Destruction of biofilms by surfactants synthesized by *Acinetobacter calcoaceticus* IMV B-7241 in the presence of competitive microorganisms

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Abstract

Introduction. The aim of this study was to investigate the role of surfactants synthesized by *Acinetobacter calcoaceticus* IMV B-7241 in media with glycerol in the presence of biological inductors in destruction of biofilms.

Materials and methods. Cultivation of *A. calcoaceticus* IMV B-7241 was carried out in a mineral medium using refined glycerol or crude glycerol, the waste of biodiesel production, as carbon sources. Biological inductors were introduced as live or inactivated cells of *Bacillus subtilis* BT-2, as well as the supernatant after strain BT-2 cultivation. Surfactants were extracted from the supernatant of the culture liquid with a modified mixture of Folch (chloroform and methanol, 2:1). The degree of biofilm destruction in the presence of surfactants was determined by spectrophotometric method.

Results and discussion. Regardless of the substrate used, the introduction of both live and inactivated cells of *B. subtilis* BT-2 into medium used for cultivation of *A. calcoaceticus* IMV B-7241 was accompanied by the synthesis of surfactants, the degree of biofilm destruction of which was higher than those obtained in the medium without an inductor. The degree of destruction of bacterial and yeast biofilms achieved by the action of *A. calcoaceticus* IMV B-7241 surfactants obtained on refined glycerol in the presence of inductor cells was 36.5–85% and was 1.5-3 times higher compared to using surfactants synthesized in medium without inductors. Note that, surfactants synthesized in the presence of biological inductors destroyed biofilms of the test cultures at fairly low (7.5–960 μg/ml) concentrations. Similar results were observed for the usage of surfactants obtained on the waste of biodiesel production. Therefore, introduction of live cells of *B. subtilis* BT-2 into the medium with the crude glycerol was accompanied by synthesis of surfactants, which at concentration 1.8-960 μg/ml caused destruction of *B. subtilis* BT-2, *Proteus vulgaris* PA-12 and *Enterobacter cloacae* C-8 biofilms at 30.1–80.7% and was higher than using similar surfactant concentrations obtained during cultivation without inductors (24.1–75%). The destruction of biofilms of *Staphylococcus aureus* BMS-1, *Candida albicans* D-6 and *Candida tropicalis* PE-2 under the action of surfactants (1.8-960 μg/ml) synthesized on crude glycerol in the presence of both live or inactivated cells of *B. subtilis* BT-2 was 1.5–8 times higher than surfactants synthesized in medium without inductor.

Conclusion. The possibility to regulate the ability to destroy bacterial and yeast biofilms of surfactants synthesized by *A. calcoaceticus I*MV B-7241 by introducing into the medium competitive bacteria *B. subtilis* BT-2 was found.

Introduction

Biofilm formation significantly contributes to microbial survival in hostile environments and it is currently considered a key virulence factor for pathogens responsible for serious chronic infections (Huigens et al., 2019). More than 90% of the studied species of bacteria are able to form biofilms. Biofilm formation was detected in more than 80% of chronic diseases of microbial etiology. Thus, about 60% of all hospital-acquired infections are caused by microorganisms located in biofilms (Parrino et al., 2019). Bacteria capable of forming biofilms are considered a major cause of chronic and acute bacterial infections. For several decades, the bacteria that cause widespread or severe infections have acquired resistance to every new antibiotic that comes on the market (D'Cunha et al., 2018).

Alternative methods for biofilm prevention and/or eradication are urgently required to modify the traditional treatments. The ability of several novel natural antimicrobial compounds (probiotics, bacteriophages, enzymes) to efficiently control biofilm formation has been identified (Algburi et al., 2017). New potential biocides (microbial surfactants, peptides) are actively investigated (Lin et al., 2021).

There is an increased need for recycling of waste products from food, wood industry and agriculture in recent years. For example, crude glycerol which is a side product of biodiesel production because the problem today is the need to dispose of large amounts of it. In recent years, there have been developments to utilize glycerol and convert it to usable biomass (Chmielarz et al., 2021). It was also tested as an additive with other waste substrates to make valuable products (Poladyan et al., 2020).

Increasing the efficiency of microbial surfactant technologies is one of the ways to use cheap industrial waste as a substrate, crude glycerol as waste of biodiesel production, in particular (Salazar-Bryam et al., 2017). The most effective way to dispose of such waste is to use them as substrates in biotechnological processes to obtain practically valuable products (Diamantopoulou et al., 2020).

