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THE EFFECT OF *ACINETOBACTER CALCOACETICUS* IMV B-7241 SURFACTANTS ON MICROBIAL ADHESION TO ABIOTIC SURFACES

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Abstract. We have studied the effect of surface-active substances (SAS, surfactants) of Acinetobacter calcoaceticus IMV B-7241 with various degree of purification (the supernatant of culture liquid, the solution of SAS, 0,001-0,036 mg/ml) for the attachment of bacteria and fungi to abiotic surfaces. The degree of adhesion of the test-cultures depended on the material's type and the concentration of SAS in the preparations. The preparation 1 (supernatant) with the concentration of SAS 0,005-0,009 mg/ml was the more effective: after treatment of the abiotic materials with this preparation the number of attached cells of bacteria and fungi decreased on average 45-60 %.

Keywords: Acinetobacter calcoaceticus IMV B-7241; adhesion; surfactants; abiotic materials.

ВЛИЯНИЕ ПОВЕРХНОСТНО-АКТИВНЫХ ВЕЩЕСТВ *ACINETOBACTER CALCOACETICUS* ИМВ В-7241 НА АДГЕЗИЮ МИКРООРГАНИЗМОВ К АБИОТИЧЕСКИМ ПОВЕРХНОСТЯМ

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Аннотация. Исследовано влияние поверхностно-активных веществ (ПАВ) Acinetobacter calcoaceticus ИМВ В-7241 различной степени очистки (супернатант культуральной жидкости, раствор ПАВ, 0,001-0,036 мг/мл) на прикрепление некоторых бактерий и грибов к абиотическим поверхностям. Степень адгезии тест-культур зависела от типа материала и концентрации ПАВ в препаратах. Более эффективным оказался препарат 1 (супернатант) с концентрацией ПАВ 0,005-0,009 мг/мл, после обработки которым наблюдали уменьшение количества прикрепленных клеток бактерий и грибов в среднем на 45-60 %.

Ключевые слова: Acinetobacter calcoaceticus ИМВ В-7241; адгезия; поверхностно-активные вещества; абиотические материалы.

Introduction. 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 [1]. It is known that most synthetic disinfectants do not penetrate deep into the biofilm; hence, disin-?ection is only partial (only the upper biofilm layer is destroyed. Microbial surfactants change the surface charge and, consequently, the cells do not adhere to the material treated with these agents [2].

Previously, we isolated oiloxidizing bacteria from oilcontaminated soil samples, which were identified as Acinetobacter calcoaceticus K-4 [3]. This strain registered at the Depository of Microorganisms of the Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, under accession numbers IMB B-7241.

The ability of *A. calcoaceticus* IMV B-7241 to synthesize the low molecular weight surface-active substances on the hydrophobic and hydrophilic substrates was established. It was shown that surfactants of IMV B-7241 strain are complex of glyco-, amino and neutral lipids [3].

Previously [4] it was found that the SAS of *A. calcoaceticus* IMV B-7241 decreased the number of the attached to medical materials cells of certain bacteria and fungi.

The purpose of this work is to investigate the effect of *A. calcoaceticus* IMV B-7241 surfactants on the microbial adhesion to the abiotic surfaces.

Research methods. *A. calcoaceticus* IMV B-7241 was grown in liquid mineral medium containing the following (g/l): NaCl – 1,0; Na_2HPO_4 – 0,6; $(NH_2)_2CO$ – 0,35; KH_2PO_4 – 0,14; MgSO4·7H2O – 0,1; 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). Ethanol at a concentration of 2 % (vol/vol) was used as a carbon source. Culture in the exponential growth phase cultivated in the respective liquid media containing 1% (vol/vol) of the

substrate were used as inoculum. The amount of inoculum (104–105 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 ℃ for 120 h.

These preparation of surfactants were used in studies:

Preparation 1 – supernatant of culture liquid, to obtain which the culture broth was centrifuged (5000 g, 45 min). The surfactant-containing supernatant was subjected to extraction with the 2:1 chloroform/methanol (Folch) mixture to isolate the surfactant (preparation 2). Extracts were evaporated using an IR-1M2 rotory evaporator (Russia) at 60 °C and absolute pressure of 0,4 atm to a constant mass.

