin, cytokinin and gibberellin nature [89–91], as well as biosurfactants with antibacterial activity against phytopathogenic bacteria [92] makes it promising for use in agriculture to increase plant yield and phytopathogenic bacteria quantity control.

Ability of *Acinetobacter* bacteria to phosphates solubilization and xenobiotics degradation

Phosphate mobilizing. Phosphate-solubilizing rhizobacteria (PSRB) improve soil fertility by converting insoluble forms of phosphorus to soluble plant-available forms [93, 94]. The source of PSRB isolation is soils that are deficient in soluble phosphates. The ability of PSRB to solubilize phosphates is due to the synthesis of organic acids, which either directly dissolve mineral phosphates as a result of solvolysis, or chelate iron, aluminum and calcium ions from phosphates [21, 22, 93, 94].

The study of *Acinetobacter* genus bacteria as phosphate-mobilizing agents began simultaneously with the study of their ability to synthesize growth-stimulating metabolites (phytohormones, siderophores, etc.) [21, 22, 87].

In the papers [22, 93] was established that *A. rhizosphaerae* BIHB 723 strain solubilized calcium triphosphate, as well as rock phosphates. During the cultivation of the BIHB 723 strain in a medium with glucose and 0.5 % of various insoluble phosphates, organic acids (gluconic, 2-keto-gluconic, oxalic, malic, formic) were synthesized, and the qualitative and quantitative content of acids varied depending on the type of phosphates [93].

Rokhbakhsh-Zamin et al. [85] showed that of the 31 representatives of the genus *Acinetobacter* isolated from the rhizosphere of African millet, 26 strains showed the ability to solubilize phosphates. The most active phosphates mobilizing agent were strains *A. calcoaceticus* PUCM1006 and *A. calcoaceticus* PUCM1005, which under cultivation in medium with glucose (10 g/L) and calcium triphosphate (5 g/L) for 7 days formed 84 and 70 mg/L of soluble phosphates, respectively.

Ability to solubilize calcium triphosphate (0.5 %) by strain *A. calcoaceticus* D10 is established in the paper [86]. During the cultivation of this strain on a tryptic soy broth medium, the degree of phosphate solubilization after 6 days was 10 mg/L.

Syed-Ab-Rahman et al. [87] investigated the phosphate-mobilizing properties of *Acinetobacter* sp. UQ154, UQ156 and UQ202 strains when grown on agar medium with dextrose (10 g/L) and calcium triphosphate (5 g/L). The phosphate solubilization index for all strains was almost the same and amounted to 2.2−2.36 (this indicator was calculated as the ratio of the sum of the diameter of the clarification zone and the colony diameter to the colony diameter).

In the paper [94] was found that *Acinetobacter* sp. CGMCC 13078 is capable of solubilizing both inorganic and organic phosphates. Strain CGMCC 13078, when grown in a liquid medium with glucose (10 g/L) and calcium triphosphate (10 g/L), synthesized formic acid in an amount of 170.4, which was accompanied by the formation of 402 mg/L of soluble phosphate after 5 days [94].

In 2020, a paper was published [95], which reports the isolation of the *Acinetobacter* sp. SK2 strain which, as a result of the formation of gluconic acid during growth on a glucose medium, solubilized 682 μg/mL of calcium triphosphate and 86 μg/mL of rock phosphate.

It should be noted that *Acinetobacter* sp. SK2 is a more effective phosphate mobilizing agent not only in comparison with representatives of the *Acinetobacter* genus [85, 86, 94], but also with other bacteria: *Burkholderia cepacia* (51 μg/mL), *Klebsiella* sp. (110–130 μg/mL) [96], *Rhizobium* sp. Td3 (423 μg/mL), SN1 (428 μg/mL) [97], *Rhizobium* sp. RM (653 μg/mL) and RS (602 μg/mL) [98].

