Synergetic antimicrobial activity of a mixture of essential oils and *Acinetobacter calcoaceticus* IMV B-7241 surfactants synthesized in the presence of the eukaryotic inducer

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Abstract

Introduction. The research was devoted to study the synergetic antimicrobial effect and role in the destruction of biofilms under the action of a mixture of tea tree or cinnamon essential oil with surfactant *Acinetobacter calcoaceticus* IMV B-7241, synthesized in the presence of a yeast inducer.

Materials and methods. Cultivation of *A. calcoaceticus* IMV B-7241 was carried out in a liquid mineral medium with purified or crude glycerol. The yeast *Saccharomyces cerevisiae* BTM-1 was used as the inducer. The concentration of the surfactant was determined after extraction from the supernatant by the gravimetric method. The antimicrobial activity of essential oils, surfactants and their mixtures was analysed according to the minimum inhibitory concentration (MIC).

Results and discussion. Surfactants was produced by the *A. calcoaceticus* IMV B-7241 strain cultivated on glycerol of various qualities in the presence of the eukaryotic inducer. The antibacterial activity against *Staphylococcus aureus* BMS-1, *Enterobacter cloacae* C-8, *Proteus vulgaris* PA-12, *Bacillus subtilis* БТ-2; antifungal activity against *Candida albicans* D-6 and *C. tropicalis* PE-2, and the ability to destruct the biofilms by a mixture of microbial surfactants and tea tree or cinnamon essential oil was studied. MIC of a mixture of tea tree or cinnamon essential oil and surfactants synthesized by the *A. calcoaceticus* IMV B-7241 strain both on purified and crude glycerol in the presence of eukaryotic inducer against studied bacteria and yeast were by 15-960 and 2.2-40 times lower, respectively, than the MICs of individual essential oils and surfactants. Using a mixture of tea tree essential oil and surfactants, synthesized by IMV B-7241 strain on purified glycerol in the presence of live yeast cells, increased the degree of bacterial biofilm destruction by 1.1–4.2 times, respectively, compared with individual compounds. Using a complex of tea tree essential oil and surfactants obtained in medium with crude glycerol with a yeast inducer increased the degree of bacterial biofilms destruction by 1.2–4.1 times compared with individual compounds. Destruction of yeast biofilms under the action of a mixture of cinnamon essential oil and surfactants, synthesized in the presence of the inducer in a medium with glycerol of different quality, was 1.2–2.1 and 1.1–2.4 times higher, respectively, than this indicator established for individual compounds.

Conclusions. The results showed the synergistic effect biological activity including the destruction of biofilms of mixture of tea tree or cinnamon essential oil with surfactants *A. calcoaceticus* IMV B-7241, obtained in the presence of live cells *S. cerevisiae* BTM-1 in producer culture medium.

Introduction

Every year, the availability of antibiotic therapy provokes an increase in the incidence of resistance to commercial antibiotics and, as a result, leads to the emergence of multi-resistant microorganisms (Huemeret al., 2020). Difficulties in treating infections caused by multidrug-resistant pathogens force researchers to search for new antimicrobial substances with different mechanisms of action, and, if possible, with fewer side effects on human health (Gan et al., 2023). Essential oils are a source of new natural antimicrobial molecules with a wide spectrum of effects on gram-positive and gram-negative bacteria, fungi, and viruses. The use of such natural compounds complicates or eliminates the possibility of resistance in microorganisms, which is due to the absence of corresponding mutations to all active molecules of various chemical natures simultaneously present in essential oils (Feudjieu et al., 2023).

Microbial surfactants with controlled biological activity (antimicrobial, antiadhesive) are also effective natural antimicrobial agents. An effective mechanism for regulating the activity of such compounds is the introduction of competitive microorganisms (inducers) into the cultivation medium of their producers. Thus, in the literature, the majority of studies are devoted to the use of bacterial inducers to increase the concentration of surfactants (Alves et al., 2019; Chen et al., 2022) and/or their biological activity (Alves et al., 2019; Gómezet al., 2016; Hamza et al., 2018). At the same time, the inducer bacteria used by most researchers are pathogenic or opportunistic, while there is no information on the use of non-pathogenic representatives, in particular yeast of the genus *Saccharomyces*, as inducers for regulating the biological activity of surfactants.

It should be noted that the use of a mixture of two or more natural antimicrobial agents opens up promising opportunities for their use in the treatment of infectious diseases, since combinations of synergistic compounds can reduce the likelihood of resistance while showing effective pharmacological results (Cheesman et al., 2017).

