Effect of competitive eukaryotic microorganisms on anti-adhesive activity of *Acinetobacter calcoaceticus* IMV B-7241 surfactants

Tetyana Pirog[1,2], Mykyta Ivanov[1]

1 – National University of Food Technologies, Kyiv, Ukraine
2 – D.K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine, Kyiv, Ukraine

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Corresponding author:

Tetyana Pirog
E-mail: tapirog@nuft.edu.ua

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Abstract

Introduction. The aim of the study was to investigate the effect of eukaryotic inductors on the antiadhesive activity and biofilm destruction ability of the microbial surfactants synthesized by *Acinetobacter calcoaceticus* IMV B-7241.

Materials and methods. Cultivation of *A. calcoaceticus* IMV B-7241 was carried out in liquid mineral media containing purified glycerol or waste of biodiesel production (crude glycerol) as substrates. The biological inductors were live and inactivated cells of the yeast *Saccharomyces cerevisiae* BTM-1, as well as the supernatant after cultivation of the BTM-1 strain. Microbial surfactants were extracted from the culture supernatant using a modified Folch mixture. The anti-adhesive activity and the degree of biofilm destruction were determined by spectrophotometric method.

Results and discussion. Surfactants synthesized in the presence of all the studied inducers in a medium with glycerol of different quality were more effective anti-adhesive agents compared to surfactants obtained without an inductor. Thus, the adhesion of *Proteus vulgaris* PA-12, *Bacillus subtilis* BT-2 and *Candida albicans* D-6 on steel, tiles and linoleum treated with surfactant solutions synthesized by introducing into the medium with both substrates of all types of inductors was on average 10-70% lower than under the action of surfactants formed without an inductor. The introduction of live *S. cerevisiae* BTM-1 cells and the corresponding supernatant into a medium with glycerol of different degrees of purification was accompanied by the formation of surfactants, under the influence of which the destruction of biofilms of bacterial test cultures was on average 5-30% higher compared to the use of surfactants synthesized without an inductor. An increase of 12-35% in the destruction of *Candida* yeast biofilms was observed only under the action of surfactants synthesized in the presence of an inductor in a medium with waste of biodiesel production.

Conclusion. The results demonstrate the potential to regulate the anti-adhesive activity of *A. calcoaceticus* IMV B-7241 surfactants and their ability to destroy biofilms by adding live or inactivated *S. cerevisiae* BTM-1 yeast cells and the corresponding supernatant to the producer culture medium.

Introduction

In the modern world, increasing attention is being paid to the study of interactions between different microorganisms, particularly the impact of co-cultivation on the biosynthesis of various metabolites. Examples of such metabolites include antibiotics, immunosuppressive agents, anticancer medicines, and other bioactive compounds synthesized by microbial cultures. In the context of current biotechnological research, much focus is placed on the potential of microbial surfactants due to their unique antimicrobial, anti-adhesive properties, and ability to disrupt biofilms (Hamza et al., 2018). To date, most studies on the regulation of biological activity of microbial surfactants have utilized prokaryotic inductors, and there are only a few reports on the impact of competitive eukaryotic microorganisms on the synthesis and biological activity of surfactants (Song et al., 2020).

It was found that co-cultivation of surfactant-producing microorganisms with competitive microorganisms can significantly increase the antimicrobial, anti-adhesive and biofilm-destroying activity of the synthesized metabolites (Pirog et al., 2023a). Our earlier results demonstrated that the presence of live and inactivated cells of *Bacillus subtilis* BT-2 in the culture medium of *Acinetobacter calcoaceticus* IMV B-7241 provided a significant increase in the antimicrobial and antiadhesive activity of surfactants (Pirog & Ivanov, 2022a; Pirog et al., 2023a). It is important to mention that the effect of biological inductors on synthesis and biological activity depends on the type of microorganism and its physiological state.

Recently, there has been a growing number of publications highlighting the positive impact of eukaryotic microorganisms, particularly yeast, on the ability of prokaryotic microorganisms to synthesize metabolites with antimicrobial activity (Bai et al., 2023; Shen et al., 2024). However, there is no information in the literature on the use of eukaryotic cells as inductors to increase the antiadhesive properties of secondary metabolites or the destruction of biofilms.

