Dependence of biological activity of surfactants synthesized by *Rhodococcus erythropolis* IMV Ac-5017 on physiological state of yeast inducer

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Abstract

Introduction. Surfactants synthesized by *Rhodococcus erythropolis* IMV Ac-5017 can be used as antibiofilm agents due to their antimicrobial activity. The antimicrobial activity of surfactants can be increased by adding inducers to the *R. erythropolis* cultivation medium.

Materials and methods. *R. erythropolis* IMV Ac-5017 was cultivated in ethanol-containing medium with *Saccharomyces cerevisiae* BTM-1 as an inducer. Surfactant concentrations were determined gravimetrically. The minimum inhibitory concentration was used to assess the antimicrobial activity of surfactants, and their ability to disrupt biofilms was determined by the spectrophotometric method.

Results and discussion. The antimicrobial activity of surfactants synthesized by *R. erythropolis* IMB Ac-5017 and their ability to destroy biofilms can be significantly increased by introduction of *S. cerevisiae* BTM-1 cells being at different physiological states into the medium for cultivation. The surfactants synthesized by *R. erythropolis* IMV Ac-5017 in the presence of live *S. cerevisiae* BTM-1 cells, as well as the corresponded supernatant, exhibited the highest biological activity. Under these conditions, surfactants synthesized demonstrated the minimum inhibitory concentrations, which were 3.6–240 times lower compared to the values reported for surfactants produced in the medium without an inducer.

Surfactants synthesized in the presence of inducers destroyed single- and dual-species biofilms by 9-59% and 1-29% more effectively, respectively, than those obtained in the medium without them.

Surfactants of *R. erythropolis* IMV Ac-5017 obtained with inactivated inducer cells were less active, probably because proteins of inducer cells could be denatured during autoclaving. Biological activity of the surfactants produced in the presence of the yeast inducer was more specific towards yeast test cultures, which can be explained by the nature of the studied inducer.

Conclusion. The addition of the yeast inducer into the medium for cultivation of *R. erythropolis* IMV Ac-5017 contributed to significant increase of biological activity of surfactants compared to those synthesized in the medium without an inducer.

Introduction

Microbial biofilms pose a significant challenge to humanity as they can cause diseases associated with chronic and acute infections, as well as food spoilage and equipment damage (Shineh et al., 2023). Most often, several species of microorganisms are involved in the formation of biofilms, and such biofilms exhibit significantly higher resistance to disinfectants and antimicrobials than single-species ones (Yuan et al., 2020).

Natural surfactants are promising biofilm destructors due to their antimicrobial activity. Microbial surfactants can be considered as a potential component of antimicrobial drugs containing antibiotics (Pirog and Kliuchka, 2024) or as an alternative to antibiotics, which is currently relevant due to the emergence of multidrug-resistant strains.

The surfactants produced by *Rhodococcus erythropolis* IMV Ac-5017 are characterized by lower antimicrobial activity compared to known surface-active amino-, rhamno-, and sophorolipids. However, it was established that the biological activity of *R. erythropolis* IMV Ac-5017 surfactants could be significantly increased by introducing into the medium for cultivation live *Escherichia coli* IEM-1 and *Bacillus subtilis* BT-2 cells (Pirog et al., 2020). Limited literature data indicate that the biological activity of microbial surfactants can be increased not only by introduction in medium for cultivation of bacterial inducers, but also by eukaryotic ones.

Cultivation with biological inducers is one of the approaches to regulate the activity of secondary metabolites during their synthesis (Sharma et al., 2017).

The challenge in regulating the biological properties of secondary metabolites by the method of cultivation with other microorganisms is that every combination of microorganisms requires separate investigation. It is currently impossible to predict the result in advance, as there are no clear rules for the metabolites production, and the induction mechanism during co-cultivation remains poorly studied and unclear (Liang, et al., 2020; Song et al., 2020).

Therefore, the aim of this study was to determine the antimicrobial and antibiofilm activity of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of cells of *Saccharomyces cerevisiae* BTM-1 being at different physiological states as a biological inducer.

Materials and methods

Objects of research

The main object of research was a strain of oil-oxidizing bacteria *Rhodococcus erythropolis* EK-1, isolated from a soil sample contaminated with oil. The EK-1 strain is registered in the Microorganisms Depository of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, under the number IMV Ac-5017. Chemically, the extracellular *R. erythropolis* IMV Ac-5017 surfactants are a complex of glycolipids (trehalose mono- and dimycolates), neutral lipids (cetyl alcohol, palmitic acid, methylpentadecanoic acid, triglycerides, mycolic acids), and aminolipids (Pirog et al., 2023). The biological inducer used in this research was *Saccharomyces cerevisiae* BTM-1 yeast.

