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EXPERIMENTAL ARTICLES

Antiadhesive Properties of the Surfactants of *Acinetobacter calcoaceticus* IMB B-7241, *Rhodococcus erythropolis* IMB Ac-5017, and *Nocardia vaccinii* IMB B-7405

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Abstract—Attachment of the cells of some bacteria, yeasts, and micromycetes to various surfaces (catheters, dentures, plastic, polyvinyl chloride, tiles, and steel) treated with the surfactants from *Acinetobacter calcoaceticus* IMB B-7241, *Rhodococcus erythropolis* IMB Ac-5017, and *Nocardia vaccinii* IMB B-7405 was studied. Adhesion of microorganisms to all the studied surfaces depended on the surfactant concentration and purity, kind of surface, and the test culture. Treatment with the surfactants from *N. vaccinii* IMB B-7405 (0.005–0.05 mg/mL), *A. calcoaceticus* IMB B-7241 (0.003–0.036 mg/mL), and *R. erythropolis* IMB Ac-5017 (0.03–0.12 mg/mL) resulted in adhesion decreased respectively by 35–75, 60–75, and 25–90% for bacteria (*Escherichia coli* IEM-1, *Bacillus subtilis* BT-2, etc.), by 80–85, 55–90, and 15–60% for yeasts *Candida albicans* D-6, and by 40–50, 35–35, and 10–20% for micromycetes (*Aspergillus niger* P-3 and *Fusarium culmorum* T-7).

Keywords: *Acinetobacter calcoaceticus* IMB B-7241, *Rhodococcus erythropolis* IMB Ac-5017, *Nocardia vaccinii* IMB B-7405, surfactants, microbial adhesion, abiotic surfaces

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Formation of microbial biofilms on different surfaces of equipment used in the food industry and medicine is dangerous, since microorganisms in biofilms possess enhanced resistance to various biocides [1–3]. In recent years, particular attention has been paid to the study of microbial surfactants as antiadhesive agents capable of preventing biofilm formation [4–7].

Previously, we isolated oil-oxidizing bacteria from oil-contaminated soil samples, which were identified as *Acinetobacter calcoaceticus* K-4 (IMB B-7241), *Rhodococcus erythropolis* EK-1 (IMB Ac-5017), and *Nocardia vaccinii* K-8 (IMB B-7405) [8]. These strains were found to be able to synthesize metabolites with surfactant and emulsifying properties. The methods for intensification of surfactant synthesis on different carbon substrates, including industrial waste [9–13], were developed, and their possible practical application in environmental protection technologies and as antimicrobial agents was shown [11, 12, 14].

The goal of the present work was to investigate the effects of surfactants from *Acinetobacter calcoaceticus* IMB B-7241, *Rhodococcus erythropolis* IMB Ac-5017, and *Nocardia vaccinii* IMB B-7405 on attachment of bacterial, yeast and micromycete cells to various surfaces.

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MATERIALS AND METHODS

Subjects of research. The major research subjects were the strains *Rhodococcus erythropolis* EK-1, *Acinetobacter calcoaceticus* K-4 and *Nocardia vaccinii* K-8 registered at the Depository of Microorganisms of the Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, under accession numbers IMB Ac-5017, IMB B-7241 and IMB B-7405, respectively.

Chemically, the extracellular surfactants of *R. erythropolis* IMB Ac-5017 are a complex of glycolipids (trehalose mono- and dimycolates), neutral lipids (cetyl alcohol, palmitic acid, methyl ether of *n*-pentadecanoic acid, mycolic acids), and phospholipids (phosphatidylglycerol and phosphatidylethanolamine) [9]. The extracellular surfactants of *A. calcoaceticus* IMB B-7241 were shown to contain glyco- (trehalose mono- and dimycolates, trehalose mono- and diacelates) and amino lipids [9]. The strain IMB B-7405 synthesizes a complex of extracellular neutral, glyco- and amino lipids [9]. Neutral lipids are represented by mycolic and *n*-alkanic acids; glycolipids are represented by trehalose diacelates and trehalose dimycolates.