The dry remnant was diluted in sterile phosphate buffer (0,1 M; pH 7,0). The preparations 1 and 2 were sterilized at 112 ℃, 30 min.

The SAS concentration in preparations 1 and 2 was established by the weight technique after extraction with a Folch mixture [3].

The strains of bacteria (Bacillus subtilis БТ-2, Escherichia coli IEM-1) and fungi (Candida albicans Д-6, Aspergillus niger P-3, Fusarium culmorum T-7) used in this work were obtained from the collection of the living microorganisms of the department of biotechnology and microbiology, National university of food technologies.

Antiadhesive properties were investigated as follows: the purified plates of materials (Dutch tile, stainless steel, plastic, polyvinylchloride) of the same size (1 cm^2) were sterilized at 112 ℃ for 40 min and then wer e immersed into the solution of preparations 1-2 and dried for 24 h in a thermostat at 30 ℃. 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 1-2 and untreated (control) samples were placed into the suspension, incubated for 2 h in a thermostat at 30 ℃, and rinsed with 10 mL

of sterile tap water to remove non-adherent cells [5]. Then the degree of cell adhesion was determined by two methods.

Spectrophotometric method [6; 7]. 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 1–2 to the optical density of the control samples (100 %).

Koch's method [8]. 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 1-2 to the cells on the control samples (100 %).

All experiments were performed in triplicate. Statistical analysis of experimental data was performed by Lakin [9]. The differences between the average values were considered reliable at a confidence level $p < 0,05$.

Results and discussion. Adhesion of microorganisms to certain surfaces is known to depend on the nature of their surface structures and the properties of the material [10]. In the work [11] 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) [11].

At the first stage our research was directed on determining antiadhesive properties of preparations 1 and 2 (0,036-0,001 mg/mL) for *B. subtilis* vegetative cells БТ-2 (14 h growth) (Table 1). The results presented in Table 1 show that decline of SAS concentration in preparations 1 and 2 was accompanied by decreasing the adhesion of БТ-2 strain cells.

Our studies (Table 1) revealed that preparation 1 (0,005 mg/mL) was a more effective antiadhesive agent than preparation 2: after the treatment of surfaces with supernatant adhesion of vegetative cells of *B. subtilis* БТ-2 on plastic, polyvinylchloride, Dutch tile and steel was 15, 20, 14 and 12 %, respectively. Further experiments showed that the surfactant preparations from *A. calcoaceticus* IMB B-7241 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 1

The adhesion vegetative cells of *B. subtilis* БТ-2 to abiotic surfaces after the treatment with surfactants of *A. calcoacetius* IMV B-7241

Preparation	Concentration, mg/mL	Adhesion, %			
		Plastic	Polyvinyl-chloride	Dutch tile	Steel
1 (supernatant)	0,036	62	78	76	75
	0,018	57	63	42	70
	0,009	38	45	32	39
	0,005	**15**	**20**	**14**	**12**
	0,003	28	35	23	24
	0,001	33	38	42	39
2 (surfactant solution)	0,036	72	68	59	69
	0,018	46	53	52	49
	0,009	41	48	48	45
	0,005	**23**	**35**	**35**	**33**
	0,003	31	40	41	37
	0,001	38	45	51	44

Note. Tables 1-3: the adhesion error was no more than 5 %.

On the next step the effect of preparation 1 and 2 from *A. calcoaceticus* IMV B-7241 on adhesion cells of *E. coli* IEM-1 to the abiotic materials were investigated (Table 2). Both preparations at low concentrations showed an antiadhesive effect. Preparation 2 (surfactant solution, 0,005 mg/mL) was a more effective antiadhesive agent than preparation 1: the number of attached cells of *E. coli* IEM-1 to abiotic surfaces treated with preparation 2 decreased by 65-70 %.

