

SYNERGISM OF ANTIMICROBIAL AND ANTI-ADHESIVE ACTIVITY OF *NOCARDIA VACCINII* IMV B-7405 SURFACTANTS IN A MIXTURE WITH ESSENTIAL OILS

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An increase in the antibiotic resistance of pathogenic microorganisms has stimulated the search for alternatives to antibiotics substances of natural origin, which are essential oils (EO) and non-toxic biodegradable microbial surfactants. **Aim.** To investigate the antimicrobial and anti-adhesive activity of a mixture of EO and surfactants of *Nocardia vaccinii* IMV B-7405 synthesized on various oil-containing media. **Methods.** *N. vaccinii* IMV B-7405 was grown in medium containing as carbon source refined sunflower oil, oil after frying french fried potatoes, potato wedges and meat. The surfactants were extracted from supernatant of cultural liquid by modified Folch mixture. The antimicrobial action of tea tree, cinnamon and lemongrass EO, surfactants and their mixtures was determined by index of the minimum inhibitory concentration (MIC). Synergistic effect of surfactants and EO was evaluated by indicator of fractional inhibitory concentration. The degree of bacteria and fungi biofilms destruction under the action of surfactants, EO and their mixtures was determined by spectrophotometric method. **Results.** It was found that *N. vaccinii* IMV B-7405 surfactants synthesized on all oil-containing substrates showed a synergistic antimicrobial and anti-adhesive activity with the investigated EO. MIC of a surfactants and EO mixture against bacteria (*Bacillus subtilis* BT-2 (spores), *Escherichia coli* IEM-1, *Staphylococcus aureus* BMS-1) and yeast (*Candida albicans* D-6, *Candida utilis* BVS-65 and *Candida tropicalis* RE-2) were 2–20 µg/ml and were significantly lower than each compound separately (156–625 and 8–80 µg/ml for EO and surfactants, respectively). The destruction of bacterial and yeast biofilms under the action of a mixture of surfactants (20–40 µg/ml) and EO (20–40 µg/ml) was 1.3–2.9 times higher compared with using of each component separately at similar concentrations. **Conclusions.** The data presented the possibility of using a mixture of EO and surfactants not only to reduce their MIC, but also as effective antimicrobial and anti-adhesive agents.

Keywords: *Nocardia vaccinii* IMV B-7405, surfactants, essential oils, synergistic action, antimicrobial activity, destruction of biofilms.

Back in 2011, the World Health Organization (WHO) published a list of priority research areas aimed at solving the problem of the spread of antibiotic-resistant causative agents of infectious diseases, 60 % of which are associated with biofilms formation [1]. One of these areas involves the usage of natural compounds such as essential oils (EO) as an alternative to synthetic antimicrobial substances. Interest in the EO is due to several reasons. Firstly, a wide component composition, in particular the presence of aldehydes, alcohols and phenols [2–3], which makes it possible to use EO as antimicrobial and anti-adhesive agents. Secondly, a different level of antimicrobial activity of oils depends on

the type and variety of the plant, methods of EO isolation, from which part of the plant and at what time of year they are obtained, and on the type of test culture [4].

However, the minimum inhibitory concentration (MIC) of such natural compounds is quite high (up to several mg/ml), which can cause severe damage of the central nervous system and aspiration pneumonia [5]. Given this, it is relevant to search for antimicrobial agents that can show a synergistic effect with EO, which will reduce the concentration of the latter. One of these perspective compounds is microbial surfactants.

Surfactants of microbial origin are biodegradable and less toxic, effective at extreme temperatures or pH values [6]. The advantage of microbial surfactants is the possibility of their biosynthesis on industrial wastes, for example, waste (fried) sunflower oil. The use of such oil as a substrate for the synthesis of microbial surfactants makes it possible to utilize these toxic wastes and reduce the cost of the final product.

The cheaper technology of microbial surfactants and a wide range of biological properties (antibacterial and antifungal activities and ability to destruct biofilm) makes them competitive in the market of synthetic substances, which leads to their usage in many applications ranging from environmental, food, and biomedical, to cosmetic and pharmaceutical industries [7, 8].

In previous studies [9–12] it was established the ability of *Nocardia vaccinii* IMV B-7405 to synthesize surfactants with high antimicrobial and anti-adhesive activity both on traditional substrates (glycerol, sunflower oil) and industrial waste (fried sunflower oil, waste from biodiesel production, molasses). It was shown that the biological properties of such metabolites depend on the nature of the carbon source (glycerol, sunflower oil), the degree of purification (purified and waste production of biodiesel) and the quality of the substrate (waste sunflower oil after frying meat, potatoes).