The strains of bacteria (*Escherichia coli* IEM-1, *Bacillus subtilis* BT-2, *Proteus vulgaris* BT-1, *Staphylococcus aureus* BMS-1, *Pseudomonas aeruginosa*

P-55, *Enterobacter cloacae* AC-22), yeasts (*Candida albicans* D-6) and micromycetes (*Aspergillus niger* P-3, *Fusarium culmorum* T-7) used in this work were obtained from the collection of living cultures of the Department of Biotechnology and Microbiology, the National University of Food Technologies, Kiev, Ukraine.

Medium composition and cultivation conditions. *R. erythropolis* IMB Ac-5017 was grown in liquid mineral medium containing the following (g/L): $NaNO_3$, 1.3; $MgSO_4 \cdot 7H_2O$, 0.1; NaCl, 1.0; Na_2HPO_4, 0.6; KH_2PO_4, 0.14; $FeSO_4 \cdot 7H_2O$, 0.01; pH 6.8–7.0. Sunflower oil at a concentration of 2% (vol/vol) was used as a substrate.

A. calcoaceticus IMB B-7241 was cultivated in a nutrient medium containing the following (g/L): $(NH_2)_2CO$, 0.35; $MgSO_4 \cdot 7H_2O$, 0.1; NaCl, 1.0; Na_2HPO_4, 0.6; KH_2PO_4, 0.14; pH 6.8–7.0. The medium was additionally supplemented with yeast autolysate, 0.5% (vol/vol), and solution of trace elements, 0.1% (vol/vol) [9]. Ethanol at a concentration of 2% (vol/vol) was used as a carbon source.

The strain *N. vaccinii* IMB B-7405 was grown in a synthetic nutrient medium containing the following (g/L): $NaNO_3$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.1; $CaCl_2 \cdot 2H_2O$, 0.1; KH_2PO_4, 0.1; $FeSO_4 \cdot 7H_2O$, 0.1; yeast autolysate, 0.5% (vol/vol). Glycerol at a concentration of 1.0% (vol/vol) was used as a carbon and energy source.

Cultures in the exponential growth phase cultivated in the respective liquid media containing 0.5–1% (vol/vol) of the substrate were used as inocula. The amount of inoculum (10^4–10^5 cells/mL) was 5–10% by volume of the nutrient medium. The bacteria were cultivated in 750-mL flasks with 100 mL of the medium on a shaker (320 rpm) at 28–30°C for 120 h.

Isolation of surfactants. The surfactant-containing culture liquid (preparation 1) was subjected to extraction with the 2 : 1 chloroform/methanol (Folch) mixture to isolate the surfactant (preparation 2). The aqueous phase remaining after surfactant extraction was conditionally denoted as preparation 3.

The grown cells were separated by centrifugation (5000 *g*, 45 min), and the supernatant (preparation 1) was exposed to further treatment. For this purpose, 50 mL of the Folch mixture was added to 50 mL of the supernatant and lipids were extracted for 5 min. After phase separation, the bottom fraction (organic extract 1) was removed and the aqueous phase was subjected to repeated extraction as described above. After phase separation, the bottom fraction was removed to obtain organic extract 2. At the third stage, 50 mL of the Folch mixture was added to the aqueous phase, and extraction was performed to obtain organic extract 3. Extracts 1–3 were combined and evaporated in an IR-1M2 rotary evaporator (Russia) at 50°C and an absolute pressure of 0.4 atm to a constant mass. The solid residue was dissolved in sterile tap water to the initial volume. All preparations were sterilized at 112°C for 30 min.

Surfactant concentrations in preparations 1 and 2 were determined by the gravimetric method after Folch extraction procedure.

Investigation of antiadhesive properties of the surfactant. Antiadhesive properties were investigated as follows [15–17]: the purified plates of materials under study (dental prostheses, Dutch tile, stainless steel, plastic, linoleum (polyvinylchloride)) of the same size (1 cm^2) were sterilized at 112°C for 40 min; the fragments of male urogenital catheters (silicon) were immersed into 75% alcohol solution for 20 min and then into the solution of preparations 1–3 and dried for 24 h in a thermostat at 30°C. One-day bacterial and yeast test cultures and three-day micromycete cultures grown on meat peptone agar (MPA) and glucose potato agar (GPA), respectively, were suspended in 100 mL of sterile tap water; the materials pretreated with preparations 1–3 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.