Given that the mechanisms of biofilm destruction and antiadhesive activity are based on their antimicrobial activity, it was assumed that the antiadhesive activity of microbial surfactants could be increased by introducing yeasts as a biological inductor.

In view of the above, the aim of this study was to investigate the antiadhesive activity and biofilm destruction ability of *A. calcoaceticus* surfactants IMV B-7241 synthesized in the presence of *Saccharomyces cerevisiae* BTM-1.

Materials and methods

Acinetobacter calcoaceticus and its cultivation

The main object of the study was a strain of petroleum-oxidising bacteria, *Acinetobacter calcoaceticus*, isolated from an oil-contaminated soil sample and 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 IMB B-7241 number.

A. calcoaceticus IMV B-7241 was grown in liquid mineral medium as described (Pirog et al., 2023b). The carbon sources used were (%, volume fraction): purified glycerol - 3, waste of biodiesel production (crude glycerol) - 5. Glycerol concentrations of different quality are equimolar in carbon.

Cultivation of *A. calcoaceticus* IMV B-7241 was carried out in 750 ml flasks with 100 ml of medium on a shaker (320 rpm) at 30°C for 7 days.

Saccharomyces cerevisiae and its cultivation

For the cultivation of the yeast strain *Saccharomyces cerevisiae* BTM-1, a liquid mineral medium similar to that used for the cultivation of surfactant producers was used, but with the replacement of the carbon source with glucose (0.5%). Cultivation was carried out for 24 hours on a shaker at 320 rpm. At the end of this period, the culture liquid was centrifuged in sterile Eppendorf microtubes (10,000 g, 10 minutes) to obtain a supernatant, which was used as an inductor by adding 2.5 ml per 100 ml of surfactant production medium. After centrifugation, the biomass (live inducer cells) was resuspended in sterile tap water to the original volume, and then 2.5 ml of this suspension was added per 100 ml of culture medium.

A portion of the resuspended biomass was sterilised at 131°C for one hour to obtain inactivated cells and 10 ml of the suspension was added to 100 ml of culture medium.

Determination of extracellular surfactant concentration

Extracellular surfactants were isolated from the culture supernatant by extraction with a mixture of chloroform and methanol (2:1) as described in (Pirog et al., 2023a). The culture liquid was centrifuged at 5000 g for 20 minutes to obtain the supernatant. Due to the fact that *A. calcoaceticus* IMV B-7241 produces a complex of various lipids, the classical Folch method (chloroform and methanol, 2:1 ratio) was modified by adding 1 N HCl (chloroform-methanol-water = 4:3:2). The described system allows the maximum separation of both polar and non-polar lipids.

Obtaining surfactant preparations

In the studies, solutions of surfactants of various concentrations were used as preparations. For this purpose, 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 biofilm destruction under the action of surfactants

To determine the anti-adhesion activity of surfactants, as well as their ability to destroy microbial biofilms, bacteria (*Staphylococcus aureus* BMC-1, *Enterobacter cloacae* C-8, *Proteus vulgaris* PA-12, *Bacillus subtilis* BT-2) and yeasts (*Candida albicans* D-6 and *Candida tropicalis* PE-2) from the collection of live cultures of microorganisms of the Department of Biotechnology and Microbiology of the National University of Food Technologies were used.

The effect of surfactants on biofilm destruction was evaluated by the method described in (Pirog and Ivanov, 2022a). The spectrophotometric method was used to estimate the number of adherent cells. The degree of biofilm destruction was determined as a percentage by comparing cell adhesion in untreated and surfactant-treated wells on a polystyrene plate.

Study of the anti-adhesive activity of surfactants

The determination of the surfactant anti-adhesion properties was carried out as described in (2014). Identical plates (1 cm^2) of the tested materials were pre-cleaned with detergent, rinsed with distilled water, air-dried, and sterilised (steel plates, tiles - at 121 °C, linoleum - at 112 °C for 30 min). After sterilisation, the abiotic surfaces were treated with a surfactant solution (sterile phosphate buffer in the control variant) and kept at 30 °C for 18-24 hours. Next, the control and surfactant-treated materials were rinsed with sterile phosphate buffer or distilled water to remove the remaining surfactant.