Degradation of xenobiotics. Bacteria of the *Acinetobacter* genus are known for their ability

Table 6

Destruction of xenobiotics by members of the *Acinetobacter* genus

Strain	Xenobiotics as substrates for cultivation	Initial concentration in the medium	Degree of destruction, %	Duration of cultivation, days	References
1	2	3	4	5	6
A. calcoaceticus IMV B-7013	Oil	0.5 % (v/v)	63.5	5	[111]
A. baumannii MKS2	Oil	10 g/L	85	14	[39]
Acinetobacter sp. D3-2	Oil	0.5 % (v/v)	82	14	[35]
A. baumannii MN3	Oil	19 g/L	78	7	[38]
A. pittii ABC	Oil	1 % (v/v)	47.15	5	[41]
			56.53	20	
A. pittii H9-3	Oil	10 g/L	36.8	21	[112]
A. halotolerans sp. nov.	A mixture of octadecane, eicosane and docosane	300 ppm of each of the hydrocarbons	51.7	14	[113]
	A mixture of kerosene, diesel fuel and gasoline	500 ppm of each of the hydrocarbons	45.8	14	
A. haemolyticus SA	Diesel fuel	1 % (v/v)	70	21	[114]
Acinetobacter baumannii	Diesel fuel	2 % (v/v)	58.1	10	[115]
Acinetobacter sp. Y2	Diesel fuel	2 % (v/v)	80	10	[116]
A. calcoaceticus CA16	Diesel fuel	2 % (v/v)	88	21	[117]
Acinetobacter sp. BS8Y	Phenol	600 mg/L	99.2	1	[118]
A. calcoaceticus PA	Phenol	800 mg/L	91.6	2	[119]
Acinetobacter sp. AVLB2	4-Nitroaniline	25 mg/L	82	7	[107]
Acinetobacter sp.	Cyprodinil	200 mg/L	88	14	[108]
Acinetobacter sp. JN8	β-Cypermethrin	100 mg/L	74.1	7	[106]
Acinetobacter sp. YT-02	Cyclohexylamine	10 mM	100	2 h	[109]

to degrade hydrocarbons, including n-alkanes [99–101], aromatic compounds [25, 102–104], and diesel fuel [23, 105]. The latest information appeared on the participation of representatives of this genus in the decomposition of pesticides [106–108] and insecticides [109, 110]. It should be noted that the ability to decompose xenobiotics was established by researchers during the cultivation of *Acinetobacter* genus bacteria on media containing toxic compounds as growing media (Table 6). In addition, in the papers [35, 38, 39, 41, 111] the authors focused on the role of synthesized biosurfactants as one of the factors that ensure a sufficiently high degree of decomposition of toxic substances.

In our studies [120], the ability of *A. calcoaceticus* IMV B-7241 to decompose aromatic compounds with simultaneous synthesis of extracellular metabolites with surface-active and emulsifying properties was determined. In the presence of biosurfactants *A. calcoaceticus* IMV B−7241 in the form of postfermentation culture liquid (5–10 %) the degree of degradation of complex oil pollutants with heavy metals (Ci^{2+}, Cd^{2+}, Pb^{2+}, 0.01–0.5 mM) in water (3–6 g/L) and soil (20 g/kg) after 20 days was 82–92 %.

The data given in Table 6 indicate that the level of decomposition of oil, diesel fuel and aromatic compounds by representatives of the *Acinetobacter* genus is comparable to the potential of actinobacteria of the *Rhodococcus* genus, which are among the most active xenobiotic decomposer among microorganisms [9, 121].

* * *

Analysis of literature data has shown that since the end of the 70s of the twentieth century and over the next 20–25 years, the main interest in *Acinetobacter* genus bacteria was due to producers of high-molecular emulsifiers. However, the situation has changed dramatically in the last decade. Currently, it is established that *Acinetobacter* genus representatives are capable not only of the synthesis of high-molecular emulsifiers, but also of low-molecular biosurfactants, promising for use in environmental technologies, practically valuable enzymes in particular, agarase and chondroitinase, and the ability to form phytohormones and solubilize phosphates determines the possibility of their practical use in crop production to increase crop yields. In addition, *Acinetobacter* bacteria are effective decomposers of aliphatic and aromatic hydrocarbons, as well as pesticides and insecticides. Every year the number of publications on the practically valuable properties of non-pathogenic bacteria of the *Acinetobacter* genus and the metabolites synthesized by them increases, which indicates their potential as promising an objects of biotechnology.

