

# CALCIUM AND MAGNESIUM CATIONS INFLUENCE ON ANTIMICROBIAL AND ANTIADHESIVE ACTIVITY OF *Acinetobacter calcoaceticus* IMV B-7241 SURFACTANTS

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Received 23.09.2016

The aim of the work was to study the effect of calcium and magnesium cations on NADP<sup>+</sup>-dependent glutamate dehydrogenase activity (key enzyme of biosynthesis of *Acinetobacter calcoaceticus* IMV B-7241 surface-active aminolipids) followed by modification of medium composition and determining antimicrobial and antiadhesive activity of synthesized surfactants.

The strain IMV B-7241 was grown in medium with ethanol. NADP<sup>+</sup>-dependent glutamate dehydrogenase activity of the cell-free extract was analyzed using the formation of glutamate in the oxidation of NADPH. Surfactants were extracted from supernatant of cultural liquid by mixture of chloroform and methanol (2:1). Antimicrobial against bacteria properties of the surfactants were determined by index of the minimal inhibitory concentration. The number of attached cells and the degree of biofilm destruction were analyzed spectrophotometrically.

It was established that in the presence of 10 mM Ca<sup>2+</sup> and Mg<sup>2+</sup> NADP<sup>+</sup>-dependent glutamate dehydrogenase activity in the cell-free extract increased to 1.5 times in comparison with that without cations. Increasing concentration of magnesium sulfate to 0.2 g/l, or adding CaCl<sub>2</sub> (0.1 g/l) into cultivation medium of IMV B-7241 strain was accompanied by rise of NADP<sup>+</sup>-dependent glutamate dehydrogenase activity in 2.4 and 3.0 times respectively, as well as increasing antimicrobial and antiadhesive activity of synthesized surfactants. Minimal inhibitory concentration of surfactants synthesized in modified media against some bacteria was in 1.3–3.5 times, adhesion on abiotic surfaces treated with such surfactants in an average of 5–17% lower, and the degree of biofilm destruction in 7–13% higher as compared to indicators for the surfactant produced in the base medium.

The obtained results indicate the possibility of regulating antimicrobial and anti-adhesive activity of surfactants under producer cultivation.

**Key words:** *Acinetobacter calcoaceticus* IMV B-7405, surfactants, activity of NADP<sup>+</sup>-dependent glutamate dehydrogenase, calcium and magnesium cations.

Earlier research [1, 2] has established the dependence of antimicrobial and anti-adhesive activities of surface-active substances (surfactants) produced by *Acinetobacter calcoaceticus* IMV B-7241 on the presence of growth factors and certain microelements in the culture medium. Changing yeast autolysate and microelements mixture in the medium containing ethanol to copper sulfate and iron sulfate was followed by an increase of surfactants production [3], yet their antimicrobial and anti-adhesive activity decreased [1, 2]. We supposed that the phenomenon might be caused by microbial surfactants being secondary metabolites commonly synthesized as a complex of such

compounds [4] whose ratio (and therefore, the properties of the final product) may change depending on the different conditions of the producer cultivation.

By their chemical nature, surfactants of *A. calcoaceticus* IMV B-7241 are a complex of neutral, glyco- and aminolipids [4]. According to the data from literature [5, 6], aminolipids are more efficient antimicrobial agents than glycolipids, and neutral lipids are characterized by very low antimicrobial activity. Therefore, an increased fraction of aminolipids might be accompanied by a stronger antimicrobial activity of the surfactants. However, today the influence of cultivation conditions on surfactants'

antimicrobial and anti-adhesive activity and the possibility of regulating them remain outside the researchers' attention, although the first studies concerning the link between the chemical composition of microbial surfactants and their properties go back almost fifteen years [7]. Meanwhile, according to [8], the biosynthesis of aminolipids with preformed properties is impossible, and can be only achieved by post-fermentation chemical modification of the synthesized surfactants or using respective gene-modified producer strains [9].