In recent years, a fairly large number of studies have appeared in the literature on the synergistic biological activity of essential oils with antibiotics (Asadi et al., 2023; Iseppi et al., 2023a; Parker et al., 2022) and surfactants (Haba et al., 2014), while at the same time there is no data on the use of essential oils with surface-active compounds synthesized by bacterial producer in the presence of inducers, in particular yeast, in the cultivation medium.

Therefore, the aim of this study is to investigate the synergistic antibacterial and antifungal effects and ability to destroy biofilms by a complex of essential oils with surfactants *A. calcoaceticus* IMV B-7241, synthesized in the presence of *Saccharomyces cerevisiae* BTM-1.

Materials and methods

Objects of research

The main object of research was oil oxidizing bacteria strain *Acinetobacter calcoaceticus* IMV B-7241 from Microorganisms Depositary of Institute of Microbiology and Virology, the National Academy of Sciences of Ukraine (Pirog et al., 2022). Yeast *Saccharomyces cerevisiae* BTM-1 was used as a biological inducer.

To determine the antimicrobial and antiadhesive activity of surfactants, as well as their ability to destroy microbial biofilms, bacteria (*Staphylococcus aureus* BMS-1, *Enterobacter cloacae* C-8, *Proteus vulgaris* PA-12, *Bacillus subtilis* BT-2) and yeast (*Candida albicans* D-6 and *C. 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 as test cultures.

Cultivation of the producer of surfactants

The strain *Acinetobacter calcoaceticus* IMV B-7241 was grown in a liquid mineral medium of the following composition (g/l): $(NH_2)_2CO$, 0.35, NaCl, 1.0, $Na_2HPO_4 \cdot 12H_2O$, 0.6, KH_2PO_4, 0.14, $MgSO_4 \cdot 7H_2O$, 0.1, distilled water up to 1 litre, pH 6.8–7.0. Yeast autolysate, 0.5% (v/v), and microelement solution, 0.1% (v/v), containing (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_3BO_3, 0.006; KI, 0.0001; EDTA (Trilon B), 0.5. Purified glycerol, 3% (v/v) and crude glycerol, which is a waste of biodiesel production, 5% (v/v) were used as a carbon source. The concentration of carbon in medium with pure glycerol was the same as in medium with crude glycerol (Pirog et al., 2023). Inoculum was produced by the cultivation of the strain in the basic medium with 0.5% of the appropriate substrate. The cultural liquid with a concentration of bacterial cells from 10^4 to 10^5 cells/ml was taken from the exponential growth phase and added to the medium for the strain cultivation, 5% (v/v). Cultivation was carried out in 750 ml Erlenmeyer flasks with 100 ml of medium for 120 hours in a shaking incubator at 30 °C and 320 rpm.

Cultivation of eukaryotic inducer

The yeast *Saccharomyces cerevisiae* BTM-1 was grown in a liquid mineral medium containing glucose (0.5%) as a carbon source for 24 hours in a shaking incubator at 30 °C and 320 rpm. After 24 hours, the liquid culture was poured into sterile eppendorf tubes and centrifuged at 10,000 g for 10 min using Eppendorf 5417R Refrigerated Centrifuge. The biomass was resuspended in sterile tap water to the volume taken for centrifugation. Resuspended biomass (live inductor cells) was added in the amount of 2.5 ml of suspension per 100 ml of surfactant producer culture medium at the beginning of the cultivation process. Cultivation of strains was carried out in 750 ml flasks with 100 ml of medium for 7 days in a shaking incubator at 30 °C and 320 rpm.

Determination of extracellular surfactants concentration

The surfactant concentration was determined by the Blay and Dyer method (Pirog et al., 2020a) in our modification. Since *A. calcoaceticus* IMV B-7241 synthesizes a complex of polar and non-polar lipids, and the well-known Blay and Dyer method used to isolate surfactants allows the isolation of mainly non-polar lipids, we modified the classical solvent system (Folch mixture) by adding 1 M HCl (chloroform - methanol - water = 4:3:2). This system allows to fully isolate both polar and non-polar lipids.

The supernatant, 25 ml (to obtain a supernatant, the culture broth was centrifuged at 5000 g for 20 minutes) was placed in a 100 ml cylindrical separating funnel and surfactants were extracted according to the advanced procedure described below. Firstly, 5 ml of 1M HCl was added and shaken for 5 min, then 20 ml of a modified Folch mixture (16 ml of Folch reagent and 4 ml of 1M HCl) was added immediately and shaken again for 5 min. The mixture obtained after extraction was left in a separating funnel to separate the phases, then the lower fraction was drained (organic extract 1) and the aqueous phase was re-extracted. After re-extraction, 25 ml of the modified Folch mixture was added to the aqueous phase again (but at once 16 ml of Folch reagent and 9 ml of 1M HCl) and extracted with shaking for 5 min. After phase separation, the lower fraction was poured off to obtain organic extract 2. The extraction was repeated once more using a standard Folch mixture (chloroform: methanol = 2:1), and organic extract 3 was obtained. The extracts 1-3 were combined and evaporated on an IP1-M2 rotary evaporator at 50 °C and an absolute pressure of 0.4 atm to constant weight.