Then the degree of cell adhesion was determined by two methods.

Koch's method [16, 17]. The materials were placed into flasks with 20 mL of sterile tap water and glass beads and shaken for 5 min to desorb the attached cells. The number of living cells in the resultant suspension was measured by the Koch's method on MPA (for bacteria) and GPA (for yeasts and micromycetes). The number (%) of attached cells (adhesion) was determined as a ratio of cells on the samples pretreated with preparations 1–3 to the cells on the control samples (100%).

Spectrophotometric method [15, 16]. 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 the samples treated with preparations 1–3 to the optical density of the control samples (100%).

All experiments were repeated three times, with three to five parallel measurements. The experimental data were statistically processed as described previously [10]. The differences between the average values were considered reliable at a confidence level $p < 0.05$.

RESULTS AND DISCUSSION

Adhesion of microorganisms to medical materials treated with microbial surfactants. The daily practice of intensive care presupposes numerous invasions

Table 1. Effect of the surfactants from *N. vaccinii* IMB B-7405 and *R. erythropolis* IMB Ac-5017 on microbial adhesion to urogenital catheter surfaces

Surfactant producer	Preparation	Concentration, mg/mL	Adhesion, %*				
			C. albicans D-6	*E. coli* IEM-1	*P. vulgaris* BT-13	*P. aeruginosa* P-55	*E. cloaceae* AS-22
N. vaccinii IMB B-7405	1 (supernatant)	0.08	60	45	15	79	32
		0.05	41	60	11	86	76
		0.03	50	Í.î.	43	90	89
	2 (surfactant solution)	0.08	43	21	56	20	24
		0.05	12	42	65	25	45
		0.03	20	N.d.	60	52	70
R. erythropolis IMB Ac-5017	1 (supernatant)	0.25	35	55	38	49	66
		0.12	41	19	41	32	72
		0.06	67	45	80	42	70
		0.03	78	60	85	58	93
	2 (surfactant solution)	0.25	30	52	30	93	64
		0.12	42	27	46	85	61
		0.06	70	40	83	88	73
		0.03	75	54	87	91	78

Tables 1–5: the adhesion error was no more than 5%. N.d., not determined.

* % of adhesion in the control (100%) without surfactant treatment.

(catheters, implants, prostheses, etc.) associated with impairment of the skin and mucous membrane integrity, which provokes penetration of a wide range of pathogenic microorganisms into a human organism [18]. Biofilm formation on medical materials has caused about 100000 deaths since 2000 in the USA alone [19]. According to the data of the World Health Organization, over 30 million patients in the USA use dentures and 80% of them suffer from chronic inflammatory processes of the oral cavity and concomitant diseases of the cardiovascular and digestive systems [20]. Medical personnel pays particular attention to prevention of colonization of biotic and abiotic surfaces by bacteria of the genera *Escherichia*, *Proteus*, *Staphylococcus*, *Pseudomonas*, *Enterobacter*, and yeasts of the genus *Candida*, since they provoke intoxications, pneumonias, purulent septic processes, candidosis, etc. [2]. The multiresistant bacterium *E. coli* EAEC was recently shown to be responsible for most cases of associated urogenital infections [21]. The virulence factor of this strain is the aggregated pili and adhesin proteins; moreover, the strain forms its own biofilm on different surfaces including catheters [21].

In recent years, the studies on potential medical application of microbial surfactants as antiadhesive agents have become numerous [4, 6, 15, 22, 23]. The literature data [4] demonstrate that the mechanism of antiadhesive action of microbial surfactants may be determined by enhanced permeability of the cell membrane, as well by changing cell surface charge and, as a consequence, impairment of biological functions of the cells.