In [1, 2] we suggested that in the presence of yeast extract and microelements mixture in *A. calcoaceticus* IMV B-7241 cultivation medium, the content of aminolipids in the produced surfactant complex is higher than in that obtained in the medium with copper sulfate and iron sulfate. Also, we assumed that among the microelements composing the cultivation medium for strain IMV B-7241 there can be cations activating NADP<sup>+</sup>-dependent glutamate dehydrogenase — a key enzyme of aminolipids biosynthesis in this strain [10]. Later this hypothesis was experimentally supported. Thus, later it was showed [11] that zinc cations are activators of NADP<sup>+</sup>-dependent glutamate dehydrogenase, and adding Zn<sup>2+</sup> (38 μM) into the medium containing ethanol and sulfates of copper and iron was followed by the production of surfactants with higher antimicrobial and anti-adhesive activity.

According to literature [12, 13] the activators of NADP<sup>+</sup>-dependent glutamate dehydrogenase in microorganisms are the cations of calcium and magnesium. Thus, in the archaea *Thermococcus* sp. this enzyme's activity increased in the presence of 5 mM CaCl<sub>2</sub>, MgCl<sub>2</sub> and MnCl<sub>2</sub> [12]. Later [13] it was showed that cations of calcium and magnesium also activated NADP<sup>+</sup>-dependent glutamate dehydrogenase in the archaea *Thermococcus waiotapuensis*: under 10 mM CaCl<sub>2</sub> and 10 mM MgSO<sub>4</sub> the activity rose 1.3 times compared to the control without metal cations.

Hence, the purpose of this work is to study influence of calcium and magnesium cations on the NADP<sup>+</sup>-dependent glutamate dehydrogenase activity of *A. calcoaceticus* IMV B-724 cell-free extract with the following modification of cultivation medium composition and determining antimicrobial and antiadhesive activity of synthesized surfactants.

## Materials and Methods

*Object of study.* The object of our study was strain *Acinetobacter calcoaceticus* K-4, registered in the Depository of Microorganism Strains of the Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine under IMV B-7241.

*Medium composition and cultivation conditions.* Strain *A. calcoaceticus* IMV B-7241 was grown in a liquid mineral medium of the following composition (g/l): (NH<sub>2</sub>)<sub>2</sub>CO — 0.35, NaCl — 1.0, Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O — 0.6, KH<sub>2</sub>PO<sub>4</sub> — 0.14, MgSO<sub>4</sub>·7H<sub>2</sub>O — 0.1, distilled water — up to 1 l, pH 6.8–7.0. The medium was also augmented with yeast autolysate — 0.5 % (v/v) and microelements solution — 0.1 % (v/v) containing (g/100 ml): ZnSO<sub>4</sub>·7H<sub>2</sub>O — 1.1; MnSO<sub>4</sub>·H<sub>2</sub>O — 0.6; FeSO<sub>4</sub>·7H<sub>2</sub>O — 0.1; CuSO<sub>4</sub>·5H<sub>2</sub>O — 0.004; CoSO<sub>4</sub>·7H<sub>2</sub>O — 0.03; H<sub>3</sub>BO<sub>3</sub> — 0.006; KI — 0.0001; EDTA — 0.5.

As carbon and energy source we used 2 % ethanol (v/v).

In one version of the experiment, the basic medium had MgSO<sub>4</sub>·7H<sub>2</sub>O content raised to 0.2 g/l (medium 1), in another the basic medium was augmented by additional dose of 0.1 g/l CaCl<sub>2</sub> (medium 2).

*Inoculum.* Seeding material was a culture in the middle of the exponential growth phase grown in a basic medium with 0.5 % (v/v) of ethanol. The amount of inoculate was 5 % of the mediums volume (10<sup>4</sup>–10<sup>5</sup> cells/ml). The culture was grown in 750 ml flasks with 100 ml of the medium on a shaker (320 rev/min) at 30 °C for 120 hr.