The test cultures of microorganisms were suspended in 100 ml of sterile tap water, then pre-treated and untreated (control) materials were placed in the suspension for 2 hours at 30 °C. The control and pre-treated materials were rinsed with phosphate buffer to remove non-adherent cells. The materials with adherent cells were air-dried, after which the adherent cells were fixed by immersing the materials in methanol (99%) for 15 minutes and stained with a 1% solution of crystal violet for 5 minutes. The material plates were rinsed with tap water and left to dry at room temperature. Subsequently, the adherent cells with dye were washed from the surface of the materials using 1 ml of glacial acetic acid, to which 9 ml of distilled water was added, and the optical density of the resulting suspension was measured using a photoelectric colorimeter at a wavelength of 540 nm.

The number of adherent cells (degree of adhesion) was determined spectrophotometrically as the ratio of the optical density of the suspension obtained from the surfaces treated with surfactants (tile, steel, linoleum) to the optical density of control samples (untreated with surfactants) and expressed as a percentage.

Results and discussion

The anti-adhesive activity of microbial surfactants may be associated with the reduction of material surface hydrophobicity and changes in surface tension. Additionally, the action of most microbial surfactants promotes an increase in the hydrophobicity of test culture cell surfaces. This leads to changes in the permeability of cell membranes and can affect the reduction of the microbial cell charge, resulting in the disruption of their biological functions (Patel et al., 2021).

Effect of the physiological state of eukaryotic inductor on the anti-adhesive activity of *A. calcoaceticus* IMV B-7241 surfactants

Table 1 shows the number of cells of microbial test cultures adhered to steel plates treated with solutions of surfactants synthesized *by A. calcoaceticus* IMV B-7241 on glycerol of various degrees of purification in the presence of a yeast inductor.

Studies have shown that, regardless of the physiological state of the inductor (live, inactivated cells, supernatant), surfactants (88 μg/ml) synthesized in its presence in the media with purified or crude glycerol proved to be more effective antiadhesive agents for all test cultures studied compared to preparations obtained without the inductor. The adhesion of *P. vulgaris* PA-12, *B. subtilis* BT-2, and *C. albicans* D-6 on steel treated with surfactant solutions synthesized by adding all types of inductors to the medium with both substrates was 10-43, 8-54, and 4-18 % lower, respectively, than under the action of surfactants obtained in the medium without inductor.

Table 1

The effect of surfactants synthesized in the presence of *S. cerevisiae* BTM-1 on the degree of adhesion of bacterial and yeast test cultures to steel

Substrate	Inductor	Adhesion, %		
		***Proteus vulgaris* PA-12**	***Bacillus subtilis* BT-2**	***Candida albicans* D-6**
Purified glycerol	Control (without inductor)	70	71	48
	Live cells	60	40	30
	Inactivated cells	34	40	31
	Supernatant	53	17	44
Crude glycerol	Control (without inductor)	73	85	43
	Live cells	30	70	33
	Inactivated cells	34	77	31
	Supernatant	40	72	31

Notes. Tables 1-3: the surfactant concentration was 88 μg/ml; the error in the adhesion determination did not exceed 5 %.

Similar results to those presented in Table 1 were observed in the case of adhesion of bacterial and yeast test cultures on tiles (Table 2) and linoleum (Table 3).

Thus, after treating tiles with surfactant solutions synthesized in the presence of *S. cerevisiae* BTM-1 (live, inactivated cells, supernatant) in a medium with glycerol of various degrees of purification, the adhesion of *P. vulgaris* PA-12, *B. subtilis* BT-2 and *C. albicans* D-6 decreased by 27-71, 13-63 and 11-21 %, respectively, compared to the effect of surfactants obtained without inductors.