In addition, there are more papers in the literature devoted to the cultivation of microorganisms in the the presence of biological inductors, the presence of which enhances the biological activity of final metabolites (peptides, bacteriocins, surfactants) and the synthesis of new metabolites with biological activity (Kumar et al., 2021).

It was previously found that *Acinetobacter calcoaceticus* IMV B-7241 synthesizes a complex of surfactants on a wide range of carbon substrates, including glycerol of different degrees of purification (Pirog et al., 2018). A study of the biological activity of surfactants synthesized crude glycerol showed that such surfactants were less effective biofilm destructors compared to those synthesized on purified glycerol.

Previous studies (Pirog et al., 2021) showed the possibility of regulating the antimicrobial activity of the surfactants *A. calcoaceticus* IMV B-7241 by adding into the cultivation medium of cells of competitive bacteria *B. subtilis* BT-2. It is important that the antimicrobial activity of surfactants synthesized on crude glycerol was significantly increased under such cultivation conditions.

Since one of the mechanisms for biofilm destruction under the influence of microbial surfactants is their antimicrobial activity (Sharma et al., 2019), it was suggested that cultivation the producer with competitive bacteria *B. subtilis* BT-2 to allow increase not only antimicrobial activity of surfactants, but also their ability to destroy biofilms.

In connection with the above, the aim of the work is to investigate the role in biofilm destruction of *A. calcoaceticus* IMV B-7241 surfactants synthesized in the presence of biological inductors in medium with glycerol of different degrees of purification.

Materials and methods

Object of research

The main object of research was strain *Acinetobacter calcoaceticus* K-4, registered in the Depository of Microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine under the number IMV B-7241.

Bacterial strains (*Bacillus subtilis* BT-2, *Staphylococcus aureus* BMS-1, *Proteus vulgaris* PA-12, *Enterobacter cloacae* C-8) and yeast (*Candida* D-6, *Candida tropicalis* PE-2) from the collection of live cultures of the Department of Biotechnology and Microbiology of the National University of Food Technologies were used as test cultures in determining the ability of surfactants to destroy bacterial and yeast biofilms.

Medium composition and conditions of cultivation

Strain *A. calcoaceticus* IMV B-7241 was grown in the liquid mineral medium (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. Yeast autolysate – 0.5% (v/v) and microelement solution – 0.1% (v/v) were additionally added into the medium. The micronutrient solution contained (g/100 ml): $ZnSO_4 \cdot 7H_2O$ – 1.1; $MnSO_4 \cdot H_2O$ – 0.6; $FeSO_4 \cdot 7H_2O$ – 0.1; $CuSO_4 \cdot 5H_2O$ – 0.004; $CoSO_4 \cdot 7H_2O$ – 0.03; H_3BO3 – 0.006; KI – 0.0001; EDTA (Trilon B) – 0.5.

As carbon sources used (%, v/v): refined glycerol – 3, crude glycerol – 5. Concentrations of glycerol of different quality are equimolar on carbon.

Culture in the exponential phase was used as an inoculum, grown in a medium of the above composition with 0.5 % of the corresponding substrate. The inoculum with the number of bacteria 10^4–10^5 cells/ml was added in an amount of 10% of the medium volume.

The bacterial strain *Bacillus subtilis* BT-2 was used as a biological inductor and were introduced as live, inactivated cells, as well as the supernatant after BT-2 strain cultivation. At the beginning of the cultivation process, inductor inoculums were added to *A. calcoaceticus* IMV B-7241 culture. *Bacillus subtilis* BT-2, grown on meat-peptone agar for 24 h, was suspended in 100 ml of sterile tap water and 2.5 ml of suspension per 100 ml of surfactant-producing culture medium was added. Inactivated cells (heat treated at 131 °C for 1 hour) were added, 10 ml per 100 ml of culture medium. Cell-free supernatant was added, 2.5 ml per 100 ml of culture medium.

Cultivation of *A. calcoaceticus* IMB B-7241 in the presence of supernatant, live and inactivated *B. subtilis* BT-2 cells and without inductors was carried out in 750 ml flasks with 100 ml of medium on a shaker (320 rpm) at 30 °C for 7 days.

Determination of extracellular surfactant concentration

The amount of extracellular surfactants was determined using our modified Bligh and Dyer method. The surfactants extraction was with a mixture of chloroform and methanol (2:1) from the supernatant of the culture liquid. Then, the culture liquid was centrifuged at 5000 g for 20 min to obtain the cell-free supernatant.

As *A. calcoaceticus* IMB B-7241 synthesizes a complex of non-polar and polar lipids, and the well-known Bligh and Dyer method used for surfactant isolation allows the separation of mainly non-polar lipids, we modified the classical solvent system (Folch mixture) by adding to it 1 M HCl (chloroform – methanol – water = 4:3:2). Such a system allows for maximum separation of both non-polar and polar lipids.

