# Antimicrobial activity of a mixture of surfactants produced by *Acinetobacter calcoaceticus* IMV B-7241 with antifungal drugs and essential oils

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## Abstract

**Introduction.** The aim of the work was to study the effect of a mixture of surfactants synthesized by *Acinetobacter calcoaceticus* IMV B-7241 under various cultivation conditions with antifungal drugs (clotrimazole and fluconazole) and essential oils (cinnamon and lemongrass) on yeast of genus *Candida*.

**Material and methods.** The cultivation of *A. calcoaceticus* IMV B-7241 was carried out in a basic medium that did not contain NaCl (medium 1), contained NaCl, 2.0 g/l (medium 2), contained NaCl, 2.0 g/l, and KCl, 1.0 g/l (medium 3). The surfactants were extracted from supernatant of cultural liquid by modified Folch mixture. Antimicrobial properties of the surfactants, antifungal drugs and essential oils were determined by index of the minimum inhibitory concentration (MIC). To assess the synergistic effect of a mixture of surfactants with antifungal drugs or essential oils the fractional inhibitory concentration index was used.

**Results and discussion**. Surfactants synthesized by *A. calcoaceticus* IMV B-7241 on the basic medium were the most effective antimicrobial agents against the yeasts strains *Candida albicans* D-6, *C. tropicalis* RE-2 and *C. utilis* BVS-65 with MIC 22.5–45 μg/ml that were 2.6–17 times lower than the values determined for surfactants synthesized on modified media. At the same time, regardless of the strain cultivation in different media, all surfactants showed synergism of antifungal activity with clotrimazole, fluconazole, cinnamon or lemongrass essential oils. Thus, in the presence of surfactants synthesized on basic and modified media in a mixture with antifungal drugs, MIC of clotrimazole and fluconazole against the studied yeast test cultures decreased by 4–32 times. The use of a mixture of essential oils with surfactants synthesized by *A. calcoaceticus* IMV B-7241 growing in different media made it possible to reduce MIC of cinnamon and lemongrass oils against yeasts of *Candida* genus 4–18 and 8–32 times, respectively. At the same time, the index of fractional inhibitory concentration did not exceed 0.5, which indicates the synergism of anifungal activity between the studied compounds.

**Conclusion**. The results confirm the possibility to reduce the minimum inhibitory concentrations of antifungal drugs or essential oils against members of genus *Candida* by their mixture with microbial surfactants.

## Introduction

The number of publications devoted to the study of yeast of genus *Candida,* which are causative agents of nosocomial infectious diseases, is increasing every year. This is due, first of all, to the spread of their resistant forms, arising against the background of prolonged use of broad-spectrum antibiotics, immunosuppressive therapy, and prolonged catheterization of patients (Singh et al., 2020).

Compared to antibacterial, the amount of antifungal agents is much less. This is mainly due to the fact that fungi are eukaryotes, so the development of new drugs for the selective control of such pathogens without toxic effects on humans is long-term and problematic (Pappas et al., 2016), So, at present, there are only five classes of drugs available for the treatment of fungal infections: azoles (fluconazole, miconazole, clotrimazole), polyenes (amphotericin, nystatin), echinocandins (micafungin, aspofungin, anidulafungin), allimines (terbinafine) and pyrimidine analogs (flucytosine) (Tsui et al., 2016), Compared to antibacterial agents, the number of antifungal agents is much smaller, and most clinical isolates of the genus *Candida* (in particular *C. albicans, C. tropicalis* and *C. glabrata*) are resistant to azoles, which are currently the most common medicine to treat fungal infections (Bhattacharya et al., 2020),

One of the approaches to increase the effectiveness of the use of existing antifungal compounds is the application of several drugs at once (for example, caspofungin and mycofungun) (Cui et al., 2015), zinc oxide nanoparticles and nystatin (Hosseini et al., 2020) and combination of antifungal drugs with essential oils or plant extracts (Jafri and Ahmad, 2020), At the same time, the concentration of such natural components should be minimal, which is associated with the ability of essential oils, when ingested, to cause severe damage to the central nervous system and aspiration pneumonia. This led to the search for methods to reduce the concentration of essential oils while maintaining their properties, in particular, their use in a mixture with other natural compounds, which can be microbial surfactants.