Table 2

Adhesion of bacteria and yeast on tiles after treatment with *A. calcoaceticus* IMV V-7241 surfactants synthesized in the presence of *S. cerevisiae* BTM-1

Substrate	Inductor	Adhesion, %		
		***Proteus vulgaris* PA-12**	***Bacillus subtilis* BT-2**	***Candida albicans* D-6**
Purified glycerol	Control (without inductor)	96	88	50
	Live cells	25	25	30
	Inactivated cells	36	37	29
	Supernatant	35	28	36
Crude glycerol	Control (without inductor)	77	78	50
	Live cells	31	N.d.	30
	Inactivated cells	50	55	39
	Supernatant	29	65	38

Note. Tables 2 and 3: n.d. - not determined

The introduction of live, inactivated yeast cells or supernatant into the media with purified or crude glycerol was accompanied by the formation of surfactants that reduced the number of bacterial cells of *P. vulgaris* PA-12 and *B. subtilis* BT-2 by 22-50 and 20-64 %, respectively, compared to the effect of surfactants synthesized without yeast inductors (Table 3).

Table 3

Adhesion of bacterial and yeast cells to linoleum treated with surfactant preparations synthesized in the presence of *S. cerevisiae* BTM-1

Substrate	Inductor	Adhesion, %		
		***Proteus vulgaris* PA-12**	***Bacillus subtilis* BT-2**	***Candida albicans* D-6**
Purified glycerol	Control (without inductor)	74	74	50
	Live cells	24	10	30
	Inactivated cells	41	54	49
	Supernatant	52	12	48
Crude glycerol	Control (without inductor)	71	75	50
	Live cells	N.d.	33	30
	Inactivated cells	N.d.	42	49
	Supernatant	31	38	48

It should be noted that the adhesion of *C. albicans* D-6 yeast on linoleum was minimal (30 %) after treatment of this surface with solutions of surfactant synthesized in the presence of live inductor cells in the medium with both substrates. The presence of inactivated *S. cerevisiae* BTM-1 cells or supernatant had practically no effect on the antiadhesive activity of surfactants against *C. albicans* D-6. The adhesion of strain D-6 cells was 48-50 % on linoleum treated with surfactant preparations synthesized both in the medium without yeast inductor and in the presence of its live cells or supernatant.

Based on the data presented in Tables 1-3, it can be concluded that the addition of yeast inductors in media with purified or crude glycerol enhances the synthesis of surfactants with high antiadhesive activity.

Destruction of biofilms under the action of *A. calcoaceticus* IMV B-7241 surfactants synthesized in the presence of eukaryotic inductor

One of the mechanisms of antiadhesive activity of microbial surfactants and the ability to destroy biofilms is their antimicrobial activity. It is known from the literature that the destruction of biofilms by lipopeptides can occur due to disruption of cytoplasmic membranes, which leads to cell lysis and metabolic leakage, and by changing the conformation of proteins, which critically affects important membrane functions (Ohadi et al., 2019).

Tables 4-6 show the summary results of the effect of surfactants synthesized with the addition of *S. cerevisiae* BTM-1 cells as an inductor during the cultivation of *A. calcoaceticus* IMV B-7241 in media with purified or crude glycerol on the destruction of bacterial and yeast biofilms.

Studies have shown that the introduction of live *S. cerevisiae* BTM-1 cells and the corresponding supernatant into the medium with glycerol of various degrees of purification led to the synthesis of surfactants that were significantly more effective in destroying biofilms of Gram-negative bacteria compared to surfactants synthesized without an inductor (Table 4). The destruction of biofilms of *E. cloacae* C-8 and *P. vulgaris* PA-12 by surfactants synthesized in the presence of live yeast cells and supernatant was 9-24 and 6-14 % higher, respectively, than by surfactants formed without inductors. The use of inactivated *S. cerevisiae* BTM-1 cells as an inductor was less effective than live cells and supernatant: the destruction of Gram-negative bacterial biofilms after treatment with such surfactants was almost the same as under the action of surfactants obtained without an inductor.