In a 100 ml cylindrical separation glass-stoppered funnel, 25 ml of supernatant was placed, 1 M HCl solution was added until the pH value is reached 4.0–4.5 (about 5 ml), the funnel was covered with a stopper and shaken for 3 min, then 15 ml of chloroform and methanol mixture (2:1) was added and shaken (lipid extraction) for 5 min. The mixture obtained after extraction was left in a separating funnel to separate the phases, after which the lower fraction was drained (organic extract 1) and the aqueous phase was re-extracted. During the second extraction, 1 M HCl solution was added to the aqueous phase to reach a pH 4.0–4.5 (about 5 ml), 15 ml chloroform-methanol mixture (2:1) and the lipids were extracted for 5 min. At the third stage, 25 ml of chloroform-methanol mixture (2:1) was added to the aqueous phase and extracted as described above to obtain organic extract 3. Extracts from 1 to 3 were combined and evaporated on IP-1M2 rotary evaporator at 50 °C and absolute pressure of 0.4 atm to constant weight.

Obtaining surfactant preparations

A. calcoaceticus IMB B-7241 surfactant solutions of various concentrations were used in the research. The dry surfactant residue was dissolved in sterile phosphate buffer (0.1 M, pH 7.0) to the original volume (25 ml) and further diluted with this buffer to the required concentration. The surfactant solutions were sterilized in an autoclave at 112 °C for 30 min.

Study of the degree of the biofilm destruction under the action of surfactants

The effect of surfactants on biofilm degradation was carried out as described in (Allegrone et al., 2021). For obtaining the biofilm formation, 180 µl of meat-peptone broth (MPB) or liquid wort and 20 µl of one-day test culture suspension were added to immunoassay microplates. Then, it was incubated for 24 h at optimal temperature for the test culture, followed by draining the culture liquid, added 180 µl of fresh MPB (liquid wort) and 20 µl of test culture suspension and further incubated for 24 h. This 48-hour cultivation is decent for the formation of a biofilm in the microplate wells. After 48 h, the culture liquid was drained and 200 µl of surfactant preparations (0.005–1.28 mg/ml) were added to the microplate wells (with the test culture biofilm formed on them previously). Sterile tap water (200 µl) was added instead of surfactant preparations to control variants (wells). Wells were washed three times with 200 µl of distilled water after 24 h of exposure and the number of adherent cells was determined spectrophotometrically. The degree of biofilm destruction (%) was determined as the difference between cells adhesion in untreated and surfactant-treated wells of the immunoassay plate.

Statistical analysis

All experiments were performed in 3 replicates, the number of parallel determinations in the experiments was 3–5.

Results and discussion

The functionality of various types of biosurfactants as antibiofilm agents is mainly determined by their types: glycolipids, rhamnolipids, sophorolipids, lipopeptides. Mechanisms of bacterial biofilm degradation differ depending on the type of surfactant (Paraszkiewicz et al., 2021). It is reported that most microbial surfactants can increase cell

surface hydrophobicity and destabilise lipid structure (Ohadi et al., 2020), as one of the mechanisms of biofilm destruction. According to other data, these changes increase the permeability of cell membranes and reduce microbial adhesion to different surfaces (Bionda et al., 2016). According to other studies, microbial surfactants inhibit the expression of bacterial genes involved in biofilm formation (Allegrone et al., 2021). In addition, a paper (Yan et al., 2019) reported a mechanism of anti-biofilm activity against *S. aureus* CMCC 26003 of microbial surfactants synthesized by lactic acid bacteria. Surfactants were found to affect the expression of biofilm-associated genes by interfering with the release of signaling molecules.

Effect of biological inductors in a medium with crude glycerol on the ability of surfactants synthesized by *A. calcoaceticus* IMV B-7241 to destroy biofilms

The degradation of biofilms under the influence of surfactants synthesized by *A. calcoaceticus* IMV B-7241 in the medium with crude glycerol in the presence of live *B. subtilis* BT-2 cells is shown (Table 1).

Table 1

Destruction of biofilms under the action of surfactants synthesized by *A. calcoaceticus* IMV B-7241 in the medium with crude glycerol in the presence of live *B. subtilis* BT-2 cells

Test culture	Presence of inductor in the medium	Destruction of biofilm (%) under the action of surfactants at a concentration (μg/ml)				
		60	120	240	480	960
Bacillus subtilis BT-2	−	50	51	51	52	55
	+	55	55	55	61	65
Enterobacter cloacae C-8	−	65	70	70	71	71
	+	60	70	70	81	81
Proteus vulgaris PA-12	−	52	58	61	66	70
	+	76	77	77	77	77
Candida tropicalis PE-2	−	40	45	52	57	58
	+	53	54	57	62	63
Candida albicans D-6	−	26	30	31	35	38
	+	39	42	48	51	52

Note. When determining the destruction of the biofilm, the error did not exceed 5%.