In this regard, the aim of this work was to investigate the antimicrobial and anti-adhesive activity of a mixture of EO and surfactants produced by *N. vaccinii* IMV B-7405 obtained on various oil-containing media.

Materials and methods. The object of the research was *Nocardia vaccinii* K-8 strain [13], registered in Microorganisms Depository of Zabolotny Institute of Microbiology and Virology, the National Academy of Sciences of Ukraine under the number IMV B-7405.

Bacterial strains (*Escherichia coli* IEM-1, *Bacillus subtilis* BT-2, *Staphylococcus aureus* BMS-1) and yeasts (*Candida albicans* D-6, *Candida utilis* BVS-65, *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 properties and ability to destroy biofilms of EO, surfactants and their mixture.

EO of lemongrass (Aromatica LLC, Ukraine) and cinnamon (RosCosmetics LLC, Ukraine) were used.

N. vaccinii IMV B-7405 strain was grown on the synthetic nutrient medium containing (g/l): NaNO_3 – 0.5; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.1; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ – 0.1; KH_2PO_4 – 0.1; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ – 1, yeast autolysate – 0.5 % (v/v). Refined oil “Oleina” (Dnepropetrovsk oil extraction plant), sunflower oil after frying french fried potatoes, potato wedges, and meat (McDonald’s fast-food restaurants, Kyiv) at a concentration of 2.0 % (v/v) were used as substrates.

The amount of extracellular surfactants was determined using modified by us Bligh and Dyer method [14] after extraction with a Folch mixture of chloroform and methanol (2:1) from culture broth supernatant. To obtain a supernatant, the culture broth was centrifuged at 5000 g for 20 minutes.

Taking into account that the strain *N. vaccinii* IMV B-7405 synthesizes a complex of polar and non-polar lipids, and the well-known Bligh and Dyer method [14] used to isolate 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 was placed in a 100 ml cylindrical separatory funnel and the surfactant was extracted 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 to constant weight on IP1-M2 rotary evaporator (Russia) at 50° C and absolute pressure of 0.4 atm.

The antimicrobial action of EO, surfactants and their mixtures was determined by the index of MIC as in previous studies [9, 10, 12].

Synergistic effect of surfactants and EO was evaluated by indicator of fractional inhibitory concentration (FIC) – the sum of the ratio of each substance concentration in a mixture with their MIC [15]. FIC is calculated by the formula:

$$\Sigma = \text{FIC} = (C_A/\text{MIC}_A) + (C_B/\text{MIC}_B),$$

where $C_{A,B}$ is the concentration of antimicrobial substance in the mixture;

$\text{MIC}_{A,B}$ – MIC of antimicrobial substance separately.

The ratio of drugs in the mixture was 1:1, the concentration of surfactants remained unchanged, and the oil concentration was reduced by the method of two-fold serial dilutions, in another embodiment, the concentration of EO remained unchanged, and the concentration of surfactants was reduced.

To obtain biofilm, 180 μl of meat-peptone broth (MPB) or liquid wort and 20 μl of one-day test culture suspension were added into polystyrene microplates, incubated for 24 h at the optimal temperature. Then the cultural liquid was poured off and another 180 μl of fresh liquid wort or MPB and 20 μl of test culture suspension were added and again incubated for 24 h. After 48 h the cultural liquid was poured off, and 200 μl of preparations (surfactants and EO in ratio 1:1) with different concentrations were added into each of the wells of the microplates (pre-covered by the biofilm). Into the control wells, surfactant preparations were replaced with distilled tap water (200 μl). After 24 h of exposition the wells were thrice washed by 200 μl of distilled water and the amount of adherent cells was determined spectrophotometrically. The degree of biofilm destruction (%) was determined as the difference between cell adhesion in untreated and treated with surfactants, EO or their mixture wells of the polystyrene microplates.

All experiments were performed in three replications, the number of parallel determinations in experiments ranged from three to five. Statistical processing of the experimental data was as described in previous papers [9–12]. The average difference was considered significant at $P < 0.05$.

Results. At the first stage, the synergism of antimicrobial activity of surfactants synthesized on oil-containing substrates with tea tree EO against bacterial test cultures (Table 1) and cinnamon and lemongrass EO against *Candida* yeast (Table 2) was studied.