Bacteria (*Staphylococcus aureus* BMS-1, *Pseudomonas* sp. MI-2, *Bacillus subtilis* BT-2, *Escherichia* coli IEM-1) and yeast (*Candida albicans* D-6, *Candida utilis* BVS-65, *S. cerevisiae* BTM-1) from the collection of live cultures of microorganisms of the Department

of Biotechnology and Microbiology, National University of Food Technologies, were used as test cultures to determine the antimicrobial activity of surfactants, as well as their ability to destroy single- and dual-species biofilms.

Cultivation of R. erythropolis IMV Ac-5017

R. erythropolis IMV Ac-5017 was cultivated in a liquid mineral medium (Pirog et al., 2020) with 2% (*v/v*) ethanol in 750 ml Erlenmeyer flasks containing 100 ml of the medium. Cultivation was performed for 5 days in a shaking incubator at 30°C and 320 rpm.

The inoculum was prepared in the same medium with 0.5% (v/v) ethanol in 750 ml Erlenmeyer flasks containing 100 ml of the medium. It was grown for 2 days in a shaking incubator at 30°C and 320 rpm and then added to the cultivation medium at 10% (v/v).

Preparation of the yeast inducer

The strain *S. cerevisiae* BTM-1 was cultivated in a liquid mineral medium with 0.5% (v/v) glucose in 750 ml Erlenmeyer flasks containing 100 ml of the medium. It was grown for 1 day in a shaking incubator at 30°C and 320 rpm. The resulting culture liquid was poured into 1.5 ml sterile Eppendorf tubes and centrifuged in an ultracentrifuge (10 000g, 15 min). After centrifugation, the supernatant was poured into sterile test tubes and added at the rate of 2.5 ml per 100 ml of the culture medium of the surfactants producer. The biomass (sediment) remaining in the Eppendorf tubes was resuspended in sterile tap water to a final volume corresponding to the volume of culture liquid taken for centrifugation. The resuspended biomass was poured into sterile test tubes (live inductor cells were added at the rate of 2.5 ml of the suspension per 100 ml of the culture medium of the surfactants producer). The rest of the resuspended biomass was sterilized in an autoclave at 131°C for 1 hour to obtain inactivated inductor cells (which were added at the rate of 10 ml of the suspension per 100 ml of the culture medium of the surfactants producer).

Isolation and preparation of the surfactants

To obtain the supernatant, the culture liquid was centrifuged (5000 g, 20 min). Surfactants were isolated from the supernatant by the Bligh and Dyer method, with a modified Folch mixture (chloroform – methanol – hydrochloric acid = 4:3:2), as described in the article (Pirog et al., 2020). The extracts were evaporated on a IP-1M2 rotary evaporator at 50ºC and an absolute pressure of 0.4 atm to constant weight. The concentration of surfactants was determined gravimetrically.

The dry surfactant residue was dissolved in preheated distilled water (25 ml). Surfactant solutions were sterilized in an autoclave at 112 ºC for 30 min.

Determination of antimicrobial activity of surfactants

The antimicrobial activity of *R. erythropolis* IMV Ac-5017 surfactants was determined using the minimum inhibitory concentration (MIC) (Chebbi et al., 2017).

MIC was determined by the method of two-fold serial dilutions in meat-peptone broth (MPB) for bacterial and liquid wort for yeast test cultures (Pirog et al., 2020). Under sterile conditions, 90 µl of MPB or liquid wort were placed in the wells of a microplate. Then, 100 µl of surfactant solution at the initial concentration was added to the first well, mixed, and 100 µl of this liquid was taken and added to the second well, mixed, 100 µl of this liquid was

taken and added to the third well, etc. Thus, in each subsequent well, the concentration of surfactants decreases by 2 times compared to the previous one. After that, 10 µl of an aqueous suspension of a day-old test culture grown on meat-peptone agar (MPA) or wort agar was added to the wells, and 10 µl of sterile tap water was added for control. The microplates were incubated for 24 hours at 37°C for bacteria and 30°C for yeast. The results were assessed visually by the turbidity of the medium: (+) – the well in which turbidity was observed (presence of growth), (-) – turbidity was not observed (absence of growth). The MIC of surfactants was determined as the surfactants concentration in the first well in which no growth of test cultures was observed. The permissible error of the experiment was ±5%.

Determination of destruction of single-species biofilms under the action of surfactants

Biofilms of test cultures were treated with the surfactant solutions of different concentrations (1.25-640 µg/ml) in the wells of microplates as described in the work (Pirog et al., 2020). The degree of destruction of biofilms (%) was determined spectrophotometrically as the difference between cell adhesion in untreated and surfactant-treated wells. The permissible error of the experiment was ±5%.