An increase in the degree of biofilm destruction of bacterial and yeast test cultures was achieved by the action of surfactants synthesized in the presence of live inducer cells in the medium with crude glycerol. The use of inactivated cells or supernatant as inductor was accompanied by the synthesis of surfactants under the influence of which biofilm degradation was the same as preparations synthesized in medium without inductors (Tables 2 and 3). Using surfactant concentrations below 60 μg/ml obtained during cultivation of *A. calcoaceticus* IMV B-7241 on crude glycerol in the presence of live *B. subtilis* BT-2 cells, the destruction of both bacterial and yeast biofilms was not different from that in the action of preparations synthesized without inductors.

A significant difference (9–10 %) in the degradation of *B. subtilis* BT-2 and *E. cloacae* C-8 biofilms in the presence of surfactants synthesized with and without an inductor was observed only when surfactants with the highest concentrations studied (480–960 μg/mL) were used. At the same time, the degree of destruction of *P. vulgaris* PA-12 biofilm by surfactants synthesized by strain IMV B-7241 in medium with inductor was 76–77 %, regardless of surfactant concentration, and was 7–24 % higher than those obtained without inductor. The highest difference (19–24 %) in the degradation of *P. vulgaris* PA-12 biofilm under the influence of surfactants synthesized with and without inductor was observed when using low concentrations of preparations (60–120 μg/ml).

The degree of destruction of yeast biofilms under the action of surfactants synthesized in the presence of *B. subtilis* BT-2 cells was 5–17% higher than that under the influence of preparations obtained without an inductor. In the case of surfactants obtained under cultivation of IMV B-7241 strain with the inductor, the destruction of *C. tropicalis* PE-2 biofilm was rather high, 53–63% in the whole range of surfactant concentrations tested. The degree of destruction of *C. albicans* D-6 biofilm exceeded 50% only under the influence of high concentrations (480–960 μg/ml) of the preparations obtained in the presence of the inductor.

In contrast to other bacterial biofilms (see Table 1), an increasing *S. aureus* BMS-1 biofilm destruction was observed when surfactants synthesized in the presence of all inductors (supernatant, live and inactivated *B. subtilis* BT-2 cells) were used: the degree of biofilm destruction was 10–19% higher than under the action of preparations obtained during *A. calcoaceticus* IMB B-7241 cultivation in medium without inductors (Figure 1).

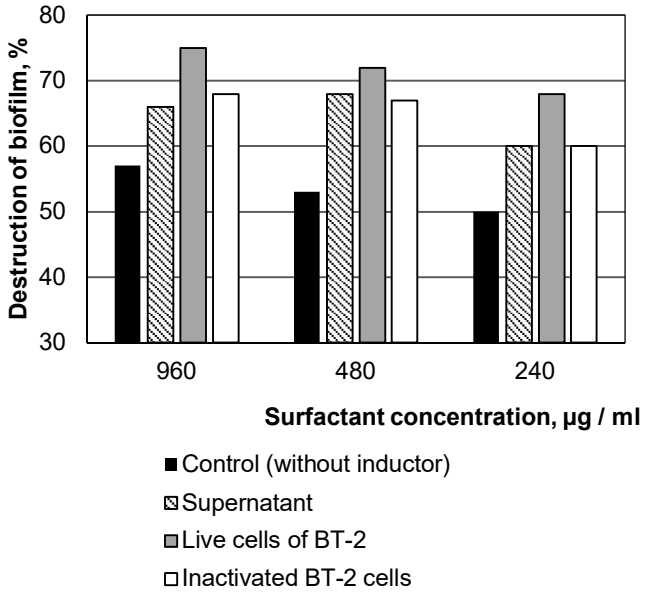

Figure 1. Effect of biological inductors in *A. calcoaceticus* IMV B-7241 in a medium with the waste of biodiesel production on the ability of synthesized surfactants to destroy *Staphylococcus aureus* BMS-1 biofilm

Under the action of lower concentrations of surfactants (7.5–120 μg/ml) synthesized on crude glycerol in the presence of supernatant, live and inactivated *B. subtilis* BT-2 cells, the destruction of *S. aureus* BMS-1 biofilm was at the same level as that caused by surfactants obtained in medium without inductors.