*Determining antimicrobial and antiadhesive activity of surfactants.* In the research we used surface-active substances in the form of cultural liquid supernatant and solutions of surfactants extracted from supernatant by the Folch mixture (chloroform and methanol, 2:1) as described in [1, 2, 3, 11].

The antimicrobial properties of surfactants were analyzed according to their minimum inhibitory concentration (MIC). MIC was determined by two-fold serial dilutions in meat-peptone broth (MPB) [2, 11]. In aseptic conditions, MPB was added to ten test tubes at 1 ml. In the first test tube, we added 1 ml of sterile solution of the surfactant a certain concentration, mixed it, 1 ml was taken and placed into the next tube. Similarly, we carried out the dilution in the next nine test tubes. From the last test tube, we pipetted out 1 ml. Therefore, the final volume in every test tube was 1 ml (MPB and the surfactant solution), but the concentration of the surfactant was

twice reduced at every step. As a control we used 1 ml of MPB without surfactants solution. Next, into every test tube 0.1 ml of the test culture suspension ( $10^5$ – $10^6$  CFU/ml) was added and the contents were mixed. The test tubes were incubated for 24 hours at 30 °C. The results were estimated visually by the cloudy or transparent medium: (+) flasks where the medium grew cloudy (indicating growth of test culture), (–) flasks without cloudiness (no growth). The MIC of the surfactant solution was determined as the surfactant concentrations in the first tube where the test culture failed to grow.

Antiadhesive properties were studied as follows [1, 11]: the purified plates of materials (Dutch tile, stainless steel, plastic, linoleum) of the same size (1 cm<sup>2</sup>) were sterilized at 112 °C for 30 min and then were added into the surfactants solution or supernatant and dried for 24 h in a thermostat at 30 °C. One day bacterial test cultures grown on meat peptone agar (MPA) were suspended in 100 mL of sterile tap water; the materials pretreated with surfactants and untreated (control) samples were placed into the suspension, incubated for 2 h in a thermostat at 30 °C, and rinsed with 10 mL of sterile tap water to remove non-adherent cells.

The plates of materials were treated with methanol (99%) for 15 min to fix the attached cells, dried at room temperature, placed for 5 min into 1% gentian violet solution, and rinsed with tap water. After drying, the materials were treated with 10 mL of 33% acetic acid solution and the optical density of the resultant suspension of desorbed cells was measured. The number (%) of attached cells (adhesion) was determined as a ratio of the optical density of the suspension obtained from surfactant-treated (supernatant, surfactant solution) materials to that of the controls (without surfactant treatment) and measured in %.

The study of surfactants' action on the biofilm destruction was carried out after [14]. To obtain biofilm, 180 µl of MPB or liquid wort and 20 µl of one-day test culture suspension were added into polystyrene microplates, incubated for 24 h at the optimal temperature. Then the cultural liquid was poured off and another 180 µl of fresh MPB (liquid wort) and 20 µl of the suspension of the test culture were added and again incubated for 24 h. In the study [14] it was established that such 48 h culture is sufficient for biofilm formation in the wells of microplate. After 48 h the cultural liquid was poured off, and into each of the wells of the microplate (pre-covered by the biofilm) 200 µl of preparations with

different surfactant concentrations (0.005–1.28 mg/ml) were added. Into the control wells, surfactant preparations were replaced with distilled tap water (200 µl). After 24 h of exposition the wells were thrice washed by 200 µl of distilled water and the amount of adherent cells was determined spectrophotometrically just as it was done for the anti-adhesive research [1, 11]. The degree of biofilm destruction (%) was determined as the difference between cell adhesion in untreated and surfactant-treated wells of the polystyrene plate.

As test cultures to evaluate the biological properties of surfactants we used bacterial strains *Escherichia coli* IEM-1, *Bacillus subtilis* BT-2, *Enterobacter cloacae* C-8, *Staphylococcus aureus* BMC-1, *Proteus vulgaris* PA-12 from the living cultures collection of the Department of Biotechnology and Microbiology of the National University of Food Technologies.