Determination of antimicrobial activity

The antimicrobial effect of essential oil, surfactants and their mixtures was analysed using the minimum inhibitory concentration (MIC) as described in (Pirog et al., 2020b). Essential oils of tea tree and cinnamon were dissolved in 5% ethyl alcohol to a concentration of 500 μg/ml. MIC determination was carried out by the method of two-fold serial dilutions in meat-peptone broth (MPB) for bacteria and liquid wort for yeast.

Under sterile conditions, 1 ml of the medium was added to 10 tubes, 1 ml of an antimicrobial substance (surfactant or essential oils) of a certain concentration was added to the first tube, after which it was mixed, 1 ml was taken and transferred to the next tube. Similarly, the dilution was carried out for the next nine tubes. 1 ml was taken from the last tube. Thus, the final volume in each test tube was 1 ml, and the concentration of surfactants, antifungal substances, or essential oils in each subsequent tube decreased by 2 times. As a control, 1 ml of meat-peptone broth or liquid wort without the addition of a solution of antimicrobial substances was used. Then, 0.1 ml of the test culture suspension (10^5–10^6 CFU/ml) was added to each of the tubes and mixed. The tubes were incubated for 24 hours at 24–26°C. The results were evaluated visually by the turbidity of the medium: (+) - test tubes in which the turbidity of the medium was observed (growth of the test culture), (−) - there was no turbidity (no growth), The minimum inhibitory concentration of antimicrobial substances was determined as the value of the concentration of the studied substances in the first test tube, where there was no growth.

When determining the MIC of a mixture of drugs, their ratio was 1:1, while in one of the options the concentration of surfactants remained unchanged, and the concentration of essential oil was reduced by the method of successive two-fold dilutions, in the other, the concentration of essential oil remained unchanged, and the concentration of surfactant reduced.

Synergistic effect of surfactants with essential oils was evaluated by indicator of fractional inhibitory concentration (FIC), which is the sum of the ratio of the concentration of each substance in a mixture with their minimum inhibitory concentration (Hallander et al., 1982). FIC is calculated by the formula

$$FIC = (C_A/MIC_A) + (C_B/MIC_B),$$

where C_A and C_B are the concentrations of the antimicrobial substance in the mixture;

MIC_A and MIC_B are minimum inhibitory concentrations of antimicrobial substance.

Determination of the degree of biofilms destruction

The study of the effect of surfactant, essential oil and their mixture on biofilm destruction was carried out as described in (Pirog et al., 2020b). To form a biofilm, 180 μl of meat-peptone broth or liquid wort and 20 μl of a suspension of a one-day test culture were added to polystyrene microplates and incubated for 24 hours at the temperature optimal for the test culture. After this, the culture liquid was drained and 180 μl of fresh MPB (liquid wort) and 20 μl of test culture suspension were added and re-incubated for the next 24 hours.

After 48 hours, the culture liquid was drained, and 200 μl of biocidal preparations (surfactant, essential oil and their mixture) of different concentrations (15.6–500 μg/ml) were added to the wells of the microplate (with the biofilm of the test culture previously formed on them). To study synergism, use surfactants and essential oil in a 1:1 ratio. Instead of drugs, sterile tap water (200 μl) was added to the control wells. After 24 hours of exposure, the wells were washed three times with 200 μl of distilled water and the number of adhered cells was determined by the spectrophotometric method. The degree of biofilm destruction (%) was

determined as the difference between cell adhesion in untreated and treated surfactant, essential oil or their mixture wells of a polystyrene tablet.

Results and discussion

Antimicrobial and antifungal activity of a complex of surfactants and essential oils

At the first stage, the effect of live inducer cells on the synergism of the antimicrobial activity of surfactants synthesized by the *A. calcoaceticus* IMV B-7241 strain on glycerol of various qualities was studied (Table 1).