Destruction of biofilms by surfactants synthesized by *A. calcoaceticus* IMV B-7241 on purified glycerol in the presence of biological inductors

The data presented in Table 2 show that in contrast to the cultivation of *A. calcoaceticus* IMV B-7241 on crude glycerol, the growth of surfactant producer on purified glycerol in the presence of both live and inactivated inducer cells as well as supernatant showed the synthesis of surfactants under the influence of which the destruction of bacterial biofilms was on average 10–20% higher compared to the action of preparations synthesized without inductor.

Table 2

Effect of surfactants synthesized by *A. calcoaceticus* IMV B-7241 in the medium with purified glycerol in the presence of *B. subtilis* BT-2 cells on bacterial biofilms destruction

Test culture	Biological inductor	Destruction of biofilm (%) under the action of surfactants at a concentration (μg/ml)					
		240	120	60	30	15	7,5
Bacillus subtilis BT-2	Control	55	54	43	40	38	35
	Supernatant	54	52	41	40	36	36
	Live cells	64	60	58	50	50	48
	Inactivated cells	59	58	50	45	43	40
Staphylococcus aureus BMS-1	Control	38	33	31	30	30	28
	Supernatant	56	52	48	40	34	33
	Live cells	65	65	50	46	40	36
	Inactivated cells	55	53	48	40	35	33
Enterobacter cloacae C-8	Control	55	55	50	40	35	30
	Supernatant	60	60	55	55	45	40
	Live cells	80	75	73	70	70	65
	Inactivated cells	76	68	65	60	55	51
Proteus vulgaris PA-12	Control	38	38	38	36	32	29
	Supernatant	47	45	45	40	40	36
	Live cells	58	55	55	50	43	42
	Inactivated cells	58	52	50	48	40	40

Note. When determining the destruction of the biofilm, the error did not exceed 5%.

The second fundamental difference between surfactants synthesized on purified glycerol in the presence of inductors and those obtained under similar conditions of strain cultivation on crude glycerol is a lower effective concentration ensuring maximum (over 50%) degradation of bacterial biofilms (30–240 and 60–960 μg/ml respectively (Tables 1 and 2). The most effective inductor under *A. calcoaceticus* IMV B-7241 cultivation on purified glycerol was *B. subtilis* BT-2 live cells: the surfactants synthesized in their presence were characterized by a higher ability to destroy most bacterial biofilms than those obtained using inactivated cells or cell-free supernatant. Data about the destruction of yeast biofilms by surfactants synthesized in the presence of inductors are shown in Table 3.

Table 3

Destruction of yeast biofilms by surfactants synthesized by *A. calcoaceticus* IMV B-7241 in the medium with purified glycerol in the presence of live and inactivated *B. subtilis* BT-2 cells

Test culture	Biological inductor	Destruction of biofilm (%) under the action of surfactants at a concentration (μg/ml)					
		240	120	60	30	15	7,5
Candida tropicalis PE-2	Control (without inductor)	43	42	40	39	33	30
	Live cells of *Bacillus subtilis* BT-2	57	57	56	54	53	42
	Inactivated *Bacillus subtilis* BT-2 cells	51	50	45	45	43	43
Candida albicans D-6	Control (without inductor)	41	41	41	36	28	23
	Live cells of *Bacillus subtilis* BT-2	64	60	55	46	46	45
	Inactivated *Bacillus subtilis* BT-2 cells	48	48	42	40	34	30

Note. When determining the destruction of the biofilm, the error did not exceed 5%.

Increased destruction of yeast biofilms by surfactants synthesized by *A. calcoaceticus* IMV B-7241 on purified glycerol in the presence of inductors was observed only when live and inactivated *B. subtilis* BT-2 cells, except supernatant were used as inductors, and besides live cells were been more effective compared to inactivated cells.

The degree of destruction of yeast biofilms by surfactants produced by IMV B-7241 strain in medium with live cells of inductor was 6–16% and 10–23% higher compared to those established for surfactants synthesized with *B. subtilis* BT-2 inactivated cells and without inductor, respectively.

Both bacterial biofilm destruction (see Table 2) and destruction of *C. tropicalis* PE-2 and *C. albicans* D-6 biofilms were at the maximum level (40–64%) when surfactants produced in the presence of an inductor were used at 30–240 μg/ml concentrations. Similar degree destruction of yeast biofilms under the influence of surfactants synthesized in the medium with crude glycerol and inductors was achieved at a higher surfactant concentration (60–960 μg/ml) (Table 1).

Note that, there is limited data in the literature on the ability of surfactants synthesized in the presence of biological inducers (live or inactivated cells, or supernatant) to destroy microbial biofilms.