The data of Table 1 show that adhesion of yeasts and bacteria to the silicon surface of urogenital catheters depended both on the type of surfactant and the degree of its purification and concentration, as well as on the type of test cultures, which was in agreement with the literature data [15, 22, 24, 25].

Adhesion of microorganisms to certain surfaces is known to depend on the nature of their surface structures and the properties of the material [24]. In the work [22] fraction-1 of the lipopeptide surfactants from *B. licheniformis* V9T14 at a concentration of 0.08 mg/mL was shown to inhibit the adhesion of *E. coli* CFT073 to polystyrene plates by 50%, while fraction-2 (at the same concentration) inhibited it by 90–95%. A 100% decrease in adhesion of *E. coli* CFT073 cells was obsreved in the presence of two fractions of lipopeptides from *B. subtilis* V19T21 (35 μg/mL) [22]. Rufisan (0.75 mg/L) inhibited adhesion of *E. coli*, *P. aeruginosa*, and *C. albicans* to polystyrene to 92, 87, and 92%, respectively; when the surfactant concentration increased to 12 mg/L, adhesion decreased to 50–70% [15]. When the concentration of the surfactant from *Trichosporon montevideense* CLOA72 decreased from 16 to 0.5 mg/mL, adhesion of *C. albicans* CC decreased from 13 to 98% [25].

Our studies (Table 1) revealed that preparation 2 from *N. vaccinii* IMB B-7405 (surfactant solution) was a more effective antiadhesive agent than preparation 1 (supernatant): adhesion of the yeast *C. albicans* D-6 and most bacteria (except for *P. vulgaris* BT-13) after the treatment of catheter surface with preparation 2

was lower by 20–30% on the average than after the treatment with preparation 1. A more substantial difference (40–60%) was shown for *P. aeruginosa* P-55 adhesion to the catheters treated with preparations 1 and 2 from *N. vaccinii* IMB B-7405 (Table 1). At the same time, after treatment of the catheter surfaces with both preparation 1 and preparation 2 from *R. erythropolis* IMB Ac-5017, adhesion of most test cultures under study (except for *P. aeruginosa* P-55) was actually the same (Table 1).

Similar patterns were demonstrated for surfactant preparations 1 and 2 from *A. calcoaceticus* IMB B-7241, which actually had the same effect on the attachment of test microorganisms to catheter surfaces. Figure 1 shows the dependence of *E. coli* IEM-1 and *C. albicans* D-6 adhesion on surfactant concentration in preparation 2 from *A. calcoaceticus* IMB B-7241. The results demonstrate the high antiadhesive activity of low concentrations of this preparation: bacterial and yeast adhesion to catheter surfaces was only 10–15% at a surfactant concentration of 0.018 mg/mL.

It is known [26] that the surfactants from *P. aeruginosa* DS10-129 decreased the number of bacterial and yeast cells (including *C. albicans* GBJ 13/4A) attached to the silicon surface of urethral catheters by 30–40% at a rather high concentration (4 mg/mL).

The results presented in Table 2 show that preparations 2 from the strains IMB B-7405, IMB Ac-5017 and IMB B-7241 at low concentrations (0.03–0.12 mg/mL) efficiently (by 60–80%) decreased adhesion of *E. coli* IEM-1 and *C. albicans* D-6 to denture silicon base and acrylic material. It should be noted that the antiadhesive properties of preparations 2 from all strains under study actually did not differ from those of preparations 1.

Investigation of the effect of preparation 1 from *R. erythropolis* IMB Ac-5017 (0.12 mg/mL) on attachment of *P. vulgaris* BT-1, *S. aureus* BMS-1, *P. aeruginosa* P-55, and *E. cloaceae* AC-22 cells to denture materials showed that their adhesion decreased by 50–85% after the treatment with surfactants (Fig. 2).