*Enzyme analysis.* To obtain cell-free extracts the culture liquid was centrifuged (5000 g, 20 min, 4 °C). The remnants of the medium were twice washed out of the obtained cell pellet with 0.05 M K<sup>+</sup>-phosphate buffer (pH 7.0) using centrifugation (4000 g, 15 min, 4 °C). The washed cells were resuspended in 0.05 M K<sup>+</sup>-phosphate buffer (pH 7.0) and destroyed with ultrasound (22 kHz) thrice for 20 s at 4 °C on UZDN-1 ultrasonic disperser. The resulting mush was centrifuged again (12000 g, 30 min, 4 °C), the pellet was discarded, and the supernatant was used in further research as cell-free extract.

NADP<sup>+</sup>-dependent glutamate dehydrogenase activity of the cell-free extract (EC 1.4.1.4) was analyzed by measuring glutamate synthesis during NADPH oxidation at 340 nm [11]. To study the effect of cations on the enzyme activity we added to the reagents mixture 0.01–10 mM Ca<sup>2+</sup> and Mg<sup>2+</sup> as solution of CaCl<sub>2</sub> and MgSO<sub>4</sub>·7H<sub>2</sub>O.

The enzyme activity was measured as the number of product nmol per 1 min of reaction per 1 mg protein. The protein content in cell-free extracts was determined after Bradford. The enzymatic activity was analyzed at 28–30 °C — the optimal temperature for *A. calcoaceticus* IMV B-7241 growth.

All experiments were carried out in triplicate, the number of parallel measurements in the experiments was 3–5. The statistical treatment of the experimental data was carried out as previously described [1–3]. The differences between the means were considered significant at  $P < 0.05$ .

## Results and Discussion

To begin with, we analyzed NADP<sup>+</sup>-dependent glutamate dehydrogenase activity of *A. calcoaceticus* IMV B-7241 cell-free extract depending on Ca<sup>2+</sup> and Mg<sup>2+</sup> content in the reaction mixture (Table 1). Experiments showed that at 10 mM Ca<sup>2+</sup> and Mg<sup>2+</sup> the activity increased 1.5 times compared to the cation-less control.

Next, NADP<sup>+</sup>-dependent glutamate dehydrogenase activity of the cell-free extract obtained from *A. calcoaceticus* IMV B-7241 cells grown in a liquid media with increased content of the enzyme activators was determined (Table 2).

We established that twice higher magnesium sulfate concentration or addition of CaCl<sub>2</sub> into cultivation medium of IMV B-7241 strain was followed by 2.4- and 3 times rise in NADP<sup>+</sup>-dependent glutamate dehydrogenase activity, respectively.

The data on antimicrobial activity of the surfactants, synthesized by *A. calcoaceticus* IMV B-7241 in basic medium, and in medium with increased concentration of calcium and magnesium cations are presented in Table 3.

The research showed that for all studied bacteria (except for *S. aureus* BMC-1), the MIC of surfactants produced in the medium 2 was 1.3–3.5 times lower than that of surface-active substances produced in the basic medium. Previous research [11] showed that adding zinc cations into the cultivation medium of *A. calcoaceticus* IMV B-7241 (another activator of NADP<sup>+</sup>-dependent glutamate dehydrogenase in this strain) was followed by synthesis of surfactants, the MIC of which against *E. coli* IEM-1, *E. cloacae* C-8, *S. aureus* BMC-1 and *P. vulgaris* PA-12 was 14, 28, 7 and 14 µg/ml, respectively, which is practically same as we obtained in our current work (Table 3).