The work (Kimelman and Shemesh, 2019) showed that in the presence of supernatant after co-cultivation of *B. subtilis* with *Lactobacillus plantarum* the degree of *S. aureus* biofilm destruction reached up to 61%, whereas supernatant after growing *L. plantarum* monoculture inhibited the formation of biofilm by only 40% after 24 h. The authors found that the increasing in biofilm destruction by the supernatant after co-cultivation of the two strains was due to the synthesis of antimicrobial lipopeptides by *Bacillus subtilis* under these conditions.

It was found by other researchers (Hamza et al., 2018) that the supernatant after co-cultivation of *Staphylococcus lentus* SZ2 and *Vibrio harveyi* MTCC 7771 was able to inhibit the biofilm of *V. harveyi* MTCC 7771 pathogen. In addition, the degradation of the *V. harveyi* biofilm after 24 h treatment with the supernatant obtained after *Staphylococcus lentus* SZ2

monoculture cultivation was 40%, while under the action of the supernatant after the strains co-cultivation reached 79%.

In research (Mohamed et al., 2020), two bacterial strains of *Micromonospora* sp. UR56 and *Actinokineospora* sp. EG49 produced new metabolites that were not typical for monocultures. The induced metabolites were phenazine derivatives and *they* showed the ability to destroy biofilms of *B. subtilis*, *S. aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa*. Phenazine at a concentration of 10 µl destroyed 24-hour biofilms of *E. coli* by an average of 54%, *S. aureus* by 50%, *B. subtilis* by 18% and *P. aeruginosa* by 42%.

Previously (Pirog et al., 2020a), it waas found that regardless of the time of introduction of competitive bacteria (*E. coli* IEM-1 and *B. subtilis* BT-2) into medium of *Nocardia vaccinii* IMV B-7405 cultivation and their physiological state (live, inactivated cells) the synthesis of surfactants was observed, after treatment with which the degree of *B. subtilis* BT-2, *S. aureus* BMS-1, *Pseudomonas* sp. MI-2 biofilm destructions were 10–35% higher compared to those established for surfactants obtained in medium without competitive microorganisms.

Our other studies (Pirog et al., 2020b) it was shown that the degree of destruction of bacterial (*B. subtilis* BT-2, *S. aureus* BMS-1, *Pseudomonas* sp. MI-2) and yeast (*C. albicans* D-6 and *Candida utilis* BBC-65) biofilms under the influence of surfactants synthesized by *Rhodococcus erythropolis* IMV Ac-5017 in the presence of live *E. coli* IEM-1 and *B. subtilis* BT-2 cells, reached up to 40-94 % and was higher compared to those surfactants synthesized by IMV Ac-5017 strain in a medium without inductors (32–65%).

To compare the degree of biofilm destruction by our and other well-known microbial surfactants (lipopeptides, rhamnolipids, sophorolipids) synthesized on glycerol, the literature data on the ability of such microbial surfactants to destroy microbial biofilms was analysed.

It is shown that under action of *B. subtilis* VS16 lipopeptides at fairly high concentrations (3000–5000 µg/ml) destruction of *S. aureus* ATCC 29523 biofilms was 67.4%, *E. coli* MTCC 65 – 63.9%, *S. typhimurium* ATCC 19430 – 61.1% (Giri et al., 2019). In addition, in the work (Sen et al., 2020) the authors showed that the destruction of *Trichophyton rubrum* MTCC 8477 and *Trichophyton mentagrophytes* NCCPF 800049 biofilms after treatment with rhamnolipids synthesized by *P. aeruginosa* SS14 reached 80-85% at the surfactant concentration of 2000 and 250 µg/ml, respectively.

In research (Borah et al., 2019) was found that rhamnolipids synthesized by *P. aeruginosa* SS14 on waste of alcohol production reached the highest (90–95%) degree of *C. tropicalis* MTCC 1000 destruction at concentrations of 500–1000 µg/ml, respectively.

The results of our studies showed that the introduction of inductors in *A. calcoaceticus* IMB B-7241 cultivation medium allowed to obtain surfactants that effectively destroy bacterial and yeast biofilms at much lower (several orders of magnitude) concentrations (7.5-480 µg / ml), than described in the literature.

Note that at present we could not find information in the available literature on the increased efficiency of yeast biofilm destruction (including *Candida* yeast) in the presence of microbial metabolites synthesized during co-cultivation of microorganisms.