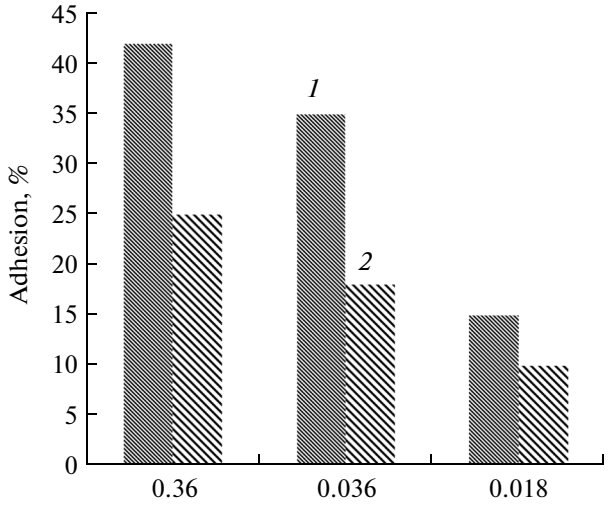

Fig. 1. Adhesion of *E. coli* IEM-1 (*1*) and *C. albicans* D-6 (*2*) to urogenital catheter silicon surfaces after their treatment with the surfactant from *A. calcoaceticus* IMB B-7241 (preparation 2) at different concentrations.

The work [23] showed that the treatment of denture materials with surfactants of plant origin from *Robinia pseudoacacia* (AC5, AC7) and *Nerium oleander* (OC5) (78.1–321.5 μg/mL) was accompanied by a decrease in the number of adherent cells of *C. albicans*. The most efficient antiadhesive agent proved to be the AC5 preparation (312.5 μg/mL), which inhibited adhesion of the yeast test culture to the silicon and acrylic surfaces of dental prostheses by 45 and 40%, respectively. Importantly, the number of cells attached to the silicon surface increased by 20% at a surfactant concentration of 78.1 μg/mL. Thus, the tested microbial surfactants at lower concentrations were more efficient antiadhesive agents compared to those described in [23].

Table 2. Microbial adhesion to dentures after the treatment with surfactant solutions (preparations 2) from *A. calcoaceticus* IMB B-7241, *R. erythropolis* IMB Ac-5017, and *N. vaccinii* IMB B-7405

Surfactant producer	Concentration, mg/mL	Adhesion, %			
		silicon base		acrylic dental material	
		C. albicans D-6	*E. coli* IEM-1	*C. albicans* D-6	*E. coli* IEM-1
N. vaccinii IMB B-7405	0.12	75	60	52	47
	0.06	50	49	33	45
	0.03	21	40	27	20
R. erythropolis IMB Ac-5017	0.25	82	85	67	61
	0.12	30	43	33	40
	0.06	79	90	57	78
A. calcoaceticus IMB B-7241	0.36	13	12	15	10
	0.036	32	34	20	24
	0.018	79	19	85	42

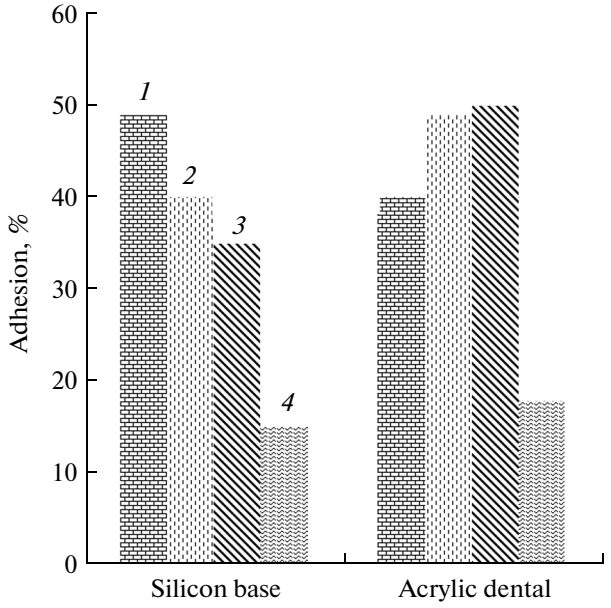

Fig. 2. Effect of preparation 1 (supernatant, surfactant concentration 0.12 mg/mL) from *R. erythropolis* IMB Ac-5017 on bacterial adhesion to denture materials: *P. vulgaris* BT-13 (*1*), *P. aeruginosa* P-55 (*2*), *E. cloaceae* AS-22 (*3*), and *S. aureus* BMS-1 (*4*).