БІОТЕХНОЛОГІЧНИЙ ПОТЕНЦІАЛ БАКТЕРІЙ РОДУ *ACINETOBACTER*

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Резюме

Донедавна літературні відомості про біотехнологічний потенціал непатогенних бактерій роду *Acinetobacter* були небагаточисельними, хоча перші повідомлення про практично цінні властивості цих бактерій датувалися 70–80-ми роками XX ст. і стосувалися синтезу біоемульгатора емульсану. В останнє десятиліття інтерес до представників роду *Acinetobacter* як об'єктів біотехнології суттєво підвищився. В огляді наведено сучасні дані літератури про синтез бактеріями цього роду високомолекулярних емульгаторів, низькомолекулярних поверхнево-активних речовин гліко- і аміноліпідної природи, ферментів (ліпази, агарази, хондроїтінази), фітогормонів, а також їх здатність до солюбілізації фосфатів і деструкції різних ксенобіотиків (аліфатичних та ароматичних вуглеводнів, пестицидів, інсектицидів). Біоемульгатори бактерій роду *Acinetobacter* залишаються об'єктом наукових досліджень і до теперішнього часу. Це, насамперед, зумовлене тим, що на відміну від інших продуктів мікробного синтезу, синтезованих широким колом мікроорганізмів, ці емульгатори є «своєрідною візитівкою» представників роду *Acinetobacter.* Так, крім добре відомих і достатньо повно досліджених емульсанів, дисперсану, алазану, упродовж останніх десяти років описано нові перспективні для біоремедіації довкілля емульгатори, які за хімічною природою є глікопротеїнами, ліпополісахаридами і полісахаридами. Низькомолекулярні поверхнево-активні гліко- та ліпопептиди бактерій роду *Acinetobacter* досліджуються упродовж всього десяти років, тому вони не є конкурентно спроможними на ринку цих продуктів мікробного синтезу. Основною причиною цього є той факт, що утворення поверхнево-активних

речовин розглядається як додаткова властивість мікроорганізмів-деструкторів гідрофобних токсичних сполук. Крім того, тільки останніми роками стали з'являтися повідомлення про біологічні (антимікробні, антиадгезивні та протипухлинні) властивості поверхнево-активних речовин представників роду *Acinetobacter*. Серед ферментів бактерій роду *Acinetobacter* найбільша увага приділена одержанню ліпаз, хоча за синтезувальною здатністю вони поступаються відомим у світі продуцентам. У той же час штам *Acinetobacter* sp. AG LSL-1 як продуцент агарази (активність 3 Од/мл) є перспективнішим за *Alteromonas sp.* C-1 (2,3 Од/мл), який був відкритий понад 15 років тому і вважався одним з найактивніших продуцентів цього ферменту. Штам *Acinetobacter* sp. C26 є перспективнішим продуцентом унікального ферменту хондроїтинази (8,5 Од/мл) порівняно з рекомбінантним штамом *Pichia pastoris* X33 (2,72 Од/мл). Дослідження бактерій роду *Acinetobacter* як фосфатмобілізувальних агентів розпочалося одночасно з вивченням їх здатності до синтезу ріст-стимулювальних метаболітів (фітогормонів, сідерофорів та ін.). *Acinetobacter* sp. SK2 є ефективнішим фосфатмобілізуючим агентом (утворення 682 мкг/мл розчинного фосфату) не тільки порівняно з представниками роду *Acinetobacter*, а й симбіотичними азотфіксаторами роду *Rhizobium*, які солюбілізують 423−653 мкг/мл фосфатів. Наведено дані власних експериментальних досліджень про синтез та біологічну активність (антимікробну, антиадгезивну, здатність до руйнування біоплівок) поверхнево-активних речовин, синтезованих штамом *A. calcoaceticus* IMB B-7241, та їх роль у деструкції нафтових, у тому числі й комплексних з важкими металами, забруднень. Здатність *A. calcoaceticus* IMB B-7241 до одночасного синтезу фітогормонів (ауксини, цитокініни, гібереліни) та поверхнево-активних речовин з антимікробною щодо фітопатогенних бактерій активністю дає змогу розглядати цей штам як перспективний для практичного використання у рослинництві для підвищення врожайності сільськогосподарських культур.

Ключові слова: рід *Acinetobacter*, поверхнево-активні речовини, біоемульгатори, ферменти, фітогормони, деструкція ксенобіотиків.

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Received 13.01.2021