Also, the antimicrobial activity of the *A. calcoaceticus* IMV B-7241 surfactants against some test cultures was higher than of well-known aminolipids of the bacteria genera *Bacillus* and *Paenibacillus* [5]. Thus, MIC of surfactin, iturin and polypeptin against different strains of *E. coli* IEM-1 was 15, 6, >300 and 3.1–12.5 µg/ml respectively; of polypeptin and octapeptin against *P. vulgaris* — 50–100 and 6.3 µg/ml; of iturin, polipeptin and octapeptin against *S. aureus* >400, 6.3 and 50 µg/ml, respectively. The minimum inhibitory concentration of the aminolipids produced by *Streptomyces amritsarensis* sp. nov. against *B. subtilis* MTCC 619 did not exceed 10 µg/ml, and against *Staphylococcus epidermidis* MTCC 435 it was 15 µg/ml [15].

Let us note here that the antimicrobial activity of surfactants produced by *A. calcoaceticus* IMV B-7241 in the basic medium and medium 1 was practically the same (Table 3). Therefore, adding more Ca<sup>2+</sup> into the cultivation medium of strain IMV B-7241 influenced the antimicrobial activity of the produced surfactants more than raising the Mg<sup>2+</sup> concentration.

Similar results were found when studying the anti-adhesive properties of the *A. calcoaceticus* IMV B-7241 surface-active substances obtained in the media with different concentrations of Ca<sup>2+</sup> and Mg<sup>2+</sup> (Table 4). In these experiments we used surfactant solutions with base concentrations of 5 µg/ml, since earlier data [1] showed that this was the concentration with the maximal anti-adhesive effect of surface-active substances synthesized in the basic medium.

The data in Table 4 show that the adhesion of *B. subtilis* BT-2 spore cells on plastic, tile, steel and linoleum was minimal (14–26%) if the surfaces were pre-treated with the solution of surfactant produced by strain IMV B-7241 in the medium 2 with additional CaCl<sub>2</sub>. Also, unlike the practically equal antimicrobial activity of surfactants produced in the basic medium and medium 1 with increased magnesium content, their anti-adhesive properties turned out to be different (Tables 3 and 4). Treating all studied materials with the solution of surfactants produced in the medium 1, was followed by the decrease in the amount of *B. subtilis* BT-2 adherent cells by 70–84%, while the preparation obtained in the basic medium only lowered it by 52–77% compared to surfaces untreated with surfactants (Table 4). Notably, surfactants produced in the media 1 and 2 exhibited higher anti-adhesive activity at 1.25 µg/ml, while lowering the concentration from 5 to 1.25 µg/ml for surfactants obtained in the basic medium caused a 6–11% increase in *B. subtilis* BT-2 adhesion on pre-treated surfaces (Table 4).

Analysis of anti-adhesive properties of the known microbial surfactants that we reviewed in [16] showed that aminolipids produced by bacteria of the genus *Bacillus* are more efficient anti-adhesive agents compared to rhamno- and sophorolipids. Thus, the efficient concentration of aminolipids is, on average, 2–50, and sophorolipids 12–200 µg/ml. However, the available literature did not contain information on the effect of aminolipids on the *B. subtilis* adhesion to different materials. Hence we compared our results (Table 4) to those in [17], where the authors studied the efficiency of *Pseudomonas aeruginosa* LCD12 rhamnolipids in preventing

Table 1. The effect of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  cations on NADP<sup>+</sup>-dependent glutamate dehydrogenase activity of *A. calcoaceticus* IMV B-7241 cell-free extract

Cations	Concentration in the reagents mixture, mM	Activity, nmol·min <sup>-1</sup> ·mg <sup>-1</sup> of protein
No cations (control)	0	345±17
$\text{Ca}^{2+}$	0.01	N. d.
	5	431±21*
	10	517±25*
$\text{Mg}^{2+}$	0.01	310±15*
	5	345±17*
	10	517±25*

Note. \* —  $P \leq 0.05$  compared to control (enzyme activity in the absence of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in the reaction mixture). N. d. — the parameter was not determined.