Effects of the surfactants on attachment of microorganisms to manufactured abiotic surfaces. A wide range of materials is presently used in various branches of industry, such as Dutch tile, steel, plastic, and linoleum (polyvinylchloride). Biofilm formation by microorganisms contaminating food staples, revetment and coating of production areas, and packaging materials in the food industry is a considerable problem [27]. It is known that most synthetic disinfectants do not penetrate deep into the biofilm; hence, disinfection is only partial (only the upper biofilm layer is destroyed) [28]. Microbial surfactants change the surface charge and, consequently, the cells do not adhere to the material treated with these agents [28].

Effects of the surfactant preparations from *A. calcoaceticus* IMB B-7241, *R. erythropolis* IMB Ac-5017, and *N. vaccinii* IMB B-7405 on attachment of the vegetative cells of *B. subtilis* BT-2 to plastic, polyvinylchloride, Dutch tile and steel were investigated at the next stage of our work (Table 3). The most efficient antiadhesive agents were preparations 1 and 2 from *A. calcoaceticus* IMB B-7241, which decreased adhesion of *B. subtilis* BT-2 to all tested surfaces at a low concentration (0.005 mg/mL) by 80–90 and 65–80%, respectively. Treating the materials with preparations 1 and 2 from *N. vaccinii* IMB B-7405 (0.01 mg/mL) resulted in the number of adherent BT-2 cells decreasing by 60–80 and 70–85%, respectively (Table 3). In contrast to preparations 1 and 2 from *A. calcoaceticus* IMB B-7241 and *N. vaccinii* IMB B-7405 with the similar antiadhesive properties, preparation 1 from *R. erythropolis* IMB Ac-5017 showed higher antiadhesive activity than preparation 2. Moreover, the 60–90% decrease in *B. subtilis* BT-2 adhesion was observed at a higher surfactant concentration in preparation 1 from the strain IMB Ac-5017 compared to the preparations from the strains IMB B-7241 and IMB B-7405 (0.06–0.25 and 0.005–0.01 mg/mL, respectively) (Table 3).

Further experiments showed that the surfactant preparations from *A. calcoaceticus* IMB B-7241, *R. erythropolis* IMB Ac-5017, and *N. vaccinii* IMB B-7405 decreased the adhesion of *B. subtilis* BT-2 spores to the tested materials, and the degree of adhesion actually did not differ from that for the vegetative cells.

Table 4 shows the data on adhesion of *E. coli* IEM-1 and *C. albicans* D-6 cells to abiotic surfaces treated with preparations 2 from the strains IMB B-7241, IMB B-7405, and IMB Ac-5017. The minimal adhesion of the tested bacteria and yeasts (20–36%) was observed after the surfaces were treated with the surfactant preparations from *A. calcoaceticus* IMB B-7241 and *N. vaccinii* IMB B-7405, respectively, at a concentration of 0.005–0.01 mg/mL. Preparation 2 from *R. erythropolis* IMB Ac-5017 was characterized by less marked antiadhesive properties: adhesion decreased by 20–40% at a surfactant concentration in the preparation of 0.06–0.12 mg/mL.

It is known [29] that the glycolipid surfactant from *P. fluorescens* BD5 (0.25 mg/mL) decreased the adhesion of *E. coli* ATCC 25922 and *C. albicans* SC5314 to polypropylene by 35 and 2%, respectively, compared to untreated surface.

Our further studies showed that preparations 1 and 2 from *R. erythropolis* IMB Ac-5017 (0.06–0.12 mg/mL) decreased the number of *S. aureus* BMS-1 and *P. aeruginosa* P-55 cells attached to plastic and steel by 60–85 and 40–80%, respectively (Table 5). The antiadhesive properties of preparations 1 and 2 were almost the same.