Interest in surfactants as antimicrobial agents is due to the unique mechanism of their action, which consists in violating the integrity of the cytoplasmic membrane and, due to this, practically excludes the possibility of the emergence of microorganisms resistant forms (Singh and Cameotra, 2004), Meanwhile the biological activity of microbial surfactants can be changed under different cultivation conditions, which should be taken into account when developing a technology for obtaining such metabolites. It was previously shown that the strain *Acinetobacter calcoaceticus* IMV B-7241 synthesizes surfactants having antimicrobial and antifungal activity (Pirog et al., 2021a; 2022), and it is possible to regulate their biological activity changing the potassium and sodium cations concentrations in the medium for cultivation (Pirog et al., 2016). These monovalent cations at high 50 and 100 mM concentrations are inhibitors of $NADP^+$-dependent glutamatedehydrogenase, a key enzyme in the biosynthesis of lipopeptides, which are the main antimicrobial agents, which ultimately resulted in low antimicrobial and antifungal activity of surfactants (Pirog et al., 2021). It was also found that surfactants synthesized by *Nocardia vaccinii* IMV B-7405 possessed synergistic antimicrobial activity against a wide range of yeasts and bacteria in mixure with antifungals drugs (nystatin and fluconazole) (Pirog et al., 2017) and essential oils (Pirog et al., 2020). So, it was assumed that it is possible to enhance the antifungal activity of surfactants synthesized by *A. calcoaceticus* IMV B-7241 in the presence of potassium and sodium cations in a mixture with antifungal agents and essential oils. This will simultaneously increase the efficiency of using not only surfactants as antimicrobial agents, but also antifungal drugs or essential oils, as well as reduce the concentration of components in the mixture.

The purpose of the present study was to investigate the possibility of synergistic action on the yeast of Candida genus of a mixture of surfactants, synthesized by Acinetobacter calcoaceticus IMV B-7241 in a medium with different contents of monovalent cations, with antifungal drugs and essential oils.

## 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.

Yeast *Candida albicans* D-6, *Candida utilis* BVS-65 and *Candida tropicalis* RE-2 from the collection of live cultures of the Department of Biotechnology and Microbiology of the National University of Food Technology were used as test cultures in determining the antimicrobial activity of surfactants, antifungal drugs or essential oils.

Clotrimazole and fluconazole, synthetic drugs belonging to the broad-spectrum azole class; essential oils of lemongrass (manufacturer Aromatika LLC, Ukraine) and cinnamon (manufacturer RosKosmetika LLC, Ukraine) were used as antifungal drugs.

### Composition of the nutrient medium and cultivation conditions

The strain *A. 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 liter, 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, were also added to the medium (basic medium). Cultivation of the strain IMV B-7241 was carried out in a basic medium that did not contain NaCl (medium 1), contained NaCl, 2.0 g/l (medium 2), contained NaCl, 2.0 g/l, and KCl, 1.0 g/l (medium 3), Used sunflower oil after frying potatoes at a concentration of 2% (v/v) was a source of carbon and energy**.** Seed material was a culture in the middle of the exponential growth phase, grown in base medium with 0.5% (v/v) used oil. The amount of inoculum was 5% of the medium volume ($10^4$–$10^5$ cells/mL), Cultivation was *carried out* in *flasks* (*750 ml*) with *100 ml* of *medium* in under rotation with *320 rpm* at *30* ºC for *120 hours.*

### Determination of extracellular surfactants concentration

The surfactant concentration was determined by the Blay and Dyer method (Bligh and Dyer, 1959) 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.

25 ml of the supernatant (to obtain a supernatant, the culture broth was centrifuged at 5000 g for 20 minutes) was placed in a 100 ml cylindrical separatory funnel and extracted surfactant according to the advanced procedure 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 (Russia) at 50 °C and an absolute pressure of 0.4 atm to constant weight.