Table 2. NADP<sup>+</sup>-dependent glutamate dehydrogenase activity of *A. calcoaceticus* IMV B-7241 cell-free extract depending on  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations in the cultivation medium

Cultivation medium	Concentration in the medium (g/l)		Activity, nmol·min <sup>-1</sup> ·mg <sup>-1</sup> of protein
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	$\text{CaCl}_2$	
Basic	0.1	0	380±19
Medium 1	0.2	0	920±46*
Medium 2	0.1	0.1	1140±57*

Note. \* —  $P \leq 0.05$  compared to control (enzyme activity during cultivation of IMV B-7241 strain in the basic medium).

Table 3. The minimum inhibitory concentration of surfactants produced by *A. calcoaceticus* IMV B-7241 in media with different  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations

Test culture	MIC (µg/ml) of surfactants, produced on		
	basic medium	medium 1	medium 2
<i>Bacillus subtilis</i> BT-2 (spores)	14	12*	4*
<i>Enterobacter cloacae</i> C-8	42	48*	32*
<i>Staphylococcus aureus</i> BMC-1	7	12*	8*
<i>Proteus vulgaris</i> PA-12	14	12*	8*
<i>Escherichia coli</i> IEM-1	28	24*	16*

Note. When measuring the minimum inhibitory concentration of surfactants, the error did not exceed 5%. \* —  $P \leq 0.05$  compared to control (MIC of surfactants produced by IMV B-7241 strain cultured in the basic medium).

the adhesion of *B. subtilis* RI6, *E. coli* PJ3, *S. aureus* FD5, and *Staphylococcus epidermidis* LK8 to polystyrene surface. It was found that the adhesion of test cultures was 50–80% if the wells of the plate were pre-treated with rhamnolipids at 8–64 µg/ml. The data in Table 4 show that surfactants produced in the process of *A. calcoaceticus* IMV B-7241 cultivation on all studied media exhibit higher

anti-adhesive activity at significantly lower concentrations.

According to the latest research, surfactants synthesized by bacteria (*Pseudomonas*, *Lactobacillus*, *Bacillus*) and yeasts (*Saccharomyces*) are able to not only prevent microorganisms adhesion on various materials, but to destroy established biofilms [16, 18–20].

In our previous study [16] we showed that *A. calcoaceticus* IMV B-7241 surfactants (0.04–1.28 mg/ml) synthesized on ethanol, glycerol and *n*-hexadecan are able to destroy more than 21–88% of the biofilm formed by *S. aureus* BMC-1, *B. subtilis* BT-2 and *E. coli* IEM-1, and the degree of bacterial biofilms destruction the grew with increasing surfactants' content.

Table 5 provides the data on the effect of *A. calcoaceticus* IMV B-7241 surfactant preparations, synthesized in the media with different concentrations of Ca<sup>2+</sup> and Mg<sup>2+</sup> on the destruction of *B. subtilis* BT-2 biofilm. In these researches, the biofilm destruction was studied under lower concentrations of strain IMV B-7241 surfactants, than in the previously discussed research [16].

It was found that regardless of the concentration and level of purification (supernatant, surfactant solution) the preparations of surfactants synthesized in the media 1 and 2 caused more efficient destruction of *B. subtilis* BT-2 biofilm than preparations obtained in the basic medium (19–58 and

12–45%, respectively). The highest degree of biofilm destruction (57–58%) was reached by preparations with surfactant concentration of 62–124 µg/ml.

Das et al. [17] showed that rhamnolipids of *P. aeruginosa* IMP67 at a concentration of 64 µg/ml were able to destroy 50% biofilm formed on polystyrene by *B. subtilis* RI6. Surface-active substances synthesized by *Saccharomyces cerevisiae* D3 at 100 µg/ml caused destruction of *B. subtilis* BT37 biofilm by 30% [21]. Therefore, surfactants produced by strain IMV B-7241 are more efficient destructors of biofilms than those of *S. cerevisiae* D3 and rhamnolipids of *P. aeruginosa* IMP67, which supports the possibility of using them as components of novel disinfectants to destroy bacterial biofilms.