Scientific literature [30] demonstrates the intensive search of agents able to decrease adhesion of microbial cells to microporous materials (plastics). It was shown that the number of adherent cells of *Listeria monocytogenes* ATCC 19112, *S. aureus* ATCC 25923, *Micrococcus luteus* ATCC 4698, *L. monocytogenes* ATCC 1911225, *S. aureus* ATCC 25923, *M. luteus* ATCC 4698, *L. monocytogenes* ATCC 191124, *S. aureus* ATCC 25923, and *M. luteus* ATCC 4698 was 20–30% after the treatment of plastic surfaces with the solution of purified surfactants synthesized by *P. aeruginosa* LBI (0.4 mg/mL) [30].

Investigation of the effects of preparations 2 from *A. calcoaceticus* IMB B-7241 and *N. vaccinii* IMB B-7405 at a concentration of 0.009 mg/mL on attachment of the micromycete *A. niger* P-3 cells to plastic, polyvinylchloride, Dutch tile, and steel showed that they had nearly the same antiadhesive

Table 3. Effects of surfactant preparations from *A. calcoaceticus* IMB B-7241, *R. erythropolis* IMB Ac-5017, and *N. vaccinii* IMB B-7405 on adhesion of *B. subtilis* BT-2 vegetative cells to abiotic surfaces

Surfactant producer	Preparation	Concentration, mg/mL	Adhesion, %			
			plastic	polyvinyl chloride	dutch tile	steel
IMB B-7241	1 (supernatant)	0.009	38	45	32	39
		0.005	15	20	14	12
		0.003	28	35	23	24
	2 (surfactant solution)	0.009	41	48	48	45
		0.005	23	35	35	33
		0.003	31	40	41	37
IMB Ac-5017	1 (supernatant)	0.25	23	25	40	47
		0.12	18	41	25	45
		0.06	10	67	12	66
	2 (surfactant solution)	0.25	75	55	70	53
		0.12	75	50	65	67
		0.06	70	47	57	82
IMB B-7405	1 (supernatant)	0.02	63	38	47	55
		0.01	40	30	36	22
		0.005	53	40	51	64
	2 (surfactant solution)	0.02	43	35	36	48
		0.01	26	27	14	17
		0.005	40	38	30	50

Table 4. Effects of surfactant solutions (preparations 2) from *A. calcoaceticus* IMB B-7241, *R. erythropolis* IMB Ac-5017, and *N. vaccinii* IMB B-7405 on *E. coli* IEM-1 and *C. albicans* D-6 adhesion to abiotic surfaces

Surfactant producer	Concentration, mg/mL	Adhesion (%) to the surface of							
		plastic		polyvinylchloride		Dutch tile		steel	
		E. coli IEM-1	*C. albicans* D-6	*E. coli* IEM-1	*C. albicans* D-6	*E. coli* IEM-1	*C. albicans* D-6	*E. coli* IEM-1	*C. albicans* D-6
IMB B-7241	0.009	52	33	48	36	43	40	49	42
	0.005	28	28	30	25	28	30	36	32
	0.003	59	37	59	39	51	43	45	45
IMB Ac-5017	0.25	80	84	65	80	75	23	88	90
	0.12	70	80	70	83	78	46	75	85
	0.06	63	80	68	80	50	63	87	85
IMB B-7405	0.02	34	42	30	22	36	35	42	35
	0.01	23	25	20	26	25	24	26	30
	0.005	38	32	40	29	47	43	53	43

effects: adhesion to all materials was 50–65% of the control (Fig. 3). On the other hand, after the surfaces were treated with preparation 2 from the strain IMB Ac-5017 (0.06 mg/mL), the number of attached cells of *A. niger* P-3 decreased only by 10–20%. A similar patterns were also observed for the cells of *F. culmorum* T-7: the degree of cell adhesion was 60–65% after the treatment of tested materials with preparations 2 from *A. calcoaceticus* IMB B-7241 and *N. vaccinii* IMB B-7405 (0.009 mg/mL) and 80–90% of the control after their treatment with the preparation from *R. erythropolis* IMB Ac-5017 (0.06 mg/mL).