**Determination of antimicrobial activity**

The antimicrobial activity of surfactants, antifungal drags, essential oils and their mixtures on yeast was determined by index of the minimum inhibitory concentration (MIC) (Andrews, 2001), Determination of MIC was carried out by the method of two-fold serial dilutions in liquid wort. Under sterile conditions, 1 ml of the medium was added to 10 tubes, 1 ml of an antimicrobial substance (surfactant, antifungal substances 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 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 antifungal drugs or oil was reduced by the method of successive two-fold dilutions, in the other, the concentration of essential oil or antifungal drugs remained unchanged, and the concentration of surfactant reduced.

**Determination of synergy of antifungal activity**

Synergistic effect of surfactants with antifungal drugs or essential oils and essential oils was evaluated by indicator of fractional inhibitory concentration (FIC) – 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 $C_A$ or $C_B$ are the concentrations of the antimicrobial substance in the mixture;

$MIC_A$ or $MIC_B$ are minimum inhibitory concentrations of antimicrobial substance.

## Results and discussion

### Determination of synergistic antifungal action of a mixture of surfactants produced by *A. calcoaceticus* IMV B-7241 and antifungal agents

Synergistic antifungal action of a mixture of surfactants produced by *A. calcoaceticus* IMV B-7241 mixed with clotrimazole (Table 1) or flucanazole (Table 2) was studied. These drugs were chosen due to the availability of data on the spread of resistant yeast of *Candida* genus against the background of the widespread use of azoles (Bhattacharya et al., 2020; Cui et al., 2015) and the possibility of combining antifungal drugs with natural compounds (Carbone et al., 2019; Kumar et al., 2013; Tabbene et al., 2015) including those of microbial origin (Tabbene et al., 2015),

It has been found that surfactants synthesized by *A. calcoaceticus* IMV B-7241 on the basic medium were the most effective antifungal agents, and the values of the minimum inhibitory concentrations in relation to the test yeast cultures were 22.5–45 μg/ml, which were lower than MIC of surfactants synthesized using modified media 1–3 (Table 1 and 2).

**Table 1**

**Antifungal activity of surfactants produced by *Acinetobacter calcoaceticus* IMV B-7241, clotrimazole and their mixtures**

| Media for surfactants synthesis | Test culture – yeast of genus *Candida* | MIC (μg/ml) of | | | ***FIC |
|---|---|---|---|---|---|
| | | Surfactants | *Surfactants mixed with clotrimazole | **Clotrimazole mixed with surfactants | |
| Basic | *C. albicans* D-6 | 22.5 | 5.6 | 1.9 | 0.28 |
| | *C. tropicalis* RE-2 | 22.5 | 5.6 | 3.9 | 0.30 |
| | *C. utilis* BVS-65 | 45 | 11.2 | 1.9 | 0.30 |
| Medium 1 | *C. albicans* D-6 | 608 | 38 | 3.9 | 0.06 |
| | *C. tropicalis* RE-2 | 304 | 19 | 3.9 | 0.12 |
| | *C. utilis* BVS-65 | 304 | 38 | 3.9 | 0.18 |
| Medium 2 | *C. albicans* D-6 | 118 | 29.5 | 15.6 | 0.49 |
| | *C. tropicalis* RE-2 | 118 | 14.7 | 15.6 | 0.36 |
| | *C. utilis* БМС-65 | 59 | 14.7 | 7.8 | 0.49 |
| Medium 3 | *C. albicans* D-6 | 769 | 96.1 | 3.9 | 0.18 |
| | *C. tropicalis* RE-2 | 384 | 48 | 7.8 | 0.24 |
| | *C. utilis* BVS-65 | 384 | 48 | 7.8 | 0.24 |

Notes:

*the concentration of clotrimazole was unchanged and equaled their ½ MIC, and the concentration of surfactants was reduced by sequential double dilutions in the concentration range of 180 – 0.17 μg/ml for surfactants synthesized on the basic medium; 608–1.1 μg/ml on medium 1; 236 – 0 .92 μg/ml on medium 2, and 768 – 1.5 μg/ml on medium 3.

**the concentration of surfactants was unchanged and equaled their ½ MIC, and the concentration of clotrimazole was reduced by sequential double dilutions in the concentration range of 250 – 0.9 μg/ml.