Thus, our work showed that adding Ca<sup>2+</sup> or increasing the concentration of Mg<sup>2+</sup> in the cultivation medium of *A. calcoaceticus* IMV B-7241 was followed by synthesis of surfactants with higher antimicrobial and anti-adhesive activity than surfactants obtained in the basic medium.

Table 4. The effect of surfactants synthesized by *A. calcoaceticus* IMV B-7241 in media with different Ca<sup>2+</sup> and Mg<sup>2+</sup> concentrations on the adhesion of *B. subtilis* BT-2 spore cells to various surfaces

Cultivation medium	Surfactant concentration, µg/ml	Materials,% adhesion			
		plastic	tile	steel	linoleum
Base	5	23	37	24	30
	1.25	29	48	30	36
Medium 1	5	21*	30*	15*	25*
	1.25	16*	20*	16*	19*
Medium 2	5	19*	26*	14*	25*
	1.25	16*	21*	N.d.	14*

Note. When measuring the adhesion the error did not exceed 5%. \* —  $P \leq 0.05$  compared to control (adhesion after treatment with surfactants, produced by IMV B-7241 in the basic medium). N.d. — not determined.

Table 5. Destruction of *B. subtilis* BT-2 biofilm under the action of *A. calcoaceticus* IMV B-7241 surfactants produced in the media of various composition

Cultivation medium	Preparations	Destruction (%) of the biofilm after treatment with surfactant (µg/ml)					
		3.9	7.8	15.5	31	62	124
Basic	Supernatant	26	28	29	31	33	35
	Surfactant solution	12	17	29	31	36	45
Medium 1	Supernatant	33*	43*	45*	45*	55*	57*
	Surfactant solution	19*	24*	40*	42*	58*	58*
Medium 2	Supernatant	39*	43*	45*	50*	52*	57*
	Surfactant solution	29*	33*	45*	48*	51*	58*

Note. During the measurement of biofilm destruction, the error did not exceed 5%. \* —  $P \leq 0.05$  compared to control (destruction of the biofilm after treatment with surfactant preparations, synthesized by strain IMV B-7241 in the basic medium).

The data are in agreement with our previous results [11] and support the

possibility of regulating antimicrobial and anti-adhesive activity of surfactants under producer cultivation.

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**ВПЛИВ КАТІОНІВ КАЛЬЦІЮ І МАГНІЮ  
НА АНТИМІКРОБНУ ТА  
АНТИАДГЕЗИВНУ АКТИВНІСТЬ  
ПОВЕРХНЕВО-АКТИВНИХ РЕЧОВИН  
*Acinetobacter calcoaceticus* IMB B-7241**

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Метою роботи було дослідити вплив  $\text{Ca}^{2+}$  і  $\text{Mg}^{2+}$  на НАДФ<sup>+</sup>-залежну глутаматдегідрогеназну активність — ключовий ензим біосинтезу поверхнево-активних аміноліпідів *Acinetobacter calcoaceticus* IMB B-7241 — з наступною модифікацією складу середовища і визначенням антимікробної та антиадгезивної активності поверхнево-активних речовин.

Штамм IMB B-7241 вирощували у середовищі з етанолом. НАДФ<sup>+</sup>-залежну глутаматдегідрогеназну активність екстракту аналізували за утворенням глутамату під час окиснення НАДФН. Поверхнево-активні речовини екстрагували із супернатанта культуральної рідини сумішшю Фолча. Антиадгезивну активність визначали спектрофотометричним методом, антимікробну — за показником мінімальної інгібуючої концентрації.