Conclusion

Consequently, as a result of this work, the possibility of regulating the biological activity of *A. calcoaceticus* IMV B-7241 surfactants has been established by introducing into the cultivation medium the competitive bacteria *B. subtilis* BT-2 cells, which are inductors of synthesis of surfactant with a higher ability to destroy both bacterial and yeast biofilms.

References

Algburi A., Comito N., Kashtanov D., Dicks L., Chikindas M.L. (2017), Control of biofilm formation: antibiotics and beyond, *Applied and Environmental Microbiology Journal*, 83(3), DOI: 10.1128/AEM.02508-16.

Allegrone G., Ceresa C., Rinaldi M., Fracchia L. (2021), Diverse effects of natural and synthetic surfactants on the inhibition of *Staphylococcus aureus* biofilm, *Pharmaceutics*, 13(8), pp. 1172, DOI: 10.3390/pharmaceutics13081172.

Bionda N., Fleeman R.M., Fuente-Núñez C., Rodriguez M.C., Reffuveille F., Shaw L.N., Pastar I., Davis S.C., Hancock R., Cudic P. (2016), Identification of novel cyclic lipopeptides from a positional scanning combinatorial library with enhanced antibacterial and antibiofilm activities, *European Journal of Medicinal Chemistry*, 108, pp. 354–363, DOI: 10.1016/j.ejmech.2015.11.032.

Borah S.N., Sen S., Goswami L., Bora A., Pakshirajan K., Deka S. (2019), Rice based distillers dried grains with solubles as a low cost substrate for the production of a novel rhamnolipid biosurfactant having anti-biofilm activity against *Candida tropicalis*, *Colloids and surfaces. B, Biointerfaces*, 182, pp. 110358, DOI: 10.1016/j.colsurfb.2019.110358.

Chmielarz M., Blomqvist J., Sampels S., Sandgren M., Passoth V. (2021), Microbial lipid production from crude glycerol and hemicellulosic hydrolysate with oleaginous yeasts. *Biotechnology for Biofuels*, 14(1), pp. 65, DOI: 10.1186/s13068-021-01916-y.

Diamantopoulou P., Filippousi R., Antoniou D., Varfi E., Xenopoulos E., Sarris D., Papanikolaou S. (2020), Production of added-value microbial metabolites during growth of yeast strains on media composed of biodiesel-derived crude glycerol and glycerol/xylose blends, *FEMS Microbiology Letters*, 367(10), DOI: 10.1093/femsle/fnaa063.

D'Cunha N.M., Peterson G.M., Baby K.E., Thomas J. (2018), Impetigo: a need for new therapies in a world of increasing antimicrobial resistance, *Journal of Clinical Pharmacy and Therapeutics*, 43(1), pp. 150–153, DOI: 10.1111/jcpt.12639.

Giri S.S., Ryu E., Sukumaran V., Chang Park S. (2019), Antioxidant, antibacterial, and antiadhesive activities of biosurfactants isolated from Bacillus strains, *Microbial Pathogenesis*, 132, pp. 66−72, DOI: 10.1016/j.micpath.2019.04.035.

Hamza F., Kumar A.R., Zinjarde S. (2018), Coculture induced improved production of biosurfactant by *Staphylococcus lentus* SZ2: Role in protecting *Artemia salina* against *Vibrio harveyi*, *Enzyme and Microbial Technology*, 114, pp. 33–39, DOI: 10.1016/j.enzmictec.2018.03.008.

Huigens R.W., Abouelhassan Y., Yang H. (2019), Phenazine antibiotic-inspired discovery of bacterial biofilm-eradicating agents, *Chembiochem: a European journal of chemical biology*, 20(23), pp. 2885–2902, DOI: 10.1002/cbic.201900116.

Kimelman H., Shemesh M. (2019), Probiotic bifunctionality of *Bacillus subtilis*—rescuing lactic acid bacteria from desiccation and antagonizing pathogenic *Staphylococcus aureus*, *Microorganisms*, 7, 407, DOI: 10.3390/microorganisms7100407.

Kumar A., Singh S.K., Kant C., Verma H., Kumar D., Singh P.P., Modi A., Droby S., Kesawat M. S., Alavilli H., Bhatia S.K., Saratale G.D., Saratale R.G., Chung S.M., Kumar M. (2021), Microbial biosurfactant: a new frontier for sustainable agriculture and pharmaceutical industries, *Antioxidants (Basel, Switzerland)*, 10(9), pp. 1472, DOI: 10.3390/antiox10091472.

Lin Z., Yuan T., Zhou L., Chen, S., Qu X., Lu P., Feng Q. (2021), Impact factors of the accumulation, migration and spread of antibiotic resistance in the environment, *Environmental Geochemistry and Health*, 43(5), pp. 1741−1758, DOI: 10.1007/s10653-020-00759-0.