*** FIC ≤ 0.5 indicates synergism.

The minimum inhibitory concentration of clotrimazole against *C. albicans* D-6 and *C. tropicalis* RE-2 was 62.5 μg/ml, against *C. utilis* BVS-65 it was 31.2 μg/ml. Addition of all surfactants synthesized by *A. calcoaceticus* IMV B-7241 to the solution of clotrimazole reduced the MIC of this drug against *C. albicans* D-6, *C. tropicalis* RE-2 and *C. utilis* BVS-65 by 4-32 times. The value of the fractional inhibitory concentration did not exceed 0.5, which indicates synergism between the compounds.

Surfactants synthesized by *A. calcoaceticus* IMV B-7241 on different media also showed synergism of antifungal activity in mixture with fluconazole (Table 2).

**Table 2**

**Antifungal activity of surfactants produced by *Acinetobacter calcoaceticus* IMV B-7241, fluconazole and their mixtures**

| Media for surfactants synthesis | Test culture – yeast of genus *Candida* | MIC (μg/ml) of | | | ***FIC |
|---|---|---|---|---|---|
| | | Surfactants | *Surfactants mixed with fluconazole | **Fluconazole mixed with surfactants | |
| Basic | *C. albicans* D-6 | 22.5 | 5.6 | 4.6 | 0.36 |
| | *C. tropicalis* RE-2 | 22.5 | 5.6 | 1.1 | 0.30 |
| | *C. utilis* BVS-65 | 45 | 5.6 | 1.1 | 0.18 |
| Medium 1 | *C. albicans* D-6 | 608 | 76 | 4.6 | 0.24 |
| | *C. tropicalis* RE-2 | 304 | 38 | 2.3 | 0.18 |
| | *C. utilis* BVS-65 | 304 | 38 | 2.3 | 0.18 |
| Medium 2 | *C. albicans* D-6 | 118 | 14.7 | 9.3 | 0.38 |
| | *C. tropicalis* RE-2 | 118 | 7.3 | 9.3 | 0.32 |
| | *C. utilis* BVS-65 | 59 | 7.3 | 4.6 | 0.24 |
| Medium 3 | *C. albicans* D-6 | 769 | 192.2 | 9.3 | 0.51 |
| | *C. tropicalis* RE-2 | 384 | 192.2 | 9.3 | 0.76 |
| | *C. utilis* BVS-65 | 384 | 96.1 | 9.3 | 0.71 |

Notes:

*the concentration of fluconazole was unchanged and equaled their ½ MIC, and the concentration of surfactants was reduced by sequential double dilutions in the concentration range of 180 – 0.17 μg/ml for surfactants synthesized on the base medium; 608 – 1.1 μg/ml on medium 1; 236 – 0.92 μg/ml on medium 2, and 768 – 1.5 μg/ml on medium 3.

** the concentration of surfactants was unchanged and equaled their ½ MIC, and the concentration fluconazole was reduced by sequential double dilutions in the concentration range of 284 – 0.55 μg/ml.

*** FIC ≤ 0.5 indicates synergism.

The minimum inhibitory concentration of fluconazole against *C. albicans* D-6, *C. tropicalis* RE-2 and *C. utilis* BMS-65 was 35.5 μg/ml. Addition of surfactants synthesized by *A. calcoaceticus* IMV B-7241 to the solution of fluconazole reduced the MIC of this drug against *C. albicans* D-6, *C. tropicalis* RE-2 and *C. utilis* BVS-65 35.5 μg/ml to 1.1–9.3 μg/ml. Despite the higher FIC values of the mixture of surfactants synthesized on medium 3 with fluconazole (FIC 0.51-0.76), the minimum inhibitory concentrations of the latter were reduced by almost 4 times (from 35.5 to 9.3 μg/ml) (Table 2). Only a few reports were published on the synergism of antifungal compounds with microbial surfactants (Tabbene et

al., 2015) and other natural compounds (Carbone et al., 2019; Kumar et al., 2013; 2015). Thus, surface-active lipopeptides synthesized by *Bacillus subtilis* B38 showed synergistic antifungal activity with amphotericin B against *C. albicans* ATCC 10231 and clinical isolates of *C. albicans* and *C. tropicalis* (strains not specified) (Tabbene et al., 2015). At the same time, the minimum inhibitory concentrations of monopreparations of lipopeptides and amphotericin B were in the range of 12.5–25 μg/ml and 0.25–1 μg/ml, respectively. The use of the mixture made it possible to reduce both the concentration of lipopeptides to 0.39 μg/ml against *C. albicans* ATCC 10231 and to 0.78–1.56 μg/ml for clinical isolates and amphotericin B to 0.06 μg/ml against strain ATCC 10231 and 0.25 μg/ml against clinical isolates.