Table 1

Minimum inhibitory concentrations of surfactants *A. calcoaceticus* IMV B-7241 synthesized on glycerol of different quality in the presence of live cells of *S. cerevisiae* BTM-1, tea tree essential oil and their mixture

Test culture	Glycerol as substrate	Presence of inductor in the medium	MIC (μg/ml) of			
			Surfactants	*Surfactants mixed with essential oil	**Essential oil mixed with surfactants	***FIC
Enterobacter cloacae C-8	purified	−	100.0	25	16.87	0.38
		+	16.8	2.1	31.25	0.37
	crude	−	185.0	2.9	3.90	0.05
		+	157.5	2.4	3.90	0.05
Proteus vulgaris PA-12	purified	−	200.0	25	31.25	0.62
		+	33.7	4.2	15.63	0.37
	crude	−	185.0	5.7	15.63	0.28
		+	78.7	1.2	15.63	0.26
Bacillus subtilis BT-2	purified	−	100.0	3.1	31.25	0.15
		+	67.5	0.53	15.63	0.07
	crude	−	315.0	11.5	15.63	0.10
		+	315.0	1.23	15.63	0.07
Staphylococcus aureus BMS -1	purified	−	25.0	0.78	31.25	0.50
		+	16.8	0.26	31.25	0.52
	crude	−	92.5	1.44	15.63	0.27
		+	78.7	1.23	15.63	0.27

Notes: The MIC of tea tree essential oil against *B. subtilis* BT-2 and *E. cloacae* C-8 was 250 and 125 μg/ml, respectively, against *P. vulgaris* PA-12 and *S. aureus* BMS-1–62.5 μg/ml;

* the concentration of tea tree essential oil was unchanged and equalled their ½ MIC, and the concentration of surfactants was reduced by sequential double dilutions in the concentration range of 200–0.19 and 268–0.26 μg/ml for surfactants synthesized on purified glycerol by presence and without inductor respectively; 315–0.30 and 370–0.36 μg/ml for surfactants synthesized on crude glycerol by presence and without inductor respectively.

**the concentration of surfactants remained unchanged and equalled their ½ MIC, and the concentration of tea tree essential oil was reduced by sequential double dilutions in the concentration range of 500–0.48 μg/ml.

*** - FIC ≤ 0.5 indicates synergism

It was found that surfactants synthesized in the presence of *S. cerevisiae* BTM-1 cells in the cultivation medium of *A. calcoaceticus* IMV B-7241 with both purified glycerol and crude glycerol showed a synergistic antibacterial effect with tea tree essential oil. For example, the MIC of surfactants synthesized in the presence of inducer cells in medium with purified glycerol against *P. vulgaris* PA-12 and *S. aureus* BMS-1 was 33.7 and 16.8 μg/ml, with tea tree essential oil it was 62.5 μg/ml, and with their mixtures it was 15.6 and 31.2 μg/ml, respectively. The use of surfactants obtained after cultivation of *A. calcoaceticus* IMV B-7241 in the presence of live cells of *S. cerevisiae* BTM-1 on crude glycerol in combination with an essential oil makes it possible to reduce the MIC of the latter against *P. vulgaris* PA-12 and *S. aureus* BMS-1 from 62.5 μg/ ml to 15.6 μg/ml (Table 1).

It is worth noting that the index of the fractional inhibitory concentration did not exceed 0.5 (with the exception of the FIC for the mixture of surfactants synthesized on purified glycerol without an inducer with tea tree oil for strain PA-12), which indicates the synergism of the antimicrobial action of such compounds.

Table 2 presents the results the antifungal action of surfactants synthesized in the presence of live *S. cerevisiae* BTM-1 cells, cinnamon essential oil, and their mixture.

Table 2

Antifungal activity of surfactants *A. calcoaceticus* IMV B-7241 synthesized on glycerol of different quality in the presence of live cells of *S. cerevisiae* BTM-1, cinnamon essential oil and their mixture

Test culture	Glycerol as substrate	Presence of inductor in the medium	MIC (μg/ml) of			
			Surfactants	*Surfactants mixed with essential oil	**Essential oil mixed with surfactants	***FIC
Candida albicans D-6	purified	−	12.5	0.39	62.5	0.25
		+	8.4	0.26	31.2	0.12
	crude	−	185	1.44	62.5	0.25
		+	157.5	0.62	31.2	0.12
Candida tropicalis PE-2	purified	−	12.5	0.39	62.5	0.25
		+	8.4	0.26	15.6	0.07
	crude	−	46.2	1.44	62.5	0.25
		+	39.4	0.15	15.6	0.07

Notes: The MIC of cinnamon essential oil against *Candida albicans* D-6 and *Candida tropicalis* PE-2 was 250 μg/ml.

*the concentration of cinnamon essential oil was unchanged and equaled their ½ MIC, and the concentration of surfactants was reduced by sequential double dilutions in the concentration range of 200–0.19 and 268–0.26 μg/ml for surfactants synthesized on purified glycerol by presence and without inductor respectively; 315–0.30 and 370–0.36 μg/ml for surfactants synthesized on crude glycerol by presence and without inductor respectively.