The data in Table 1 showed that regardless of the type of substrate used (refined and waste sunflower oil), surfactants of *N. vaccinii* IMV B-7405 strain were effective antimicrobial agents against all test cultures. Also, it was found that the adding of surfactants synthesized by *N. vaccinii* IMV B-7405 on all oil-containing substrates into a mixture with tea tree oil made it possible to reduce its MIC by orders magnitude against all bacterial test cultures. The index of the fractional inhibitory concentration did not exceed 0.5, which indicates a synergism of the antimicrobial activity of such mixture.

The synergistic effect of EO of cinnamon and lemongrass and surfactant of *N. vaccinii* IMV B-7405 strain against yeast of the *Candida* genus was analyzed in Table 2. It was found that surfactants synthesized in medium with refined and waste sunflower oil showed a synergistic antimicrobial effect in a mixture with cinnamon and lemongrass EO. At the same time, the use of such a mixture against *Candida albicans* D-6 and *Candida tropicalis* RE-2 reduced not only the MIC of surfactants (from 16–78 to 4–19 $\mu\text{g}/\text{ml}$), but also reduced the MIC of EO in 8–130 times (Table 2).

The degree of destruction of bacterial (Table 3) and yeast (Table 4) biofilms under the action of surfactants, EO, and mixtures was determined. As well as for the synergy of antimicrobial activity determining, tea tree EO was used to study the ability to destroy bacterial biofilms, and cinnamon and lemongrass EO were used for yeast. The degree of bacterial biofilms destruction was studied in the concentration range (5–160 $\mu\text{g}/\text{ml}$), but the maximum degree of destruction (26.5–49%) obtained at a concentration of 40 $\mu\text{g}/\text{ml}$ of each compound (tea tree EO and surfactants). Therefore, the synergistic effect was determined for a mixture with the same concentration.

It was found that the degree of biofilms destruction under the action of surfactants, synthesis in medium with refined and waste oil in a mixture with tea tree oil was in 1.5–2.5 times higher than degree of biofilms destruction when using only EO or surfactant solutions separately. Similar patterns were obtained when studying the effect of surfactants with cinnamon and lemongrass EO against yeast biofilms (Table 4). Thus, the degree of test cultures biofilms destruction when using a mixture of surfactants with cinnamon or lemongrass EO was in 1.5–2 times higher than when using each of the preparations separately. Moreover, the effective concentration of both single antimicrobial compounds and their mixture was 20 $\mu\text{g}/\text{ml}$.

Table 1

Antimicrobial activity of *N. vaccinii* IMV B-7405 surfactants, tea tree EO and their mixtures

Oil for surfactant synthesis	Test-culture	MIC ($\mu\text{g/ml}$)				Tea tree EO in a mixture with surfactants**	FIC, FIC $\leq 0,5$ – synergy
		Surfactants	Tea tree EO	Surfactants in a mixture with tea tree EO*	Tea tree EO		
Refined	<i>Escherichia coli</i> IEM-1	16	625	4	39	0.24	
	<i>Staphylococcus aureus</i> BMS-1	16	156	2	4.8	0.15	
After french fried potatoes frying	<i>Bacillus subtilis</i> BT-2 (spores)	32	156	8	2.4	0.26	
	<i>Escherichia coli</i> IEM-1	12	625	3	19.5	0.27	
	<i>Staphylococcus aureus</i> BMS-1	24	156	6	4.8	0.28	
	<i>Bacillus subtilis</i> BT-2 (spore)	48	156	12	19.5	0.37	
After potato wedges frying	<i>Escherichia coli</i> IEM-1	16	625	2	19.5	0.15	
	<i>Staphylococcus aureus</i> BMS-1	8	156	4	2.4	0.49	
After meat frying	<i>Bacillus subtilis</i> BT-2 (spore)	64	156	16	9.5	0.3	
	<i>Escherichia coli</i> IEM-1	40	625	10	4.8	0.25	
	<i>Staphylococcus aureus</i> BMS-1	20	156	5	9.5	0.19	
	<i>Bacillus subtilis</i> BT-2 (spore)	80	156	10	19	0.37	

Legends: the error of the analysis of MIC did not exceed 5%; tab. 1 and 2: * – the concentration of EO remained unchanged, and the concentration of surfactants was reduced by the method of two-fold serial dilutions; ** – the concentration of surfactants remained unchanged, and the concentration of EO was reduced by the method of two-fold serial dilutions.