It was established in (Kumar et al., 2013) that diketopiperazines are cyclic dipeptides synthesized by *Bacillus* sp. N, in combination with clotrimazole, showed synergism of antimicrobial activity against *C. albicans* MTCC 277. Thus, the minimum inhibitory concentrations of the Cyclo-(L-Pro-L-Leu) dipeptide and clotrimazole against the MTCC 277 strain were 64 and 8 mg/ml, and in the mixture decreased to 2 and 1 μg/ml, respectively. The value of the fractional inhibitory concentration did not exceed 0.15, which indicates synergism. Similar results were observed when using a mixture of Cyclo(D-Pro-L-Leu) and Cyclo(L-Pro-L-Tyr) dipeptides with clotrimazole (Kumar et al., 2013). The MICs of these mixtures against *C. albicans* MTCC 277 were 2–4 μg/ml, respectively, while the minimum inhibitory concentrations of dipeptides were in the range of 16–32 μg/ml, and the MIC of clotrimazole was 8 μg/ml. Note that the cytotoxic effect of diketopiperazines in relation to fibroblast and epithelial cell lines was observed at a concentration of more than 200 μg/ml, which indicates the safety of using such a natural metabolite.

There is information in the literature about the use of essential oils (Carbone et al., 2019) and extracts (Kumar et al., 2015) of plant extracts in combination with clotrimazole. In the article (Carbone et al., 2019) it was found that the MIC of clotrimazole against *C. albicans* ATCC 10231 was 128 μg/ml, but in a mixtures with lavender or rosemary essential oils (ratio 1:1, concentration of essential oils 0.5–2%, v/v) decreased to 78 and 62.5 μg/ml, respectively. It should be noted that a mixture of clotrimazole with essential oils was used in the form of nanostructured lipid carriers, which made it possible to reduce the cytotoxicity of essential oils. It was shown that α- and β-asarans, the main active components of calamus (*Acorus calamus*), and showed antifungal activity against representatives of the *Candida* genus not only in the form of monodrugs, but also in combination with azoles (clotrimazole, fluconazole) (Kumar et al., 2015). When using their mixture with clotrimazole and fluconazole, there was a decrease in the minimum inhibitory concentrations against *C. tropicalis* MTCC 184 of both antifungal drugs (from 1 and 4 μg/ml to 0.06 and 0.25 μg/ml, respectively) and natural compounds (with 500 and 8 μg/ml to 64 and 2 μg/ml for α- and β-assarones, respectively).

Our results (see Tables 1 and 2) showed that the MICs of clotrimazole and fluconazole mixed with surfactants synthesized by *A. calcoaceticus* IMV B-7241 on basic or modified media are comparable and in some cases even lower than described in the literature (Kumar et al., 2013; Carbone et al., 2019; Kumar et al., 2013; 2015; Tabbene et al., 2015).

**Determination of synergistic antifungal action of a mixture of surfactants produced by *Acinetobacter calcoaceticus* IMV B-7241 and essential oils**

The synergism of the antifungal activity of a mixture of surfactants synthesized by *A. calcoaceticus* IMV B-7241 on different media and cinnamon and lemongrass essential oils was studied. The choice of essential oils was due to the following reasons: (a) the main

component of cinnamon essential oil, cinnamaldehyde, prevents the synthesis of ergosterol by binding to enzymes involved in the formation of the cytoplasmic membrane in yeast cells (da Nóbrega Alves et al., 2020); (b) geraniol, citral, citronellal and citronellol, which are the main components of lemongrass essential oil, inhibit the formation of hyphae as one of the virulence factors in the members of *Candida* genus (de Toledo et al., 2020).