**the concentration of surfactants remained unchanged and equaled their ½ MIC, and the concentration of cinnamon essential oil was reduced by sequential double dilutions in the concentration range of 500–0.48 μg/ml.

*** FIC ≤ 0.5 indicates synergism.

It was found that the minimum inhibitory concentrations of surfactants obtained by introducing live yeast cells into the medium of A. calcoaceticus IMV B-7241 with purified glycerol against C. albicans D-6 and C. tropicalis PE-2 were 8.4 μg/ml, with cinnamon essential oil it was 250 μg/ml, and with their mixtures it was 31.2 and 15.6 μg/ml,

respectively. In particular, using surfactants in combination with essential oil, the MIC of the latter was reduced to 0.26 μg/ml. Similar patterns were observed in the case of the use of surfactants synthesized in a medium containing crude glycerol in the presence of a eukaryotic inducer. The MIC values of such surfactants in a mixture with essential oil against yeast of the genus *Candida* were by 2.5–5 times lower than the minimum inhibitory concentrations of monosurfactant preparations.

The FIC index relative to yeast test cultures did not exceed 0.5, which indicates the synergism of the antifungal effect of surfactants synthesized by introducing a eukaryotic inducer into the environment of *A. calcoaceticus* IMV B-7241 with cinnamon essential oil against yeast of the genus *Candida*.

As noted above, there are no data in literature on the synergistic effect of essential oils with microbial secondary metabolites synthesized in the presence of biological inducers. However, there is work on the synergistic antimicrobial effects of essential oils or surfactants with other antimicrobial secondary metabolites such as antibiotics (Aleksic Sabo et al., 2021; Asadi et al., 2023; El Atki et al., 2019; Gan et al., 2023; Iseppi et al., 2023b; Özel et al., 2022;) and antifungal agents (Angiolella, 2021; Essid et al., 2017; Parkeret al., 2022; Sharifzadeh et al., 2017). For example, in a study (Asadi et al., 2023), it was found that a mixture of carvacrol, a component of cumin essential oil, and cefixime have a synergistic antimicrobial effect against *E. coli*, which was confirmed by the FIC index (≤ 0.5).

The study (Özelet al., 2022) showed a synergistic effect of the components of cumin essential oil with tigecycline, linezolid, gentamicin and tetracycline against bacterial test cultures. Thus, for the complex of tigecycline with carvacrol, a synergistic effect was established against both *E. coli* and *P. aeruginosa* (FIC value did not exceed 0.25). When using a mixture of linezolid with both thymol and eugenol relative to *E. coli*, the fractional inhibitory concentration value was 0.375. Complex preparations of carvacrol with gentamicin and eugenol with tigecycline demonstrated a synergistic effect against *P. aeruginosa*, the FIC value was 0.375.

It was shown the synergistic antimicrobial activity of tea tree and eucalyptus essential oils with oxacillin against various strains of *S. aureus* (Iseppi et al., 2023b). Thus, under the action of a complex of tea tree essential oil and oxacillin against *S. aureus* 13 and *S. aureus* 20, the FIC was 0.19. At that time, the value of the fractional inhibitory concentration of a mixture of both oxacillin with tea tree oil and eucalyptus oil relative to *S. aureus* 32 did not exceed 0.5.

The work (Gan et al., 2023) recorded the synergism of antimicrobial activity (FIC 0.375) of a mixture of thymol essential oil with both streptomycin and gentamicin against *S. aureus*, as well as this ester with chloramphenicol against *Acinetobacter baumannii*. The authors also noted that the MIC of antibiotics decreased from 62.5 to 7.8 μg/ml.

El Atki et al. (2019) showed a synergistic antibacterial effect of cinnamon essential oil with antibiotics against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. Thus, the FIC value for a mixture of cinnamon essential oil with chloramphenicol was 0.5 for each test culture. The complex of this essential oil with ampicillin demonstrated a synergistic effect against *S. aureus* ATCC 25923, with an inhibitory fractional concentration of 0.38. In addition, the minimum inhibitory concentrations of each antimicrobial compound in the mixture were 2-4 times lower compared to MICs for both the essential oil and antibiotics used alone.

The synergistic effect of essential oils with antifungal agents was studied in the numerous research (Angiolella, 2021; Essid et al., 2017; Parker et al., 2022; Sharifzadeh et al., 2017). Thus, in the work (Angiolella, 2021) it was shown that when using a mixture of pelargonium essential oil with fluconazole, a synergistic antifungal effect was observed against all tested strains of *C. albicans* with FIC values of 0.5. It was also reported that the

MIC of each antimicrobial compound in such a mixture decreased to 390 mg /ml and 0.0625 mg/ml, respectively.