Determination of destruction of dual-species biofilms under the action of surfactants

To form biofilms 100 µl of MPB (for two species of bacteria) or 50 µl of MPB and 50 µl of liquid wort (for bacteria and yeast), as well as 10 µl of aqueous suspensions of test cultures (proportions selected experimentally), were placed into polystyrene microplates and incubated for 24 hours at the optimal temperature for the test culture. After that, the culture liquid was drained, the medium and suspensions of microorganisms were reintroduced, and the microplates were incubated for another 24 hours. The culture liquid was drained again, the wells of microplates (with dual-species biofilms preformed in them) were washed twice with sterile tap water, and then 100 µl of the surfactant solutions of different concentrations (1.25-640 µg/ml) were added. In the control wells, 100 µl of sterile tap water was added instead of surfactants. The microplates were incubated for another 24 hours.

The degree of destruction of biofilms (%) was determined spectrophotometrically as the difference between cell adhesion in untreated and surfactant-treated wells. The permissible error of the experiment was ±5%.

Results and discussion

Antimicrobial activity of the surfactants

At the first stage of our research, the antimicrobial activity of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the medium with the yeast inducer in different physiological states was determined (Table 1).

Table 1

Influence of the physiological state of yeast inducer on antimicrobial activity of *R. erythropolis* IMV Ac-5017 surfactants

Test cultures	Minimum inhibitory concentrations (μg/ml) of surfactants synthesized in the presence of *S. cerevisiae* BTM-1:			
	live cells	inactivated cells	supernatant	without an inducer (control)
Escherichia coli IEM-1	10	85	41.3	300
Pseudomonas sp. MI-2	10	85	41.3	300
Staphylococcus aureus BMS-1	40	170	82.5	300
Bacillus subtilis BT-2	40	170	82.5	300
Candida albicans D-6	5	42.5	20.7	300
Candida utilis BVS-65	5	42.5	20.7	300
Saccharomyces cerevisiae BTM-1	1.25	21.3	10.3	300

Note**.** When determining the minimum inhibitory concentration, the error did not exceed 5 %.

The results showed that cultivation of *R. erythropolis* IMV Ac-5017 in the medium with all studied biological inducers was accompanied by synthesis of the surfactants with increased antimicrobial activity.

Thus, the introduction of live *S. cerevisiae* BTM-1 cells into the culture medium of *R. erythropolis* IMV Ac-5017 was accompanied by the synthesis of surfactants, the MICs of which in relation to test cultures of gram-negative (*E. coli* IEM-1, *Pseudomonas* sp. MI-2) and gram-positive (*B. subtilis* BT-2, *S. aureus* BMS-1) bacteria were 7.5-30 times lower than those established for surfactants produced without an inducer. Similar patterns were observed for yeast test cultures (*C. albicans* D-6, *C. utilis* BVS-65, and *S. cerevisiae* BTM-1): the minimum inhibitory concentration of surfactants synthesized in the medium with the live inducer was two orders of magnitude lower compared to the control (1.25-5 and 300 μg/ml).

In the presence of *S. cerevisiae* BTM-1 supernatant in the medium, surfactants were synthesized, the MIC values of which in relation to bacterial and yeast test cultures were, respectively, 3.6-7.3 and 14.5-29.1 times lower compared to surfactants obtained using a monoculture. The addition of inactivated inductor cells into the culture medium also affected the antimicrobial activity of the synthesized surfactants – the MICs for bacteria and yeast were 1.8-3.5 and 7.1-14.1 times lower compared to the control.

The obtained results on the higher efficiency of live inducer cells and supernatant compared to inactivated cells may indicate that induction requires both chemical and biological interaction between the surfactant producer and the inducer. It is likely that during autoclaving of the yeast cell suspension, proteins and other macromolecules of the inducer cells are denatured, which partially inhibits potential biochemical interactions.

Thus, the data presented in Table 1 indicate the possibility of increasing the antimicrobial activity of *R. erythropolis* IMV Ac-5017 surfactants against various bacterial and yeast test cultures due to the introduction of *S. cerevisiae* BTM-1 in different physiological states into the culture medium. Surfactants of *R. erythropolis* IMV Ac-5017

synthesized without inducers acted nonspecifically on test cultures. At the same time, the surfactants obtained in the presence of the yeast inducer were more effective against yeast test cultures, especially against the inducer itself, which can be explained by specific competitive interactions between *R. erythropolis* IMV Ac-5017 and *S. cerevisiae* BTM-1 in the culture medium.

In previous studies by scientists of the Department of Biotechnology and Microbiology (Pirog et al., 2020), live *E. coli* IEM-1 and *B. subtilis* BT-2 cells were used as inducers to increase the biological activity of *R. erythropolis* IMV Ac-5017 surfactants. Under such conditions, the synthesized surfactants were characterized by the MIC of 3-12 μg/ml against bacterial test cultures (*E. coli* IEM-1, *B. subtilis* BT-2, *S. aureus* BMS-1). Comparing with our data (10-40 μg/ml), it can be stated that depending on the inducer in the presence of which surfactants are synthesized, the specificity of their action on the test cultures changes.