Table 4

Destruction of biofilms of Gram-negative bacterial test-cultures under the action of surfactants synthesized *by A. calcoaceticus* IMV B-7241 in the presence of *S. cerevisiae* BTM-1

Test culture	Substrate	Inductor	Destruction of biofilm, %
Enterobacter cloacae C-8	Purified glycerol	Control (without inductor)	52
		Supernatant	61
		Live cells	76
		Inactivated cells	51
	Crude glycerol	Control (without inductor)	62
		Supernatant	84
		Live cells	81
		Inactivated cells	59
Proteus vulgaris PA-12	Purified glycerol	Control (without inductor)	55
		Supernatant	61
		Live cells	69
		Inactivated cells	54
	Crude glycerol	Control (without inductor)	61
		Supernatant	67
		Live cells	71
		Inactivated cells	59

Notes. Tables 4-6: surfactant concentration 88 µg/ml; the error in determining the degree of biofilm destruction did not exceed 5%.

Under the action of surfactants obtained with the addition of live cells of the yeast *S. cerevisiae* BTM-1 or the corresponding supernatant to the media with purified or crude glycerol, the degree of destruction of biofilms of Gram-positive bacteria *B. subtilis* BT-2 and *S. aureus* BMS-1 was higher by 5-27 and 13-30 %, respectively, compared to surfactants synthesized without the addition of inductors (Table 5). The use of inactivated cells had almost no effect on the activity of surfactant preparations in the destruction of biofilms of Gram-positive test cultures.

Table 5

Effect of surfactants synthesized by *A. calcoaceticus* IMV B-7241 in presence of *S. cerevisiae* BTM-1 on the destruction of biofilms of Gram-positive test cultures

Test culture	Substrate	Inductor	Destruction of biofilm, %
Bacillus subtilis BT-2	Purified glycerol	Control (without inductor)	70
		Supernatant	N.d.
		Live cells	95
		Inactivated cells	75
	Crude glycerol	Control (without inductor)	61
		Supernatant	76
		Live cells	89
		Inactivated cells	60
Staphylococcus aureus BMC-1	Purified glycerol	Control (without inductor)	86
		Supernatant	98
		Live cells	98
		Inactivated cells	85
	Crude glycerol	Control (without inductor)	65
		Supernatant	95
		Live cells	95
		Inactivated cells	60

Note. Tables 5 and 6: N.d. - not determined

Increased destruction of biofilms of the yeast test cultures *C. tropicalis* PE-2 and *C. albicans* D-6 was found only under the action of surfactants synthesized in the presence of yeast inductors in the medium with crude glycerol (Table 6). The destruction of yeast biofilms did not depend on type of inductor (live, inactivated cells or the corresponding supernatant).

The introduction of live, inactivated *S. cerevisiae* BTM-1 cells or the corresponding supernatant into the medium with purified glycerol was accompanied by the formation of surfactants, after action of which the destruction of *C. tropicalis* PE-2 and *C. albicans* D-6 biofilms did not differ from that established for the preparations synthesized without the inductor. Our further studies will be devoted to the analysis of such an effect of the “quality” of the substrate and the inductor on the biological activity of the synthesized surfactants.

It is known from the literature that co-cultivation of bacteria with eukaryotic inductors (micromycetes, yeast) can significantly affect the synthesis and activity of secondary metabolites. There are three different categories of results that can be identified when various strains of bioactive metabolite producers are co-cultured with eukaryotic inductors: 1) increased synthesis of biologically active compounds (Bai et al., 2023; DeFilippi et al., 2018; Fifani et al., 2022; Li et al., 2020; Liu et al., 2022 a, b; Pan et al., 2021; Ramchandran et al., 2020; Sharma et al., 2022; Song et al., 2020; Wu et al., 2018); 2) synthesis of metabolites with cytotoxic activity (Cowled et al., 2023; Meng et al., 2024; Sun et al., 2021); 3) synthesis of new secondary metabolites, or synthesis of metabolites that are not synthesized in monocultures (Cowled et al., 2023; Meng et al., 2024; Shen et al., 2024; Sun et al., 2021).