Table 2

Synergism of antimicrobial activity of *N. vaccinii* IMV B-7405 surfactants and EO against representatives of the *Candida* genus

Oil for surfactant synthesis	Test-culture	MIC ($\mu\text{g/ml}$)				Lemongrass EO in a mixture with surfactants**
		Surfactants	Surfactants in a mixture with cinnamon EO*	Surfactants in a mixture with lemongrass EO*	Cinnamon EO in a mixture with surfactants**	
Refined	<i>Candida albicans</i> D-6	16	4	8	N.d	19.5
	<i>Candida tropicalis</i> RE-2	32	8	8	N.d	9.7
After french fried potatoes frying	<i>Candida albicans</i> D-6	32	16	8	19.5	9.7
	<i>Candida tropicalis</i> RE-2	32	16	4	9.7	2.4
After potato wedges frying	<i>Candida albicans</i> D-6	24	6	6	19.5	9.7
	<i>Candida tropicalis</i> RE	48	12	12	9.7	2.4
After meat frying	<i>Candida albicans</i> D-6	76	19	9.5	9.7	9.7
	<i>Candida tropicalis</i> RE-2	38	9.5	9.5	9.7	1.2

Legends: N.d. – not determined; The MIC of cinnamon and lemongrass EO for all test cultures was 156 $\mu\text{g/ml}$.

Table 3**The biofilms destruction under the action of a mixture of *N. vaccinii* IMV B-7405 surfactants and tea tree EO**

Substrate for surfactant synthesis	Test-culture	Destruction (%) of biofilms under the action of	
		surfactants	a mixture of surfactants and EO
Refined oil	<i>Escherichia coli</i> IEM-1	43	67.9
	<i>Staphylococcus aureus</i> BMS-1	22	41.0
	<i>Bacillus subtilis</i> BT-2 (spore)	17.2	48.2
Oil after french fried potatoes frying	<i>Escherichia coli</i> IEM-1	49.4	63.3
	<i>Staphylococcus aureus</i> BMS-1	34.2	53.8
	<i>Bacillus subtilis</i> BT-2 (spore)	17.2	38.4
Oil after potato wedges frying	<i>Escherichia coli</i> IEM-1	41.8	62.5
	<i>Staphylococcus aureus</i> BMS-1	13.6	38
	<i>Bacillus subtilis</i> BT-2 (spore)	30.5	38.1
Oil after meat frying	<i>Escherichia coli</i> IEM-1	22.1	50.4
	<i>Staphylococcus aureus</i> BMS-1	22.1	47.2
	<i>Bacillus subtilis</i> BT-2 (spore)	16.5	40.6

Legends: the concentration of surfactant solutions and a mixture of surfactants with EO was 40 µg/ml. The degree of biofilms destruction under the action of tea tree EO (40 µg/ml) did not exceed 21–26.5%. The error of determining the destruction of the biofilms did not exceed 5%.

Table 4**Destruction of yeast biofilms under the action of *N. vaccinii* IMV B-7405 surfactants and EO**

Substrate for surfactant synthesis	Test-culture	Destruction (%) of biofilms under the action of		
		surfactants	a mixture of surfactants and cinnamon EO	a mixture of surfactants and lemongrass EO
Refined oil	<i>Candida albicans</i> D-6	30.8	48.2	52.2
	<i>Candida tropicalis</i> RE-2	27.4	49.2	42.8
	<i>Candida utilis</i> BVS- 65	33.0	56.2	55.0
Oil after french fried potatoes frying	<i>Candida albicans</i> D-6	34.2	53.1	57.2
	<i>Candida tropicalis</i> RE-2	49.2	71.0	51.8
	<i>Candida utilis</i> BVS- 65	25.4	60.1	52.1
Oil after potato wedges frying	<i>Candida albicans</i> D-6	40.5	58.1	55.1
	<i>Candida tropicalis</i> RE-2	31.5	55.4	58.2
	<i>Candida utilis</i> BVS- 65	38.1	44.2	60.2
Oil after meat frying	<i>Candida albicans</i> D-6	40.5	42.1	63.5
	<i>Candida tropicalis</i> RE-2	27.5	42.8	36.2
	<i>Candida utilis</i> BVS- 65	26.0	51.5	50.8

Legends: the concentration of surfactant solutions and a mixture of surfactants with EO was 20 µg/ml. The degree of biofilms destruction under the action of EO (20 µg/ml) did not exceed 17–20%. The error of determining the destruction of the biofilms did not exceed 5%.