The minimum inhibitory concentration of cinnamon essential oil against all test cultures was 156 μg/m. It has been found that the use of a mixture of cinnamon essential oil with surfactants synthesized by *A. calcoaceticus* IMV B-7241 grown on different media made it possible to reduce the minimum inhibitory concentrations of the essential oil against studied yeast of *Candida* genus by 4–18 times, from 156 to 8.5–39 μg/ml (Table 3).

**Table 3**

**Antifungal activity of surfactants produced by *Acinetobacter calcoaceticus* IMV B-7241, cinnamon essential oil and their mixture**

| Media for surfactants synthesis | Test culture – yeast of genus *Candida* | MIC (μg/ml) of | | | ***FIC |
|---|---|---|---|---|---|
| | | Surfactants | *Surfactants mixed with essential oil | **Essential oil mixed with surfactants | |
| Basic | *C. albicans* D-6 | 22.5 | 1.4 | 8.5 | 0.11 |
| | *C. tropicalis* RE-2 | 22.5 | 2.8 | 39 | 0.34 |
| | *C. utilis* BVS-65 | 45 | 1.4 | 17 | 0.11 |
| Medium 1 | *C. albicans* D-6 | 608 | 19 | 17 | 0.04 |
| | *C. tropicalis* RE-2 | 304 | 9.5 | 39 | 0.28 |
| | *C. utilis* BVS-65 | 304 | 9.5 | 17 | 0.13 |
| Medium 2 | *C. albicans* D-6 | 118 | 14.7 | 39 | 0.37 |
| | *C. tropicalis* RE-2 | 118 | 14.7 | 39 | 0.37 |
| | *C. utilis* BVS-65 | 59 | 7.3 | 39 | 0.37 |
| Medium 3 | *C. albicans* D-6 | 769 | 24 | 17 | 0.13 |
| | *C. tropicalis* RE-2 | 384 | 12 | 17 | 0.13 |
| | *C. utilis* BVS-65 | 384 | 12 | 17 | 0.13 |

Notes:

*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 180 – 0.17 μg/ml for surfactants synthesized on the base medium; 608 – 1.1 μg/ml on medium 1; 236 – 0.92 μg/ml on edimum 2, and 768 – 1.5 μg/ml on medium 3.

**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 624 – 1.2 μg/ml.

*** FIC ≤ 0.5 indicates synergism.

The FIC index did not exceed 0.5, which indicates synergism between the compounds. Similar patterns were observed when using a mixture of lemongrass essential oil and *A. calcoaceticus* IMV B-7241 surfactant. For example, the minimum inhibitory concentrations against *C. albicans* D-6 of surfactants synthesized in all media were in diapason from 22.5 to 769 μg/ml, lemongrass essential oil was 312 μg/ml, and their mixtures were only 9.7–39 μg/ml. At the same time, the value of the fractional inhibitory concentration did not exceed 0.5, which indicates the synergism of the antifungal action of surfactants and lemongrass

essential oil. It was shown that the minimum inhibitory concentration of a mixture of *Nocardia vaccinii* IMV B-7405 surfactants with cinnamon and lemongrass essential oils against yeast of *Candida* genus was 4 – 19.5 μg/ml that was significantly lower than the MIC of single surfactants, 16–76 μg/ml, or essential oils, 156 μg/ml (Pirog et al., 2020). At the same time, surfactants synthesized by strain *N. vaccinii* IMV B-7405 under different cultivation conditions were effectively being mixed with essential oils and reduced the MIC of the latter.

There are only single reports on the synergism of the antifungal activity of microbial surfactants mixed with essential oils (Pirog et al., 2020). At the same time, there is information on the use of various essential oils and plant extracts in combination with antifungal drugs (fluconazole, nisin, ketoconazole, amphotericin B) against drug-resistant strains of *Candida* genus (Herman and Herman, 2021). However, the authors do not give the values of the minimum inhibitory concentrations of monodrugs and their mixtures.

## Conclusions

The results confirm the possibility of using a mixture of microbial surfactants and antifungal drugs or essential oils to reduce the minimum inhibitory concentrations of the latter against members of *Candida* genus.