Table 6

Degree of destruction of yeast biofilms under the action of surfactants synthesized in the presence of yeast inductors

Test culture	Substrate	Inductor	Destruction of biofilm, %
Candida tropicalis PE-2	Purified glycerol	Control (without inductor)	57
		Supernatant	52
		Live cells	60
		Inactivated cells	53
	Crude glycerol	Control (without inductor)	60
		Supernatant	95
		Live cells	95
		Inactivated cells	92
Candida albicans D-6	Purified glycerol	Control (without inductor)	64
		Supernatant	59
		Live cells	64
		Inactivated cells	57
	Crude glycerol	Control (without inductor)	54
		Supernatant	N.d.
		Live cells	75
		Inactivated cells	66

At the same time, there are few reports in the literature on the effect of eukaryotic inductors on the synthesis and especially the biological activity of microbial surfactants. In recent years, several studies have been published on the effect of yeast inductors on the synthesis of surfactants and their antimicrobial activity (Bai et al., 2023; DeFilippi et al., 2018; Fifani et al., 2022; Liu et al., 2022 a, b; Pan et al., 2021; Ramchandran et al., 2020; Wang et al., 2022).

The effect of fungal inductors *Fusarium sambucinum* 2351, *Rhizopus stolonifer* 198 and *Verticillium dahliae* 175 on the synthesis of lipopeptides by *Bacillus subtilis* strain B9-5 showed an increase in the concentration of fengicin and surfactin by 15-30 % when co-cultured with *R. stolonifer* 198 (DeFilippi et al., 2018).

In the study (Pan et al., 2021), it was found that when *Bacillus amyloliquefaciens* HM618 was co-cultivated with micromycetes (*Aspergillus oryzae* BNCC338380, *Trichoderma reesei* BNCC337997, and *Aspergillus nidulans* BNCC190203), an increase in the level of surfactin synthesis by more than 2 times and an increase in its antifungal activity against the pathogenic fungi *Rhizoctonia solani* and *Botrytis cinerea* were observed. The researchers suggested that this effect may be due to the formation of hydrolytic enzymes by micromycetes.

It was found (Fifani et al., 2022) that the addition of supernatant or autoclaved mycelium of *Trichoderma harzianum* IHEM5437 to the culture medium of the lipopeptide producer *Bacillus velezensis* GA1 slightly increased the concentration of iturin, fengicin and surfactin. The researchers explained it by the ability of fungi to produce amino acids and proteins that bacteria use as a source of nitrogen.

In the works (Liu et al., 2022 a, b), it was found that the addition of live *Magnaporthe oryzae* Guy11 cells or their supernatant to the culture medium of *Streptomyces bikiniensis* HD087 led to an increase in the concentration of lipopeptides in crude extract by 107.4 % compared to cultivation without inductors. The authors noted that the inductor activates the tricarboxylic acid cycle, which in turn leads to an increase in the level of reducing equivalents (NADH and FADH), as well as ATP and pyruvate, key metabolites for fatty acid biosynthesis.

In a study (Ramchandran et al., 2020), it was shown that in the presence of autoclaved *C. albicans* SC 5314 cells in the culture medium of the surfactant producer *B. subtilis* RLID 12.1, the concentration of AF3 and AF5 lipopeptides increased by 1.4 and 2 times, respectively, compared to cultivation without an inductor. In addition, these lipopeptides demonstrated a high level of antifungal activity against *Candida auris* yeast strains (minimum inhibitory concentrations were 4-16 μg/ml).

In the study (Bai et al., 2023), it was found that co-cultivation of the recombinant *Yarrowia lipolytica* YL21 strain with *B. amyloliquefaciens* HM618 was accompanied by an increase in the synthesis of fengicin, surfactin and iturin A by 7, 12 and 3 times, respectively, compared to the monoculture of *B. amyloliquefaciens* HM618.

Wang with co-authors (2022) found that co-cultivation of the fungicin producer *B. amyloliquefaciens* HM618 with the genetically modified yeast *Pichia pastoris* GS115 led to a 2-7-fold increase in the concentration of synthesized fengicin compared to the concentration obtained in bacterial monoculture.

In recent years, the literature has reported an increase in the synthesis of secondary metabolites other than surfactants (in particular, antibiotics) using eukaryotic inductors (Li et al., 2020; Song et al., 2020; Sharma et al., 2017; Wu et al., 2018).

A study (Song et al., 2020) showed that the addition of live *Fusarium oxysporum* f. sp. *cucumerinum* cells or supernatant after cultivation of this strain to *Streptomyces rimosus* M527 culture medium led to a 1.8- and 1.5-fold increase in the concentration of the antibiotic rimocidin compared to cultivation without inductor.