Similar study (Pirog et al., 2023) was devoted to the effect of prokaryotic inducers *E. coli* IEM-1 and *B. subtilis* BT-2 in different physiological states on the biological activity of *Acinetobacter calcoaceticus* IMB B-7241 and *Nocardia vaccinii* IMB B-7405 surfactants. Thus, surfactants of the IMB B-7241 and IMB B-7405 strains synthesized in the presence of live inducer cells were characterized by the MIC values that were 3-23 and 2.9-16 times lower, respectively, in relation to bacterial test cultures compared to surfactants synthesized without inductors. We observed a more significant decrease in the minimum inhibitory concentrations of microbial surfactants produced in the medium with live *S. cerevisiae* BTM-1cells in relation to bacterial test cultures – by 7.5-30 times compared to the control.

The minimal inhibitory concentrations of *A. calcoaceticus* IMB B-7241 surfactants synthesized in the presence of live *B. subtilis* BT-2 cells against yeast test cultures were 2.8-5.6 times lower compared to surfactants obtained without any inducers. Because of our research, the MICs of *R. erythropolis* IMV Ac-5017 surfactants produced in the medium with live *S. cerevisiae* BTM-1cells against *Candida* yeast were as much as 60 times lower compared to the control. Again, the same trend is observed: in the presence of prokaryotic inducers, surfactants with more effective action against bacterial test cultures are synthesized, while in the presence of eukaryotic inducers, surfactants are more effective against yeast.

The effect of the supernatant was less effective: in its presence, the *A. calcoaceticus* IMB B-7241 surfactants were synthesized with minimum inhibitory concentrations for most test cultures of bacteria and yeast only 2 times lower than those for surfactants produced without any inducers. In contrast, the presence of *S. cerevisiae* BTM-1 supernatant in the culture medium of *R. erythropolis* IMV Ac-5017 was accompanied by the synthesis of surfactants with minimum inhibitory concentrations reduced by as much as 3.6-29 times.

In the presence of inactivated prokaryotic inducer cells in the culture media of *A. calcoaceticus* IMB B-7241 and *N. vaccinii* IMB B-7405, the surfactants were synthesized with MICs against bacterial test cultures that were 2-8 and 2-13.3 times lower compared to the controls. However, it should be noted that the authors obtained inoculum of *E. coli* IEM-1 and *B. subtilis* BT-2 on a meat-peptone agar medium, while we cultivated *S. cerevisiae* BTM-1 in a liquid medium, which is a significant advantage when scaling the technology to an industrial level.

Our data indicate that the regulation of the biological activity of *R. erythropolis* IMV Ac-5017 surfactants can be achieved by introducing biological inducers and activators of key enzymes involved in the biosynthesis of surfactant components responsible for antimicrobial activity into the medium. In the available foreign scientific literature, there is also information on secondary metabolites (including surfactants of microbial origin) synthesized in the presence of inducers in the culture medium, but the data are limited. Live inducer cells are used most often in experiments and inactivated or supernatant cells are used less frequently.

Alves et al. (2019) studies the impact of live cells of *Pseudomonas aeruginosa* ATCC 27853 and *Listeria innocua* NCTC 11288 on the concentration of rhamnolipids produced by *Pseudomonas sp.* 74. The work (DeFilippi et al., 2018) focuses on the effect of live *Rhizopus stolonifer* 198 cells on the synthesis of *B. subtilis* B9-5 lipopeptides, while in the research (Bagheri et al., 2022) the impact of live *Azospirillum oryzae* NBT506 cells and the corresponding supernatant on the biosynthesis process of *Bacillus velezensis* UTB96 surfactin was studied. However, the authors did not investigate the biological activity of the obtained surfactants. Additionally, there is also information on the influence of *S. cerevisiae* in different physiological states on the concentration of the antibiotic natamycin produced by *Streptomyces natalus* N5 (Shi et al., 2017) and *Streptomyces natalensis* HW-2 (Wang et al., 2013), valinomycin produced by *Streptomyces lavendulae* ACR-DA1 (Sharma et al., 2017), and rimocidin by *Streptomyces rimosus* M527 (Song et al., 2020), but the authors also did not investigate the biological activity of the obtained substances.

To date, there are several articles describing studies on the antimicrobial activity of microbial surfactants. Dusane et al. (2011) studied the antimicrobial activity of surface-active lipopeptides produced by *Bacillus sp.* S3, *Bacillus pumilus* S8, *Bacillus licheniformis* D1, and *Serratia marcescens* V1, obtained in the presence of live *P. aeruginosa* (strain number not specified), *B. pumilus* FJ938166, *C. albicans* (strain number not specified), and *Yarrowia lipolytica* (strain number not specified) cells. Fifani et al. (2022) studied *B. velezensis* GA1 lipopeptides synthesized in the presence of live, heat-inactivated *Trichoderma harzianum* IHEM5437 cells, as well as the corresponding supernatant, against the test cultures of the inducers themselves. However, to assess antimicrobial activity, the authors used the standard disk diffusion method and determination of the dry weight of the biomass of test cultures, which makes it impossible to directly compare their results with ours. The authors note that when cultivating producer bacteria in a medium with biological inducers, surfactants with significantly higher antimicrobial activity were synthesized: the inhibition zones of the test cultures increased by 2-4 mm, and the amount of dry biomass decreased by 50-100% compared to the effect of surfactants obtained using monocultures.