Surfactants produced by *Acinetobacter calcoaceticus* IMV B-7241 cultivated on different media showed synergism of antifungal activity of their mixture with antifungal drugs or essential oils. In the presence of surfactants synthesized on basic medium and modified media in a mixture with antifungal drugs and essential oils, made it possible to reduce minimum inhibitory concentrations of clotrimazole, fluconazole and cinnamon, lemongrass essential oils against yeasts of *Candida* genus by 4–32, 4–18 and 8–32 times, respectively. Nevertheless, the possible influence of the composition of the nutrient medium on surfactant antifungal ability should be taken into account when developing technologies for these products manufacturing.

## References

Andrews M.J. (2001), Determination of minimum inhibitory concentrations, *Journal of Antimicrobial Chemotherapy*, 48(1), pp. 5–16, DOI: 10.1093/jac/48.suppl 1.5.

Bhattacharya S., Sae-Tia S., Fries B.C. (2020), *Candidiasis* and mechanisms of antifungal resistance, *Antibiotics (Basel)*, 9(6), pp. 312, DOI: 10.3390/antibiotics9060312.

Bligh E.G., Dyer W.J. (1959), A rapid method for total lipid extraction and purification, *Canadian Journal of Biochemistry and Physiology*, 37(8), pp. 911−917

Carbone C., Teixeira M.D.C., Sousa M.D.C., Martins-Gomes C., Silva A.M., Souto E.M.B., Musumeci T. (2019), Clotrimazole-loaded mediterranean essential oils NLC: a synergic treatment of *Candida* skin infections, *Pharmaceutics,* 11(5), DOI: 10.3390/pharmaceutics11050231.

Cui J., Ren B., Tong Y., Dai H., Zhang L. (2015), Synergistic combinations of antifungals and anti-virulence agents to fight against *Candida albicans*, *Virulence*, 6(4), pp. 362–371, DOI: 10.1080/21505594.2015.1039885.

da Nóbrega Alves D., Monteiro A.F.M., Andrade P.N., Lazarini J.G., Abílio G.M.F., Guerra F.Q.S., Scotti M.T., Scotti L., Rosalen P.L., Castro R.D. (2020), Docking prediction, antifungal activity, anti-biofilm effects on *Candida* spp., and toxicity

against human cells of cinnamaldehyde, *Molecules*, 25(24), pp. 5969, DOI: 10.3390/molecules25245969.

de Toledo L.G., Dos Santos Ramos M.A., Bento da Silva P., Rodero, C. F., de Sá Gomes V., Noronha da Silva A., Pavan F. R., da Silva I. C., Bombarda Oda F., Flumignan D. L., Gonzaga Dos Santos A., Chorilli M., Gottardo de Almeida M.T., Bauab T. M. (2020), Improved in vitro and in vivo anti-*Candida albicans* activity of *Cymbopogon nardus* essential oil by its incorporation into a microemulsion system, *International Journal of Nanomedicine*, 15, pp. 10481–10497, DOI: 10.2147/IJN.S275258.

Hallander H.O., Dornbusch K., Gezelius L., Jacobson K., Karlsson I. (1982), Synergism between aminoglycosides and cephalosporins with antipseudomonal activity: interaction index and killing curve method, *Antimicrobial Agents and Chemotherapy*, 22(5), pp. 743–52.

Herman A., Herman A.P. (2021), Herbal products and their active constituents used alone and in combination with antifungal drugs against drug-resistant *Candida* sp, *Antibiotics*, 10(6), DOI: 10.3390/antibiotics10060655.

Hosseini S.S., Joshaghani H., Shokohi T., Ahmadi A., Mehrbakhsh Z. (2020), Antifungal activity of ZnO nanoparticles and nystatin and downregulation of sap1-3 genes expression in fluconazole-resistant *Candida albicans* isolates from vulvovaginal candidiasis, *Infection and Drug Resistance*, 13, pp. 385–394, DOI: 10.2147/IDR.S226154.

Jafri H., Ahmad I. (2020), Thymus vulgaris essential oil and thymol inhibit biofilms and interact synergistically with antifungal drugs against drug resistant strains of *Candida albicans* and *Candida tropicalis*, *Journal de Mycologie Medicale*, 30(1), 100911, DOI: 10.1016/j.mycmed.2019.100911.