Sharma et al. (2017) found that in the presence of live *S. cerevisiae* cells, the synthesis of valinomycin by *Streptomyces lavendulae* strain ACR-DA1 increased by 34% compared to the one without inductor.

There are also studies that report the synthesis of new secondary metabolites, including those with cytotoxic activity, with the use of eukaryotic inductors (Cowled et al., 2023; Meng et al., 2024; Shen et al., 2024; Sun et al., 2021).

In the study (Cowled et al., 2023), two new compounds with cytotoxic activity (myctospiromide A and kitrinomycin A) were identified in the co-culture of the micromycetes *Penicillium brasilianum* MST-FP1927 and *Aspergillus nomius* MST-FP2004. The compound kitrinomycin A showed significant biological activity against NS-1 murine melanoma cells (LD_{99} 7.8 μM) and against the cattle parasite *Tritrichomonas foetus* (LD_{99} was 4.8 μM).

Shen et al. (2024) showed that the co-cultivation of *A. oryzae* (strain was not shown) and *Epicoccum dendrobii* (strain was not shown) was accompanied by the synthesis of new secondary metabolites, such as epiclactone A, epiclactone B, epioxochromane and aoergostane. It was found (Meng et al., 2024) that the addition of *A. oryzae* cells to the culture of *Monascus purpureus* resulted in the formation of two new cyclohexylfurans, monaspins A and B. Monaspin B showed potent antiproliferative activity against the leukemic cell line HL-60, inducing apoptosis, with an IC_{50} of 160 nM.

At the same time, it should be noted that to date there is a limited number of publications on the effect of surfactants synthesized in the presence of inducers on the destruction of microbial biofilms (Hamza et al., 2018; Kimelman and Shemesh, 2019; Mohamed et al., 2020; Pirog and Ivanov, 2022a) or the antiadhesive activity of surfactants (Pirog and Ivanov, 2022b).

Our previous results (Pirog and Ivanov, 2022a) showed that the introduction of *B. subtilis* BT-2 (live or inactivated cells or supernatant) into the *A. calcoaceticus* IMB B-7241 cultivation medium enhanced the antibiofilm activity of the synthesized surfactants. The degree of destruction of bacterial and yeast biofilms by the *A. calcoaceticus* IMB B-7241 surfactants obtained with the introduction of a prokaryotic inducer was 1.5-3 times higher compared to the use of surfactants synthesized in a medium without inductors.

The results reported in this article showed that even in the presence of a eukaryotic inductor, surfactants were obtained with a higher ability to destroy biofilms compared to surfactants synthesized by monoculture (see Tables 4-6). However, it should be noted that the destruction of biofilms under the action of surfactants synthesized with the addition of a yeast inductor was 1.1-1.7 times higher compared to the use of preparations obtained without the addition of inductors. Therefore, the eukaryotic inductor was slightly less effective than the prokaryotic inductor, in the presence of which surfactants were synthesized that increased biofilm destruction by 1.5-3 times (Pirog and Ivanov, 2022a).

Meanwhile, the level of increase in the antiadhesive activity of *A. calcoaceticus* IMV B-7241 surfactants synthesized in the presence of both prokaryotic and eukaryotic inductors was almost the same (Pirog and Ivanov, 2022b) (Tables 1-3 of this article). In particular, when abiotic materials (steel, linoleum, tiles) were treated with surfactant preparations obtained by adding biological inductors, the adhesion of bacterial and yeast test cultures decreased by 1.1-5.9 times (Pirog and Ivanov, 2022b) and 1.1-6 times (Tables 1-3 of this article).

Conclusions

The results obtained demonstrate that it is possible to regulate not only the antimicrobial but also the antiadhesive activity of surfactants synthesized by *A. calcoaceticus* IMV B-7241 and their ability to destroy biofilms by introducing live or inactivated cells of the yeast *S. cerevisiae* BTM-1 and the corresponding supernatant into the media containing crude or purified glycerol. These studies are important for understanding the complex interactions between competing microorganisms and the effect of inductors on the biological activity of microbial secondary metabolites, which is essential for the development of new strategies in the microbiological and biotechnological industries.

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