Another study (Essid et al., 2017) also investigated the synergistic effect of various combinations of essential oils with fluconazole on *Candida* yeasts. It was found that when using complexes of fluconazole with essential oils of *Pelargonium graveolens* and *Cinnamomum verum*, the FIC value was 0.37 for each complex, respectively. Such combinations made it possible to reduce the effective concentrations of selected biocides by 4-8 times.

Parker et al. (2022) found that using a mixture of clove or lemongrass essential oils with antibiotics flucytosine, fluconazole and micafungin can achieve a synergistic antifungal effect against yeast test cultures. A complex preparation of clove essential oil with fluconazole demonstrated synergistic antifungal activity against *C. auris* AR0381, *C. lusitaniae* AR0398 with FIC values of 0.28; 0.0625 respectively.

Sharifzadeh with co-authors (2017) showed that a mixture of menthol essential oil combined with itraconazole or nystatin showed a synergistic effect against all *Candida* species tested. FIC values for combinations of menthol with itraconazole and nystatin ranged from 0.250 to 0.561 and 0.139 to 0.623 against *C. glabrata* strains and from 0.182 to 0.750 and 0.188 to 0.760 against *C. krusei*, respectively.

Only a small number of research are devoted to the study of the synergistic antimicrobial action of the surfactant complex with essential oils (Haba et al., 2014; Pirog et al., 2020b) or antibiotics (Olfa et al., 2015; Pirog et al., 2020c). Olfa et al. (2015) studied the antimicrobial activity of the complex of bacillomycin D, synthesized by *B. subtilis* B38, with amphotericin B against *Candida* yeasts. It was found that under the action of a mixture with an antibiotic, it was possible to reduce the MIC of surfactants by 8-25 times against all tested yeast strains. The FIC of bacillomycin D with amphotericin B was from 0.27 to 0.5, which indicates the synergism of the compounds.

Pirog et al. (2020b) studied the synergistic effect of cinnamon and lemongrass essential oils and the surfactant of the *Nocardia vaccinii* IMV B-7405 against yeast of the *Candida* genus. It was found that surfactants obtained in a medium with refined or waste sunflower oil have a synergistic antimicrobial effect when mixed with essential oils of cinnamon and lemongrass. The use of such a mixture against *C. albicans* D-6 and *C. tropicalis* RE-2 led to a decrease not only in the MIC of surfactants (from 16–78 to 4–19 μg/ml), but also in a decrease in the MIC values of essential oils in 8–130 times.

Consequently, the results obtained indicate that surfactants synthesized by introducing live *S. cerevisiae* BTM-1 cells into the cultivation medium of *A. calcoaceticus* IMV B-7241 with glycerol of various qualities demonstrate a synergistic antimicrobial effect in combination with essential oils. The use of a mixture of such surfactants with these biocidal compounds makes it possible to reduce the MIC of the surfactants themselves and essential oils by 2–47 and 2.5–250 times, respectively.

Destruction of microbial biofilms under the action of a complex of surfactants with essential oils

The mechanisms of biofilm destruction under the action of microbial surfactants are based on the antimicrobial action of such compounds (Sharma et al., 2019). Therefore, at the next stage of research, the ability to destroy microbial biofilms through the action of a mixture of essential oils with surfactants of *A. calcoaceticus* IMV B-7241, synthesized by introducing live cells of *S. cerevisiae* BTM-1 into a medium with glycerol of various degrees of purification was studied (Tables 3–4).

Table 3

Destruction of biofilms of bacterial test cultures under the action of surfactants synthesized by *A. calcoaceticus* IMV B-7241 in the presence of live cells of *S. cerevisiae* BTM-1, tea tree essential oil and their mixture

Test culture	Anti-microbial compound	Glycerol as substrate	Presence of inductor	Destruction of biofilm (%), concentration of antimicrobial compounds (μg/ml)					
				500	250	125	62.5	31.2	15.6
Staphylococcus aureus BMS -1	surfactants	purified	–	80	60	50	41	36	28
			+	86	71	62	54	41	22
		crude	–	63	44	30	34	26	15
			+	100	83	51	38	41	21
	mixture of surfactant and tea tree essential oil	purified	–	84	50	47	34	30	24
			+	100	88	70	59	46	40
		crude	–	86	60	52	38	33	17
			+	92	89	60	45	42	22
Bacillus subtilis BT-2	surfactants	purified	–	100	100	94	72	60	50
			+	68	78	80	90	100	100
		crude	–	75	72	70	69	55	42
			+	72	75	72	100	100	32
	mixture of surfactant and tea tree essential oil	purified	–	100	92	88	85	57	60
			+	44	63	69	78	100	100
		crude	–	100	86	72	53	40	32
			+	47	63	72	75	94	98

Note: The degree of *S. aureus* BMS-1 and *B. subtilis* BT-2 biofilms destruction under the action tea tree essential oil in the range of studied concentrations (500–15.6 μg/ml) was 13–59 and 24–72%, respectively.