There is also information in the literature regarding co-cultivation or cultivation with inducers, which results in an increase in the antimicrobial activity of other secondary metabolites. Li with co-authors (2022) showed an increase in the inhibition coefficient of the ethyl acetate extract of the metabolite complex (phytohormones, aminoglycosides, amines, macrolides, peptides, terpenoids, alkaloids, steroids, coumarins, phenolic acids) from the co-culture of *Streptomyces albireticuli* MDJK11 and *Streptomyces albofavus* MDJK44 against the fungal test cultures *Fusarium moniliforme*, *Fusarium solani*, and *Fusarium graminearum* (strain numbers not specified) by 2-43.6% compared to that of the extracts produced by the corresponding monocultures. Serna-Cock with co-authors (2019) reported an increase in the growth inhibition zone of *Listeria monocytogenes* ATCC 13932 by 1-3.5 mm due to the supernatant of the metabolite complex from the co-culture of *Lactobacillus plantarum* and *Weissella cibaria* (strain numbers not specified).

As a result of cultivating *Trichoderma asperellum* GDFS1009 with *Bacillus amyloliquefaciens* 1841 (Karuppiah et al., 2019) and *B. amyloliquefaciens* ACCC11060 (Wu et al., 2018), metabolite complexes were obtained, the antimicrobial activity of which against *F. graminearum* (strain number not specified) was an order of magnitude higher (4.5×10^2 spores/ml) compared to the action of the metabolites produced by the monoculture of *T. asperellum* GDFS1009 (7×10^3 spores/ml), and against *Botrytis cinerea* (strain number not specified), the inhibition coefficient increased by 36% (67% and 31%, respectively, for the metabolites obtained in co-culture and monoculture). The article Liu et al. (2022) is dedicated to the cultivation of *Trichoderma atroviride* SG3403 with *B. subtilis* 22, resulting in a

metabolite complex that also exhibited an increased inhibition coefficient against *F. graminearum* MN396567 by 19-57% compared to the influence of the metabolites from monocultures (62% and 5-43%, respectively).

In the study (Luti and Yonis, 2013) the author investigated the effect of live and thermally inactivated cells of *E. coli*, *B. subtilis*, and *S. cerevisiae* on the synthesis of phenazine by *P. aeruginosa* (strain numbers not specified). Although the influence of inducers in different physiological states on the antimicrobial activity of the antibiotic was not studied, we were intrigued by the suggestion that the metabolite 'might recognize' the test culture of the inducer and be specific to it. A similar thought arose during our analysis of the minimum inhibitory concentration of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of *S. cerevisiae* BTM-1 against the inducer itself.

Consequently, there is little literature on the antimicrobial activity of surfactants synthesized in the presence of inducers in different physiological states; however, our results for the minimum inhibitory concentration of *R. erythropolis* IMV Ac-5017 surfactants obtained in the presence of *S. cerevisiae* BTM-1 are comparable to similar studies. Furthermore, using the method of co-cultivation with inducers or competing microorganisms can significantly enhance the antimicrobial activity of secondary metabolites.

Determination of the degree of destruction of single-species biofilms

Besides antimicrobial activity, surfactants of microbial origin also possess the ability to disrupt biofilms. Therefore, in the next stage, we studied the degree of destruction of mono-species bacterial and yeast biofilms under the action of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of a yeast inducer in different physiological states during cultivation.

The results showed that surfactants synthesized in media with inducers were more effective at disrupting bacterial and yeast biofilms than surfactants obtained without any inducers (Tables 2–5).

Table 2

Destruction of Gram-positive bacteria biofilms under the action of the surfactants synthesized in the presence of *Saccharomyces cerevisiae* BTM-1

Test cultures	Physiological state of the inducer	Destruction (%) under the action of the surfactants (μg/ml)									
		640	**320**	**160**	**80**	**40**	**20**	**10**	**5**	**2.5**	**1.25**
Bacillus subtilis BT-2	Live cells	75.2	64.8	62.4	60.5	50.2	42.3	31.7	28.2	27.4	24.5
	Inactivated cells	71.0	63.1	56.8	38.7	29.0	26.9	28.8	21.8	20	18
	Supernatant	78.2	75.0	69.5	56.6	50.2	43.8	33.2	36.7	31.8	29.6
	Control	61.9	54.1	42.4	34.0	25.2	20.2	16.0	17.4	14.5	11.4
Staphylococcus aureus BMS-1	Live cells	79.6	66.8	58.3	57.7	46.4	48.3	35.8	23.5	22.7	26.4
	Inactivated cells	76.1	65.4	55.2	34.8	25.6	24.1	24.7	24.8	23.9	19.6
	Supernatant	80.4	73.9	62.3	59.1	52.3	42.3	31.7	34.2	34.1	23.9
	Control	63.7	53.6	37.7	33.6	20.6	17.1	16.7	17.6	15.6	10.6

Note. Tables 2–5: during the determination of the degree of biofilm destruction, the error did not exceed 5%.