Discussion. In recent years [16–18], scientists have noted that the most active components of EO are terpenes (mirene, α -terpeneol, terpinen-4-ol, 1,8-cineole, α -terpinene, citral, geraniol, menthone, β -terpinene, γ -terpinene, limonene, β -caryophyllene) and terpenes with a phenolic structure (carvacrol, eugenol, thymol). Because the specific effect of EO on bacteria or yeast depends on their component composition, we have chosen

EO that contains these components. So, tea tree EO contains four main components such as terpinen-4-ol, α - and γ -terpinene and 1,8-cineol which cause the loss of potassium ions and increase the permeability of the cytoplasmic membrane mainly in bacteria [19]. Cinnamaldehyde and limolene, which are the main components of cinnamon and lemongrass EO, respectively, inhibit electron transport, change protein translocation and

synthesis of cellular components in yeast [20, 21], mostly of *Candida* genus. The choice of such test cultures is because currently the number of drugs for the treatment of infections associated with the development of *Candida* genus representatives is limited by a small number of compounds (azoles, echinocandins, polyenes and allylamines), the use of which is accompanied by a number of side effects [3].

In previous studies [22] we showed the possibility of using of *Rhodococcus erythropolis* IMV Ac-5017 surfactants and tea tree EO, however, in this work we investigated the synergism of antimicrobial effect by the degree of cell survival in suspension culture, which does not allow us to compare our results with previous studies.

We were unable to find information in the available literature about the synergy of antimicrobial or antifungal action of the mixture of EO and microbial surfactants. However, there are publications about the synergism of antimicrobial activity of tea tree EO with antibiotics [23–26] and limited information about the use of cinnamon EO with synthetic antifungal drug fluconazole [27–29]. Thus, as in [23] it was shown that the use of a mixture of tea tree EO and chloramphenicol make it possible to reduce the MIC of the latter from 64 µg/ml to 2 µg/ml against *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538 and the MIC of EO – from 1024 to 32 µg/ml.

It was established in [24] that the use of a mixture of cloxacillin antibiotic and tea tree EO reduced the MIC of each of the antimicrobial substances: for EO decreased by one-half (from 12.5 to 6.25 µg/ml), and for the antibiotic – decreased from 0.5 to 0.125 µg/ml against various penicillin-resistant strains of *S. aureus* 13, *S. aureus* 139 and *S. aureus* 96. However, the fractional inhibitory concentration in this mixture was more than 0.5, which indicates the indifference of the components of the mixture, that is, EO and antibiotic do not enhance the antimicrobial properties of each other. Despite this, scientists argue that the combination of cloxacillin with EO can reduce the concentration of the antibiotic and avoid acquiring resistance to pathogenic staphylococci.

The possibility of using tea tree EO in combination with amikacin, oxacillin, cefazolin, vancomycin, rifampin, meropenem, colistin against methicillin-resistant *S. aureus* (MRSA) was shown in the work [25]. Synergies of antimicrobial action were shown only by mixtures of EO with amikacin, oxacillin, cefazolin and rifampicin, while the FIC

did not exceed 0.32. Notably, tea tree EO at sub-inhibitory concentrations reduced oxacillin and cefazolin MICs against MRSA from 64 to 2 µg/ml and from 32 to 1 µg/ml, respectively.

The paper [26] showed the possibility of using a mixture of tea tree EO and gentamicin. Thus, the MIC of individual preparations of EO and gentamicin against *B. cereus* ATCC 7464, *B. subtilis* ATCC 6633, *S. aureus* ATCC 2921, *S. aureus* ATCC 6538p and *E. coli* ATCC 25922 was 1.7–13.9 mg/ml and 0.25–0.5 µg/ml, respectively. At the same time, the use of the mixture made it possible to reduce the MIC of both antimicrobial compounds: the EO (to 3.45–0.68 mg/ml) and gentamicin (to 0.12–0.06 µg/ml).