Kumar S.N., Aravind S.R., Sreelekha T.T., Jacob J., Kumar B.S. (2015), Asarones from Acorus calamus in combination with azoles and amphotericin B: a novel synergistic combination to compete against human pathogenic *Candida* species in vitro, *Applied Biochemistry and Microbiology,* 175(8), pp. 3683–95, DOI: 10.1007/s12010-015-1537-y.

Kumar S.N., Nambisan B., Mohandas C., Sundaresan A. (2013), In vitro synergistic activity of diketopiperazines alone and in combination with amphotericin B or clotrimazole against *Candida albicans, Folia Microbiologica,* 58(6), pp. 475–482, DOI: 10.1007/s12223-013-0234-x.

Pappas P.G., Kauffman C.A., Andes D.R., Clancy C.J., Marr K. A., Ostrosky-Zeichner L., Reboli A.C., Schuster M.G., Vazquez J.A., Walsh T.J., Zaoutis T.E., Sobel J.D. (2016), Clinical practice guideline for the management of candidiasis: 2016 update by the infectious diseases society of America, *Clinical Infectious Diseases: an Official Publication of the Infectious Diseases Society of America*, 62(4), DOI: 10.1093/cid/civ933.

Pirog T.P., Kliuchka L.V., Kliuchka I.V., Shevchuk T.A., Iutynska G.O. (2020), Synergism of antimicrobial and anti-adhesive activity of Nocardia vaccinii IMV B-7405 surfactants in a mixture with essential oils, *Mikrobiolohichnyi Zhurnal*, 82(4), pp. 31–40, DOI: 10.15407/microbiolj82.04.031.

Pirog T., Kluchka L., Lytsai D., Stabnikov V. (2021a), Factors affecting antibiofilm properties of microbial surfactants. *Scientific Study & Research: Chemistry & Chemical Engineering, Biotechnology, Food Industry*, 21(1), pp. 27–37. https://pubs.ub.ro/?pg=revues&rev=cscc6&num=202101&vol=1&aid=5246.

Pirog T.P., Lutsay D.A., Shevchuk T.A., Iutynska G.O. (2021b), Synthesis and biological activity of *Acinetobacter calcoaceticus* IMV B-7241 surfactants depending on monovalent cations content in cultivation medium, *Mikrobiolohichnyi Zhurnal*, 83(2), pp. 20–31, DOI: 10.15407/microbiolj83.02.020.

Pirog T.P., Sidor I.V., Lutsai D.A. (2016), Calcium and magnesium cations influence on antimicrobial and antiadhesive activity of *Acinetobacter calcoaceticus* IMV B-7241 surfactants, *Biotechnologia Acta,* 9(6), pp. 50–57, DOI: 10.15407/biotech9.06.050.

Pirog T., Stabnikov V., Stabnikova E. (2022), Bacterial microbial surface- active substances in food-processing industry, In: Paredes-Lopez O., Stabnikov V., Shevchenko O., Ivanov V., eds., *Bioenhancement and Fortification of Foods for a Healthy Diet,* CRC Press, Boca Raton, London, pp. 271–294, DOI: 10.1201/9781003225287-18.

Singh D.K., Tóth R., Gácser A. (2020), Mechanisms of pathogenic *Candida* species to evade the host complement attack, *Frontiers in Cellular and Infection Microbiology*, 10, 94, DOI: 10.3389/fcimb.2020.00094.

Singh P., Cameotra S.S. (2004), Potential applications of microbial surfactants in biomedical sciences*, Trends in Biotechnoljgy*, 22(3), pp. 142–146, DOI: 10.1016/j.tibtech.2004.01.010.

Tabbene O., Di Grazia A., Azaiez S., Ben Slimene I., Elkahoui S., Alfeddy M.N., Casciaro B., Luca V., Limam F., Mangoni M.L. (2015), Synergistic fungicidal activity of the lipopeptide bacillomycin D with amphotericin B against pathogenic *Candida* species, *FEMS Yeast Research*, 15(4), DOI: 10.1093/femsyr/fov022.

Tsui C., Kong E.F., Jabra-Rizk M.A. (2016), Pathogenesis of *Candida albicans* biofilm, *Pathogens and Disease*, 74(4), DOI: 10.1093/femspd/ftw018.