It was found that when using a mixture of surfactants with essential oils of tea tree and cinnamon, it was possible to increase the degree of destruction of both bacterial and yeast biofilms of most of the test cultures studied compared to the effect of single preparations of surfactants and essential oils. For example, the degree of destruction of biofilms under the action of a tea tree essential oil complex from surfactants synthesized by adding an inducer to a medium with purified glycerol against to the bacteria S. aureus BMS-1 was 1.1 to 3.1 times higher compared to the use of monopreparations surfactants and essential oil (Table 3).

Similar patterns were observed when using a mixture of essential oil and surfactant obtained on crude glycerol in the presence of live cells of S. cerevisiae BTM-1. At the same time, the degree of destruction of the bacterial biofilm when using such a mixture was higher by 1.1 and 1.3–1.7 times for the indicators established separately for the surfactant and essential oil, respectively (Table 3).

It should be noted that with a decrease in the concentration of surfactants synthesized by adding live yeast cells to a medium with glycerol of varying degrees of quality, and used both separately and in mixture with tea tree oil, the degree of B. subtilis BT-2 biofilms destruction gradually increased (Table 3). Thus, under the influence of surfactants obtained during growth on a medium with purified glycerol in the presence of S. cerevisiae BTM-1, in a mixture with essential oil (concentration 15.6–31.25 μg/ml), the degree of destruction of the biofilm of strain BT-2 reached 100%, which was 2.4–4.2 times higher than that established for monopreparations surfactants and essential oils.

Similar patterns were observed when using a complex of essential oil and surfactants obtained during the cultivation of the producer on crude glycerol with the addition of live inducer cells. With such a mixture in the concentration range of 15.6–31.2 μg/ml, the degree of *B. subtilis* BT-2 biofilm destruction was 94–98%.

It was found that with increasing concentration of the tested antimicrobial compounds, both in the mixture and separately, the degree of destruction of *Candida* yeast biofilms increases (Table 4).

Table 4

Destruction of *Candida* biofilms under the action of surfactants synthesized by *A. calcoaceticus* IMV B-7241 in the presence of live *S. cerevisiae* BTM-1 cells, cinnamon essential oil and their mixture

Test culture	Antimicrobial compound	Glycerol as substrate	Presence of inductor	Destruction of biofilm (%) by action, concentration of antimicrobial compounds (μg/ml)					
				500	250	125	62.5	31.2	15.6
Candida, albicans D-6	surfactants	purified	−	82	68	58	45	34	33
			+	92	84	80	83	73	68
		crude glycerol	−	77	71	69	57	46	34
			+	100	100	92	69	60	51
	mixture of surfactant and cinnamon essential oil	purified	−	86	70	62	50	41	34
			+	100	100	100	94	80	76
		crude glycerol	−	70	60	50	30	19	14
			+	78	62	53	32	27	18
Candida tropicalis PE-2	surfactants	purified	−	35	37	31	25	14	15
			+	46	39	35	35	33	28
		crude glycerol	−	43	38	27	22	16	15
			+	55	41	33	23	25	17
	mixture of surfactant and cinnamon essential oil	purified	−	54	48	42	35	26	19
			+	58	65	67	56	50	40
		crude glycerol	−	60	50	47	36	21	18
			+	61	65	66	59	47	40

Note: The degree of *C. albicans* D-6 and *C. tropicalis* PE-2 biofilms destruction under the action of cinnamon essential oil in the range of studied concentrations (500–5.6 μg/ml) was 57–5 and 24–2%, respectively.

Thus, under the action of a complex of cinnamon essential oil and surfactants, synthesized in the presence of an inducer in the producer's cultivation medium with purified glycerol, at a concentration of 125–500 μg/ml, it was possible to achieve 100% destruction of C. albicans D-6 biofilms. However, under the influence of a complex of such essential oil from surfactants (15.6–500 μg/ml) formed on crude glycerol when adding live yeast cells, the degree of destruction of the *C. albicans* D-6 biofilm decreased by 1.3–2.8 and 1.2–3.2 times compared to the use of monosurfactants and essential oils, respectively (Table 4). It was found that under the influence of a mixture of surfactants synthesized in the presence of the BTM-1 strain in a medium with purified glycerol, with cinnamon essential oil, it was possible to increase the degree of *C. tropicalis* PE-2 biofilms destruction by 1.2–2.1 times, compared using each of the drugs separately (Table 4).