Table 3

Destruction of Gram-negative bacteria biofilms under the action of the surfactants synthesized in the presence of the yeast inducer

Test cultures	Physiological state of the inducer	Destruction (%) under the action of the surfactants (μg/ml)									
		640	**320**	**160**	**80**	**40**	**20**	**10**	**5**	**2.5**	**1.25**
Escherichia coli IEM-1	Live cells	85.0	86.8	72.6	67.9	67.4	62.6	61.8	58.9	50.8	50.8
	Inactivated cells	78.4	64.1	60.8	58.9	47.5	39.8	38.9	28.4	20.8	13.1
	Supernatant	88.9	74.1	71.8	57.5	54.6	52.5	51.3	54.6	48.4	32.6
	Control	63.1	57.9	40.5	38.9	26.0	20.0	16.4	16.2	14.1	11.0
Pseudomonas sp. MI-2	Live cells	82.6	79.6	70.3	66.3	59.2	58.6	55.1	57.5	50.0	42.7
	Inactivated cells	74.9	67.6	65.6	55.7	42.2	36.1	35.7	25.8	24.3	19.8
	Supernatant	81.8	76.4	66.2	53.9	51.1	50.6	53.5	46.1	49.3	35.7
	Control	60.2	56.7	41.8	37.9	25.0	17.3	15.8	15.3	13.0	12.1

Table 4

Degree of destruction of *Candida* yeast biofilms under the action of surfactants synthesized in the presence of the biological inducer

Test cultures	Physiological state of the inducer	Destruction (%) under the action of the surfactants (μg/ml)									
		640	**320**	**160**	**80**	**40**	**20**	**10**	**5**	**2.5**	**1.25**
Candida albicans D-6	Live cells	96.4	96.4	83.0	78.4	78.1	63.2	61.3	58.7	55.5	53.3
	Inactivated cells	83.9	80.0	70.0	60.8	56.1	45.8	39.5	28.3	27.7	26.7
	Supernatant	93.9	83.3	80.9	78.1	75.3	63.7	66.7	59.3	50.9	48.0
	Control	64.5	53.9	43.9	39.7	28.3	18.9	15.6	16.2	15.3	14.3
Candida utilis BVS-65	Live cells	91.7	92.8	86.7	71.7	73.5	64.2	62.4	50.9	52.4	44.7
	Inactivated cells	83.5	77.2	71.2	67.0	56.0	42.1	33.4	33.9	27.7	25.8
	Supernatant	93.0	87.5	83.6	73.1	74.8	62.7	52.4	55.3	51.5	41.0
	Control	64.6	55.8	41.7	38.7	23.9	19.5	18.5	16.8	14.4	13.7

It was established that the introduction into the culture medium of live cells of *S. cerevisiae* BTM-1, as well as the corresponding supernatant, was accompanied by the synthesis of surfactants, which in the entire studied concentration range (1.25-640 μg/ml) by 9.1-31.2 and 13.3-31.7%, respectively, more effectively destroyed single-species biofilms of gram-positive bacteria *B. subtilis* BT-2 and *S. aureus* BMS-1 than preparations obtained without an inducer.

Table 5

Degree of destruction of yeast inducer biofilms under the influence of surfactants synthesized in its presence

Test cultures	Physiological state of the inducer	Destruction (%) under the action of the surfactants (μg/ml)									
		640	**320**	**160**	**80**	**40**	**20**	**10**	**5**	**2.5**	**1.25**
Saccharomyces cerevisiae BTM-1	Live cells	99.6	98.1	91.3	80.5	80.5	74	76.3	63.1	63.2	51.1
	Inactivated cells	87.4	82.1	75.1	74.2	67.8	51.1	40.2	30.9	28.9	27.8
	Supernatant	93.6	95.9	87.4	83.3	71.0	61.6	60.0	57.7	41.3	44.0
	Control	65.5	57.5	43.7	32.3	22.4	18.5	17.4	12.8	14.6	11.0

The most pronounced increasing destruction of these biofilms was observed with surfactants (20-160 μg/ml) obtained in the presence of live yeast cells, and those synthesized in the presence of the supernatant at concentrations of 20-320 μg/ml. The maximum degree of destruction (75.2-80.4%) was observed under the influence of surfactants at the highest concentration studied (640 μg/ml).

At the same time, in the presence of inactivated inducer cells, surfactants were synthesized, under the action of which the destruction Gram-positive bacteria biofilms increased by only 3.8-14.5% (Table 2). Similar patterns were observed with surfactants (1.25-640 μg/ml) obtained in a medium with live yeast cells and supernatant on mono-species biofilms of Gram-negative bacteria.