Mohamed S.H., Hossam M., Mohammed R., Mohamed M.F., Ahmed S.M., Ahmed A.H., Sameh F.A., Mostafa E.R., Hani A.A., Ramadan A.U. (2020), Induction of antibacterial metabolites

by co-cultivation of two red-sea-sponge-associated *Actinomycetes micromonospora* sp. UR56 and *Actinokinespora* sp. EG49, *Marine Drugs*, 18(5), pp. 243, DOI: 10.3390/md18050243.

Ohadi M., Forootanfar H., Dehghannoudeh G., Eslaminejad T., Ameri A., Shakibaie M., Adeli-Sardou M. (2020), Antimicrobial, anti-biofilm, and anti-proliferative activities of lipopeptide biosurfactant produced by *Acinetobacter junii* B6, *Microbial Pathogenesis*, 138, DOI: 10.1016/j.micpath.2019.103806.

Paraszkiewicz K., Moryl M., Płaza G., Bhagat D., Satpute S., Bernat P. (2021), Surfactants of microbial origin as antibiofilm agents, *International Journal of Environmental Health Research*, 31(4), pp. 401–420, DOI: 10.1080/09603123.2019.1664729

Parrino B., Schillaci D., Carnevale I., Giovannetti E., Diana P., Cirrincione G., Cascioferro S. (2019), Synthetic small molecules as anti-biofilm agents in the struggle against antibiotic resistance, *European Journal of Medicinal Chemistry*, 161, pp. 154–178, DOI: 10.1016/j.ejmech.2018.10.036.

Pirog T.P., Lutsai D.A., Shevchuk T.A., Iutynska G.O., Elperin I.V. (2018), Antimicrobial and anti-adhesive activity of surfactants synthesized by *Acinetobacter calcoaceticus* IMV B-7241 on technical glycerol, *Mikrobiologichny Zhurnal*, 80(2), pp. 14–27, DOI: 10.15407/microbiolj80.02.014.

Pirog T.P., Skrotska O.I., Shevchuk T.A. (2020a), Influence of biological inducers on antimicrobial, antiadhesive activity and biofilm destruction by *Nocardia vaccinii* IMV V-7405 surfactants, *Mikrobiologichny Zhurnal*, 82(3), pp. 35-44, DOI: https://doi.org/10.15407/microbiolj82.03.035.

Pirog T., Kluchka L., Skrotska O., Stabnikov V. (2020b), The effect of co-cultivation of *Rhodococcus erythropolis* with other bacterial strains on biological activity of synthesized surface-active substances, *Enzyme and Microbial Technology,* 142, 109677, DOI: 10.1016/j.enzmictec.2020.109677.

Pirog T., Ivanov M., Yarova H. (2021), Antimicrobial activity of *Acinetobacter calcoaceticus* IMV B-7241 surfactants, synthesized in the presence of biological inductors, *Scientific Works of NUFT*, 27(4), pp. 43-52.

Poladyan A., Blbulyan S., Semashko T., Dziameshka V., Zhukouskaya L., Trchounian A. (2020), Application of organic waste glycerol to produce crude extracts of bacterial cells and microbial hydrogenase-the anode enzymes of bio-electrochemical systems, *FEMS Microbiology Letters*, 367(7), DOI: 10.1093/femsle/fnaa056.

Salazar-Bryam A.M., Lovaglio R.B., Contiero J. (2017), Biodiesel byproduct bioconversion to rhamnolipids: Upstream aspects, *Heliyon*, 3(6), DOI: 10.1016/j.heliyon.2017.e00337.

Sen S., Borah S.N., Bora A., Deka S. (2020), Rhamnolipid exhibits anti-biofilm activity against the dermatophytic fungi *Trichophyton rubrum* and *Trichophyton mentagrophytes*, *Biotechnology Reports*, 27, DOI: 10.1016/j.btre.2020.e00516.

Sharma D., Misba L., Khan A.U. (2019), Antibiotics versus biofilm: an emerging battleground in microbial communities, *Antimicrobial Resistance and Infection Control*, 8(76), DOI: 10.1186/s13756-019-0533-3.

Yan X., Gu S., Cui X., Shi Y., Wen S., Chen H., Ge J. (2019), Antimicrobial, anti-adhesive and anti-biofilm potential of biosurfactants isolated from *Pediococcus acidilactici* and *Lactobacillus plantarum* against *Staphylococcus aureus* CMCC26003, *Microbial pathogenesis*, 127, pp. 12–20, DOI: 10.1016/j.micpath.2018.11.039.