Note that preparations 3 (the aqueous phase after surfactant extraction) of all tested strains also possessed antiadhesive properties, albeit less marked compared to preparations 1 and 2. After extraction of the surfactants, the supernatant was shown to have no surfactant properties, suggesting the absence of surfactants in preparations 3. Obviously, *A. calcoaceticus* IMB B-7241, *R. erythropolis* IMB Ac-5017, and *N. vaccinii* IMB B-7405 synthesize other extracellular

Table 5. Adhesion of *S. aureus* BMS-1 and *P. aeruginosa* P-55 to plastic and steel surfaces after treatment with the surfactant preparations from *R. erythropolis* IMB Ac-5017

Surfactant preparation	Concentration, mg/mL	Adhesion (%) to the surface of			
		plastic		steel	
		S. aureus BMS-1	*P. aeruginosa* P-55	*S. aureus* BMS-1	*P. aeruginosa* P-55
1 (supernatant)	0.12	12	15	20	48
	0.06	32	39	45	60
2 (surfactant solution)	0.12	20	25	25	48
	0.06	30	35	50	57

metabolites with antiadhesive properties, different from the lipid surfactants. Our further research will be devoted to elucidation of this problem.

The differences in antiadhesive properties of the tested surfactants (see Tables 1–4 and Fig. 3) may result from the differences in their chemical composition [9]. The presence of amino lipids in the complex of surfactants synthesized by *A. calcoaceticus* IMB B-7241 may be the cause of their higher antiadhesive activity. It is known [6, 22] that lipopeptides synthesized by the representatives of *Bacillus* and *Pseudomonas* are efficient antimicrobial and antiadhesive agents. Note that the surfactants from *A. calcoaceticus* IMB B-7241 and *N. vaccinii* IMB B-7405 demonstrated rather high antiadhesive activity at concentrations lower by orders of magnitude than those described in the literature [22, 23, 25, 26, 29, 30].

Thus, the surfactant preparations from *A. calcoaceticus* IMB B-7241, *R. erythropolis* IMB Ac-5017, and *N. vaccinii* IMB B-7405 with different degrees of purification (both as supernatant culture liquid and as a solution of extracted surfactants) can be used for development of highly efficient preparations, decreasing microbial adhesion to the surface of different materials. Note that it would be more economically expedient to use preparation 1 (supernatant) because the technology of its production presupposes no additional stages of isolation and purification.

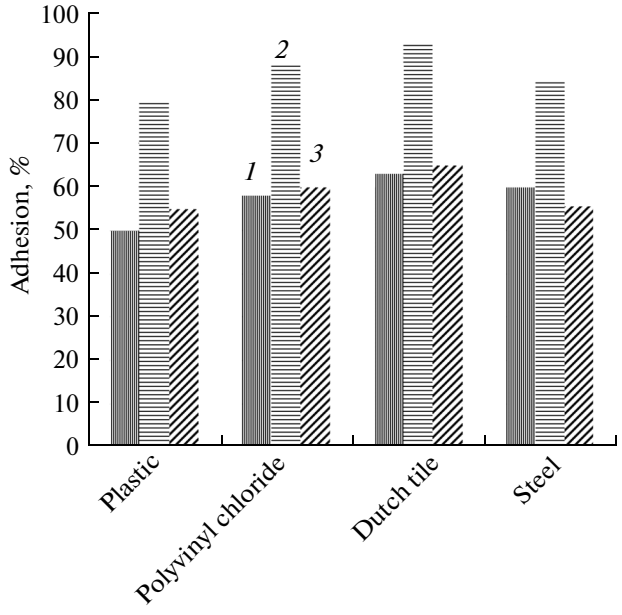

Fig. 3. Adhesion of *A. niger P*-3 to abiotic surfaces treated with surfactant solutions (preparations 2) from *A. calcoaceticus* IMB B-7241 (0.009 mg/mL) (*3*), *R. erythropolis* IMB Ac-5017 (0.06 mg/mL) (*2*), and *N. vaccinii* IMB B-7405 (0.009 mg/mL) (*1*).

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