Under these conditions, the destruction of *E. coli* IEM-1 and *Pseudomonas sp.* MI-2 biofilms increased by 16-45.4%, compared to the control. The degree of biofilm disruption increased the most under the action of surfactants in low concentrations (1.25-40 μg/ml). The destruction of these biofilms reached a maximum degree of 81.8-88.9% under the influence of surfactants at high concentrations (320-640 μg/ml).

The addition of inactivated cells to the medium was accompanied by the synthesis of surfactants, which at concentrations of 10-160 μg/ml also effectively disrupted these mono-species biofilms, with an increase in the degree of destruction ranging from 17.2 to 23.8%. At other concentrations, the increase was only 2.1-15% (Table 3).

Surfactants synthesized in the presence of all studied inducers exhibited a more specific action towards yeast test cultures across the entire concentration range studied, which can be explained by their antimicrobial activity. The degree of destruction of biofilms formed by yeast of the genus *Candida* after treatment with solutions of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of live inducer cells increased by 27.1-49.8, inactivated cells – by 12.1-32.1, and supernatant – by 27.3-50.9% (Table 4).

The most specific action of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of yeast was observed against the biofilms of the inducer itself. The destruction of *S. cerevisiae* BTM-1 biofilms under the action of surfactants obtained in the presence of live cells increased by as much as 34.1-58.9, the supernatant – by 26.7-51, and inactivated cells – by 14-45.4% (Table 5).

To summarize all of the above, the obtained experimental data (Tables 2-5) demonstrate the possibility of significantly enhancing the ability of *R. erythropolis* IMV Ac-5017 surfactants to disrupt both bacterial and yeast biofilms by the addition of live *S. cerevisiae*

BTM-1 cells, as well as the corresponding supernatant, into the cultivation medium of the producer.

The possibility of enhancing the anti-biofilm activity of *R. erythropolis* IMV Ac-5017 (Pirog et al., 2020), *N. vaccinii* IMB B-7405 (Pirog et al., 2023), and *A. calcoaceticus* IMV B-7241 (Pirog and Ivanov, 2022) surfactants was shown by the addition of live and inactivated *B. subtilis* BT-2 and *E. coli* IEM-1 cells, as well as the supernatant, to the cultivation medium. Comparing these results with ours, it can be stated that the action of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of live yeast cells was generally no less effective against single-species bacterial biofilms.

In the available foreign scientific literature, information regarding the disruption of single-species biofilms using microbial surfactants is limited; however, we managed to find several articles. Gómez et al. (2016) showed complete inhibition of single-species biofilms of *E. coli* O157*:H7* ATCC 35150, *L. monocytogenes* ATCC 7644, and *Salmonella typhimurium* ATCC 14028 under the action of surfactants synthesized in a co-culture of lactic acid bacteria *Lactococcus lactis* 368 with *Lactobacillus curvatus* MBSa3 or *Lactobacillus sakei* MBSa1. Additionally, Kimelman et al. (2019) showed strong inhibition of the formation of single-species *S. aureus* ATCC 25923 biofilms, as evidenced by a reduction in the optical density of the samples by approximately 1.6 times after treatment with the *B. subtilis* YC189 lipopeptides synthesized during co-cultivation with *L. plantarum*.

In the study (Hamza et al., 2018), the authors investigated the effect of the supernatant of *Staphylococcus lentus* SZ2 glycolipids synthesized in the presence of *Vibrio harveyi* MTCC 7771 on the mono-species biofilms of the competitive bacteria themselves. The increase in the degree of biofilm destruction ranged from 1.26% to 39.36%, depending on the duration of exposure. In contrast, *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of yeast inducer destroyed the biofilms of the inducer by several tens more efficiently.

Consequently, our results on the anti-biofilm activity of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of *S. cerevisiae* BTM-1 are on par with studies conducted by both domestic and foreign colleagues.

Determination of the degree of destruction of dual-species biofilms

At the next stage, we decided to use surfactants produced by *R. erythropolis* IMV Ac-5017 for the destruction of dual-species biofilms.

The results showed that surfactants (1.25-640 μg/mL) synthesized in the presence of an inducer were more effective in destroying bacterial and bacterial-yeast combined biofilms than surfactants synthesized without the inducer (Tables 6-7).

It was found that in the presence of living cells of *S. cerevisiae* BTM-1 in the culture medium of *R. erythropolis* IMV Ac-5017, surfactants were synthesized, which in a wide range of concentrations (1.25-640 μg/ml) by 3-29% more effectively destroyed two species of bacteria (*B. subtilis* BT-2 + *Pseudomonas* sp. MI-2 and *S. aureus* BMS-1 + *E. coli* IEM-1) and bacterial and yeast (*Pseudomonas* sp. MI-2 + *C. albicans* D-6 and *S. aureus* BMS-1 + *C. utilis* BVS-65) biofilms compared to the effect of surfactants synthesized without an inducer.