Similar results were obtained using surfactants synthesized on crude glycerol in the presence of a eukaryotic inducer. Thus, the degree of destruction of biofilms of the corresponding test culture was higher when using a complex preparation of such surfactants and cinnamon essential oil (concentration 500–15.6 μg/ml), compared with the indicators established for monopreparations of surfactants and oil in 1.1–2.5 and 1.3–2.2 times, respectively (Table 4).

Previously (Pirog et al., 2020b) synergism in the destruction of *C. albicans* D-6 and *C. tropicalis* PE-2 biofilms under the action of a mixture of surfactants synthesized by *Nocardia vaccinii* IMV B-7405 on refined and waste sunflower oil and cinnamon, lemongrass essential oils was observed. The degree of destruction of *C. albicans* D-6 and *C. tropicalis* PE-2 biofilms when using a mixture of these surfactants with essential oil was 1.5–2 times higher than the values established for individual compounds.

There are no studies in the available literature on the synergistic effect of essential oils with microbial secondary metabolites synthesized with the participation of biological inducers in the destruction of biofilms, including the ability of a mixture of surfactants with essential oils to destroy microbial biofilms. However, there are studies on the destruction of biofilms under the influence of essential oils mixed with antibiotics (Iseppi et al., 2023a; Miladi et al., 2017; Rosato et al., 2020).

Rosato et al. (2020) showed a synergistic effect of using a mixture of essential oils of cinnamon, peppermint and thymol in combination with antibiotics in the destruction of bacterial biofilms. Thus, the degree of *S. aureus* ATCC 29213 biofilms destruction under the action of oxacillin with peppermint or cinnamon essential oil was 68 and 73%, respectively, and was 16.8 or 19.2% higher than for the use of monopreparations.

Iseppi with co-authors (2023b) examined the ability to destroy bacterial biofilms under the influence of tea tree essential oil, the antibiotics cefotaxime, vancomycin, oxacillin and their mixture. It was found that when using a mixture of tea tree essential oil (32 μg/ml) and cefotaxime (16 μg/ml) or vancomycin (32 μg/ml), the degree of *E. coli* 22BT or *E. faecalis* VAN3 biofilms destruction increases by 40 or 47% compared with the use of monopreparations of these antibiotics. It is also noted that when exposed to a mixture of such essential oil with oxacillin, the degree of *S. aureus* biofilm destruction was 50% higher than when using only the antibiotic.

Miladi et al. (2017) found that thymol, eugenol and carvacrol (the main antimicrobial components of cumin essential oil) showed a synergistic effect with tetracycline in the destruction of *S. aureus* strains biofilms. Thus, the degree of *S. aureus* B193 biofilm destruction under the action of a mixture of tetracycline (12 μg/ml), as well as eugenol (250 μg/ml), carvacol (79 μg/ml) and thymol (85 μg/ml), was 50% and was greater compared to the use of single-agent essential oils and antibiotics.

Conclusions

The results obtained show the synergistic positive effect of using mixtures of microbial surfactants, synthesized by the *Acinetobacter calcoaceticus* IMV B-7241 strain on glycerol of various qualities in the presence of a eukaryotic inducer, and tea tree or cinnamon essential oils on their antimicrobial and antifungal activities and ability to destroy microbial biofilms.

Minimum inhibitory concentrations of a mixture of tea tree and cinnamon essential oils and surfactants synthesized by the *A. calcoaceticus* IMV B-7241 strain both on purified and crude glycerol in the presence of eukaryotic inducer against bacteria *S. aureus* BMS-1, *E. cloacae* C-8, *P. vulgaris* PA-12, *B. subtilis* БТ-2 and yeast *C. albicans* D-6 and *C. tropicalis*

PE-2 were by 15–960 and 2.2–40 times lower, respectively, than the MICs of monopreparations of essential oils and surfactants.

Under the action of a mixture of tea tree essential oil and surfactants synthesized by the *A. calcoaceticus* IMV B-7241 strain on glycerol of different quality in the presence of live yeast cells, it was possible to increase the degree of bacterial biofilms destruction by 1.2–4.2 times, compared to the indicators established for individual antimicrobial compounds. The degree of yeast biofilms destruction under the action of a mixture of cinnamon essential oil and bacterial surfactants was by 1.2–2.4 times higher compared to monopreparations of essential oil and surfactants.

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