In [27], the authors report the use of cinnamon EO (MIC 62.5 µg/ml) in combination with fluconazole (1000 µg/ml), which makes it possible to decrease the MIC of antibiotic against *C. albicans* ATCC 10213, the FIC value is 0.32 which indicates their synergism. The authors note that the combination of cinnamon EO with fluconazole affected on fungi membrane integrity, thus, inhibited the ergosterol biosynthesis by 69.51 %. The decreasing ability of ergosterol biosynthesis using mixture can cause the damages of the membrane, increase cell permeability and finally cell death.

The use of cinnamon EO made it possible to reduce the MIC of fluconazole against *C. albicans* PRA-06 from 625 µg/ml to 19.5 µg/ml, while the FIC value was 0.5 [28].

Synergism of fluconazole and cinnamaldehyde (main components of cinnamon EO) antifungal action was established in the work [29]. The MIC of individual preparations of fluconazole and cinnamaldehyde was 100 and 256 µg/ml, respectively; and when they were used in mixture MIC was reduced to 12.5 and 64 µg/ml (FIC value was 0.375). Similar results were observed for a mixture of cinnamaldehyde and amphotericin B. The MIC of the preparations in the mixture was 12.5 and 0.031 µg/ml, respectively, compared to the MIC of cinnamaldehyde (100 µg/ml) and the antifungal drug (0.25 µg/ml) separately.

It should be noted that the index of fractional inhibitory concentration of a mixture of tea tree EO, cinnamon and lemongrass (FIC not shown) and surfactant from *N. vaccinii* IMV B-7405, obtained on all oil-containing substrates, did not exceed 0.5, indicating their synergism. Herewith, the MICs of all EO in the mixture with surfactants were reduced by several times (see Tables 1, 2).

In previous studies we have shown that the surfactants of *N. vaccinii* IMV B-7405 also exhibit a synergistic effect in a mixture with fluconazole against *C. albicans* D-6, *C. tropicalis* RE-2 and *C. utilis* BVS- 65 [30].

It is known that in addition to antimicrobial activity, microbial surfactants also have an anti-adhesive effect, including the ability to destroy the biofilms. Since one of the mechanisms of biofilms destruction is an antimicrobial activity, we suggested that the surfactants of *N. vaccinii* IMV B-7405 will have synergies with EO in the destruction of biofilm. Notably, there is no information in the literature about the ability of surfactants and EO mixture to destroy biofilms.

The mechanism of the destruction of biofilms under the action of EO is associated with the presence of phenolic terpenoids in their composition, which are able to penetrate into the polysaccharide matrix of the biofilm and act on microorganisms in its composition. Due to their hydrophobic nature, they interact with the bilipid layer of cytoplasmic membrane, which leads to loss of integrity due to leakage of ions, ATP and nucleic acids [31].

There is no information in the literature about ability of lemongrass EO in combination with various antimicrobial agents to destroy biofilms, while at the same time, there is data about biofilms destruction under the actions of tea tree and cinnamon EO but only in combination with antibiotics or synthetic antifungal drugs. Thus, in [32], the authors established the synergistic effect of terpinen-4-ol (the main component of tea tree EO) and nisin on the biofilm destruction of *Bacillus cereus* MTCC 1272 and *Salmonella typhimurium* MTCC 3224. When terpinene-4-ol is added to nisin, the degree of biofilms destruction increased in 3–4 times (up to 65–80%) compared to using only antibiotic.

Nuryastuti et al. [33] showed that tea tree EO at a concentration of 2 % (v/v) after 24 hours of exposition completely destroyed the biofilms of various *S. aureus* strains that colonize medical equipment and devices. In the work, it was shown that under the actions of mupirocin (32 µg/ml), the degree of destruction of the biofilm of *C. albicans* ATCC 10231 was 14 %, and with the addition of cinnamon EO in a similar concentration (1:1 ratio), the degree increased to 58.6 %. The authors in the same work investigated the degree of destruction of

the biofilm in the presence of a mixture of cinnamon oil with fluconazole. The biofilm destruction was 6.05% under the actions of antifungal drug only (64 µg/ml), then the additional application of cinnamon oil in the same concentration increased the degree of destruction to 61.11 %.

In article [34], we noted that the practical use of microbial surfactants, particular in medicine, is limited. Despite the large number of study works of their antimicrobial properties, aminolipid daptomycin (manufacturer company Cubist Pharmaceuticals) called Cubicin®, remains the only one permitted in medical practice. This situation is primarily associated with high production and purification cost of microbial metabolites. In a recently published review [35], we showed the ways of practical use of a mixture of EO and other antimicrobial compounds (in particular antibiotics) in veterinary medicine and cosmetology. It can be assumed that the antibiotics in the composition of such mixtures can be replaced by microbial surfactants, in particular synthesized by *N. vaccinii* IMV B-7405, and use as a part of means for external use, which, in addition to the antimicrobial effect, have an anti-adhesive effect and ability to destruct biofilms.