Table 2

The effect of surfactant preparations from *A. calcoaceticus* IMV B-7241 on attachment of *E. coli* IEM-1 cells to abiotic materials

Preparation	Concentration, mg/mL	Adhesion, %			
		Plastic	Polyvinyl-chloride	Dutch tile	Steel
1 (supernatant)	0,036	79	70	86	92
	0,018	69	58	81	72
	0,009	66	55	72	65
	0,005	**52**	**42**	**48**	**48**
	0,003	86	58	68	88
	0,001	90	88	77	92
2 (surfactant solution)	0,036	66	61	84	79
	0,018	55	52	48	73
	0,009	52	48	43	69
	0,005	**28**	**30**	**26**	**36**
	0,003	59	79	52	56
	0,001	69	82	61	70

Table 3 shows the data on adhesion of C. albicans D-6 cells to abiotic surfaces treated with preparations 1 and 2 from the strains IMB B-7241. After treatment of studied materials with supernatant and surfactant solution (0,005 mg/mL) adhesion of C. albicans D-6 cells was 30-35 %.

Investigation of the effects of preparations 1 and 2 from *A. calcoaceticus* IMB B-7241 at a concentration of 0.009 mg/mL on attachment of the micro-mycete *A. niger* P-3 cells to plastic, polyvinylchloride, Dutch tile, and steel

showed that they had nearly the same antiadhesive effects: adhesion to all materials was 52-62 % (Figure 1).

Table 3

The adhesion of *C. albicans* cells Д-6 to different materials after the treatment with surfactants of *A. calcoacetius* IMV B-7241

Preparation	Concentration, mg/mL	Adhesion, %			
		Plastic	Polyvinyl-chloride	Dutch tile	Steel
1 (supernatant)	0,036	74	70	61	73
	0,018	65	57	51	55
	0,009	39	39	44	49
	0,005	**31**	**28**	**36**	**35**
	0,003	41	45	51	55
	0,001	72	76	78	75
2 (surfactant solution)	0,036	67	55	49	60
	0,018	61	51	46	51
	0,009	33	36	40	42
	0,005	**28**	**25**	**30**	**32**
	0,003	37	39	43	45
	0,001	87	88	85	88

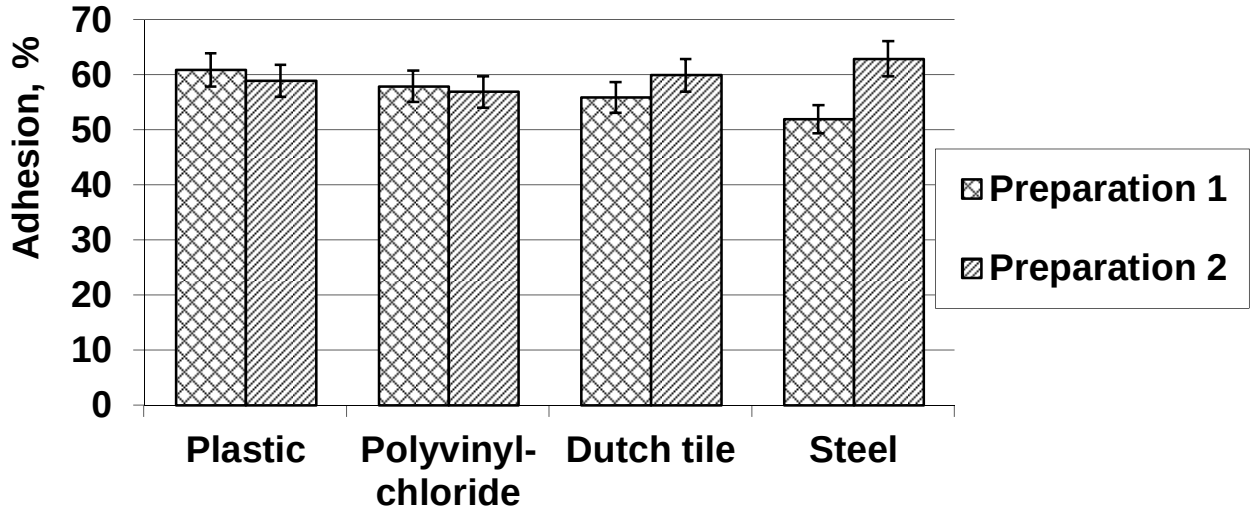

Figure 1. The effect of *A. calcoaceticus* IMV B-7241 surfactants (0,009 mg/mL) on adhesion of cells of *A. niger* P-3 to abiotic surfaces

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 1 and 2 from *A. calcoaceticus* IMB B-7241.

Conclusions. Thus, the surfactant preparations from *A. calcoaceticus* IMB B-7241 with different degrees of purification (both as supernatant of 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.

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