The addition of the supernatant was accompanied by the synthesis of surfactants, which were 1-22% more effective in destroying combined bacterial biofilms and 7–26% more effective in destroying bacterial-yeast biofilms compared to the effect of surfactants obtained using a monoculture.

Table 6

Effect of surfactants on bacterial combined biofilms

Test cultures	Physiological state of the inducer	Destruction (%) under the action of the surfactants (μg/ml)									
		640	**320**	**160**	**80**	**40**	**20**	**10**	**5**	**2.5**	**1.25**
Bacillus subtilis BT-2 + *Pseudomonas* sp. MI-2	Live cells	69	61	53	52	49	43	39	40	31	35
	Inactivated cells	58	50	48	48	48	41	31	31	31	28
	Supernatant	72	63	67	60	59	39	39	36	38	33
	Control	52	53	45	49	40	38	27	20	23	18
Escherichia coli IEM-1 + *Staphylococcus aureus* BMS-1	Live cells	68	68	56	49	46	41	40	32	35	38
	Inactivated cells	52	53	47	44	32	36	33	29	28	26
	Supernatant	67	63	54	46	47	48	37	33	33	27
	Control	51	50	44	33	32	29	23	18	19	12

Note. Tables 6-7: during the determination of the degree of biofilm destruction, the error did not exceed 5%.

Table 7

Action of surfactants on bacterial-yeast dual-species biofilms

Test cultures	Physiological state of the inducer	Destruction (%) under the action of the surfactants (μg/ml)									
		640	**320**	**160**	**80**	**40**	**20**	**10**	**5**	**2.5**	**1.25**
Pseudomonas sp. MI-2 + *Candida albicans* D-6	Live cells	79	76	61	62	54	50	46	31	27	23
	Inactivated cells	66	57	56	49	42	40	36	30	26	19
	Supernatant	80	71	67	60	61	51	49	35	39	24
	Control	56	47	41	46	40	33	26	20	20	17
Staphylococcus aureus BMS-1 + *Candida utilis* BVS-65	Live cells	68	52	48	48	47	40	38	33	30	27
	Inactivated cells	60	45	48	42	40	33	35	23	25	20
	Supernatant	65	53	42	45	43	43	42	38	35	22
	Control	55	42	42	32	30	25	23	17	18	13

The degree of destruction of dual-species biofilms under the action of surfactants obtained in the presence of inactivated yeast cells increased by only 1-15% compared to the control.

The maximum degree of destruction, 53-72% and 60-80% for bacterial and bacterial-yeast biofilms, respectively, was observed under the influence of high concentrations (320-640 μg/mL) of surfactants synthesized in the presence of the inducer. Overall, the action of surfactants synthesized in the presence of yeast was more specific to dual-species biofilms of yeast with Gram-negative bacteria, as explained by the results of studies on the destruction of single-species biofilms.

Thus, the experimental data obtained (Tables 6-7) show the possibility of significantly enhancing the ability of *R. erythropolis* IMV Ac-5017 surfactants to destroy combined biofilms by adding live *S. cerevisiae* BTM-1 cells, as well as the corresponding supernatant, to the cultivation medium of the producer.

In the available literature, there is little information regarding the use of microbial surfactants to combat combined biofilms. For example, in the study Ceresa et al. (2021), it was shown that the action of rhamnolipid AC7BS produced by *P. aeruginosa* 89 on a dual-species biofilm of *C. albicans* ATCC 10231 with *S. aureus* ATCC 25923 resulted in 94% growth inhibition. In the study Keyhanian et al. (2023), after treating biofilms of *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 with a mixture of *Bacillus cereus* and *Serratia nematodiphila* (strain numbers not specified) surfactants, their degree of destruction was 60% and 80%, respectively, while the individual components of the agent were less effective.

Consequently, after analyzing the available literature data, it can be concluded that the *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of the yeast is comparable in biofilm-inhibitory action to other antimicrobial agents.

Conclusions

The obtained results indicate the possibility of a significant increase in the biological activity of *R. erythropolis* IMV Ac-5017 surfactant, provided that it is synthesized in a medium with *S. cerevisiae* BTM-1. Furthermore, the activity of the surfactants produced in the presence of the inducer was more specific towards yeast test cultures, which can be explained by the nature of the studied inducer.

The introduction into the culture medium of *R. erythropolis* IMV Ac-5017 *S. cerevisiae* BTM-1 in different physiological states (living cells, as well as the corresponding supernatant) was accompanied by the synthesis of surfactants, the MICs of which in relation to bacterial and yeast test cultures were one to two orders of magnitude lower, the destruction of single-species bacterial and yeast biofilms by 9–59%, and of two-species bacterial-bacterial and bacterial-yeast biofilms by 1-29% higher compared to the indicators established for surfactants obtained without an inductor.

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