Встановлено, що за наявності у середовищі 10 мМ  $\text{Ca}^{2+}$  і  $\text{Mg}^{2+}$  НАДФ<sup>+</sup>-залежна глутаматдегідрогеназна активність екстракту підвищувалася в 1,5 раза порівняно з такою без катіонів. Збільшення концентрації сульфату магнію до 0,2 г/л або додавання  $\text{CaCl}_2$  (0,1 г/л) у середовище культивування супроводжувалося підвищенням цієї активності у 2,4 і 3,0 рази відповідно, а також посиленням антимікробної та антиадгезивної активності синтезованих поверхнево-активних речовин. Мінімальна інгібуюча концентрація поверхнево-активних речовин, синтезованих на модифікованих середовищах, щодо деяких бактерій була в 1,3–3,5 раза, їх адгезія на абіотичних поверхнях, оброблених такими речовинами, — у середньому на 5–17% нижчою, а ступінь руйнування біоплівки на 7–13% вищим порівняно з показниками, встановленими для поверхнево-активних речовин, утворюваних на базовому середовищі.

Наведені дані свідчать про можливість регуляції антимікробної та антиадгезивної активності поверхнево-активних речовин у процесі культивування продуцента.

**Ключові слова:** *Acinetobacter calcoaceticus* IMB B-7405, поверхнево-активні речовини, НАДФ<sup>+</sup>-залежна глутаматдегідрогеназна активність, катіони кальцію та магнію.

**ВЛИЯНИЕ КАТИОНОВ КАЛЬЦИЯ И  
МАГНИЯ НА АНТИМІКРОБНУЮ И  
АНТИАДГЕЗИВНУЮ АКТИВНОСТЬ  
ПОВЕРХНОСТНО-АКТИВНЫХ ВЕЩЕСТВ  
*Acinetobacter calcoaceticus* IMB B-7241**

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Целью работы было исследовать влияние  $\text{Ca}^{2+}$  и  $\text{Mg}^{2+}$  на НАДФ<sup>+</sup>-зависимую глутаматдегідрогеназную активність (ключевой энзим биосинтеза поверхностно-активных аминоллипидов *Acinetobacter calcoaceticus* IMB B-7241) с последующей модификацией состава среды и определением антимікробной и антиадгезивной активности поверхностно-активных веществ.

Штамм IMB B-7241 выращивали в среде с этанолом. НАДФ<sup>+</sup>-зависимую глутаматдегідрогеназную активність экстракта анализировали по образованию глутамата при окислении НАДФН. Поверхностно-активные вещества экстрагировали из супернатанта культуральной жидкости смесью Фолча. Антиадгезивную активність определяли спектрофотометрическим методом, антимікробную — по показателю минимальной ингибирующей концентрации.

Установлено, что при наличии в среде 10 мМ  $\text{Ca}^{2+}$  и  $\text{Mg}^{2+}$  НАДФ<sup>+</sup>-зависимая глутаматдегідрогеназная активність экстракта повышалась в 1,5 раза по сравнению с таковой без катионов. Увеличение концентрации сульфата магния до 0,2 г/л или добавление  $\text{CaCl}_2$  (0,1 г/л) в среду культивирования сопровождалось повышением этой активності в 2,4 и 3,0 раза соответственно, а также увеличением антимікробной и антиадгезивной активності синтезированных поверхностно-активных веществ. Минимальная ингибирующая концентрация поверхностно-активных веществ, синтезированных на модифицированных средах, по отношению к некоторым бактериям была в 1,3–3,5 раза, их адгезия на абиотических поверхностях, обработанных такими поверхностно-активными веществами — в среднем на 5–17% ниже, а степень разрушения биопленки на 7–13% выше по сравнению с показателями, установленными для поверхностно-активных веществ, образуемых на базовой среде.

Приведенные данные свидетельствуют о возможности регуляции антимікробной и антиадгезивной активності поверхностно-активных веществ в процессе культивирования продуцента.

**Ключевые слова:** *Acinetobacter calcoaceticus* IMB B-7405, поверхностно-активные вещества, НАДФ<sup>+</sup>-зависимая глутаматдегідрогеназная активність, катионы кальция и магния.