Notably, in the literature, information about the antimicrobial, anti-adhesive activity of surfactants synthesized on waste sunflower oils is limited. In this paper, we showed the possibility of using surfactants obtained on a wide range of oil-containing substrates, including various types of waste oils, as effective antimicrobial and anti-adhesive agents that are able to show synergism with EO.

Thus, the results presented in this work confirm individual literature data on the antimicrobial activity synergism of EO and surfactants of microbial origin. A distinctive feature of our results is the possibility of obtaining surfactants on oil-containing waste of various qualities. In addition, the synergistic effect of a mixture of EO and microbial surfactants on the destruction of bacterial and yeast biofilms was first shown.

The data presented the possibility of using a mixture of EO and surfactants not only to reduce their MIC, but also as effective antimicrobial and anti-adhesive agents, due to the unique mechanism of action, they prevent the formation of resistant forms of microorganisms.

СИНЕРГІЗМ АНТИМІКРОБНОЇ ТА АНТИАДГЕЗИВНОЇ АКТИВНОСТІ ПОВЕРХНЕВО-АКТИВНИХ РЕЧОВИН *NOCARDIA VACCINII* IMB В-7405 В СУМІШІ З ЕФІРНИМИ ОЛІЯМИ

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Резюме

Підвищення антибіотикорезистентності патогенних мікроорганізмів стимулювало пошук альтернативних антибіотикам речовин природного походження, якими є ефірні олії та нетоксичні біодеградабельні мікробні поверхнево-активні речовини. **Мета.** Дослідити антимікробну та антиадгезивну активність суміші ефірних олій та поверхнево-активних речовин *Nocardia vaccinii* IMB В-7405, синтезованих на різних олієвмісних середовищах. **Методи.** *N. vaccinii* IMB В-7405 вирощували у середовищі, що містило як джерело вуглецю рафіновану соняшникову олію, а також олію після смаження картоплі «фрі», картоплі селянської та м'яса. Поверхнево-активні речовини екстрагували з супернатанту культуральної рідини модифікованою сумішшю Фолча. Антимікробну дію ефірних олій чайного дерева, кориці та лемонграсу, поверхнево-активних речовин та їх суміші аналізували за показником мінімальної

інгібуючої концентрації. Для оцінки синергічної дії поверхнево-активних речовин та ефірних олій використовували показник фракційної інгібуючої концентрації. Ступінь руйнування бактеріальних і дріжджових біоплівки за дії поверхнево-активних речовин, ефірних олій та їх суміші визначали спектрофотометричним методом. **Результати.** Встановлено, що поверхнево-активні речовини, синтезовані *N. vaccinii* IMB В-7405 на усіх олієвмісних субстратах, проявляли синергічну антимікробну та антиадгезивну активність з досліджуваними ефірними оліями. Мінімальні інгібуючі концентрації суміші ПАР з оліями щодо бактерій (*Bacillus subtilis* БТ-2 (спори), *Escherichia coli* ІЕМ-1, *Staphylococcus aureus* БМС-1) і дріжджів (*Candida albicans* Д-6, *Candida utilis* БВС-65 та *Candida tropicalis* РЕ-2) становили 2–20 мкг/мл і були значно нижчими, ніж кожної сполуки окремо (156–625 і 8–80 мкг/мл для ефірних олій та поверхнево-активних речовин відповідно). Деструкція бактеріальних і дріжджових біоплівки за дії суміші поверхнево-активних речовин (20–40 мкг/мл) і ефірних олій (20–40 мкг/мл) була в 1,3–2,9 разів вищою порівняно з використанням кожного компонента окремо в аналогічних концентраціях. **Висновки.** Наведені дані засвідчують можливість використання суміші ефірних олій та поверхнево-активних речовин не лише для зниження їх мінімальних інгібуючих концентрацій, а й як ефективних антимікробних та антиадгезивних агентів.

Ключові слова: *Nocardia vaccinii* IMB В-7405, поверхнево-активні речовини, ефірні олії, синергічна дія, антимікробна активність, руйнування біоплівки.

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Received 7.04.2020