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INFLUENCE OF CULTIVATION CONDITIONS ON ANTIMICROBIAL PROPERTIES OF *Nocardia vaccinii* IMV B-7405 SURFACTANTS

T. P. Pirog[1, 2]
E. V. Panasyuk[1]
L. V. Nikityuk[1]
G. O. Iutinska[2]

[1]National University of Food Technologies, Kyiv

[2]Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, Kyiv

E-mail: tapirog@nuft.edu.ua

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The aim of the work was to study of antimicrobial effect of *Nocardia vaccinii* IMV B-7405 surfactants, synthesized in various cultivation conditions, against phytopathogenic bacteria of genera *Pseudomonas*, *Xanthomonas*, and *Pectobacterium*. The antimicrobial properties of surfactants were determined in suspension culture by Koch method and also by index of the minimum inhibitory concentration. Surfactants were extracted from supernatant of cultural liquid using mixture of chloroform and methanol (2: 1). It has been established that antimicrobial properties of surfactants depend on the nature of the carbon source in the medium (refined sunflower oil, as well as waste oil after frying potatoes and meat, glycerol), the duration of the cultivation (5 and 7 days), the degree of surfactants purification (the supernatant of cultural liquid, purified surfactants solution) and the test culture type. The highest antimicrobial activity was exhibited by purified surfactants solutions synthesized by IMV B-7405 strain on the waste oil after potato frying (decreased survival of pathogenic bacteria by 50–95%), and surfactants formed within 7 days of *N. vaccinii* IMV B-7405 cultivation on all test substrates (minimum inhibitory concentration 7–40 μg/ml, which is several times lower than the surfactant, synthesized for 5 days).

These data are promising for the development of ecologically friendly biopreparations to control the number of phytopathogenic bacteria.

Key words: *Nocardia vaccinii* IMV B-7405, surfactants.

The last decades saw an increase in pathogenic microorganisms' resistance towards established biocides, which stimulated on the search for new alternatives in antimicrobial preparations. According to [1–3], microbial surfactants are among such preparations. Due to their overall environmental safety, their applications can span medicine, agriculture and food production [1–5].

In our previous report [6] we researched the influence of surfactants synthesized by *Rhodococcus erythropolis* IMV As-5017, *Acinetobacter calcoaceticus* IMV B-7241 and *Nocardia vaccinii* K-8 on phytopathogenic bacteria. It was shown that the survival of cells (10^5–10^7 in ml) of the *Pseudomonas* and *Xanthomonas* phytopathogenic bacteria was found to be 0–33% after treatment with surfactants of the IMV Ac-5017 and IMV B-7241 strains for 2 h (0.15–0.4 mg/ml). In the presence of *N. vaccinii* IMV B-7405 surfactants (0.085–0.85 mg/ml), the number of cells of the majority of the studied phytopathogenic bacteria decreased by 95–100% [6]. The data suggest that microbial surfactants are a promising base for the development of environmentally friendly preparations limiting the abundance of bacterial phytopathogens.

Microbial surfactants belong to secondary metabolites and are, as a rule, synthesized as complexes of similar substances (amino-, glyco-, phospho- and neutral lipids) [7]. Under different conditions of the producers' cultivation, the components ratios in the secondary metabolite complexes can change, which is followed by changes in their biological properties [8]. We have also shown [9] the dependency of the qualitative composition of glycolipids synthesized by *R. erythropolis* EK-1 (IMV Ac-5017) on *n*-hexadecan, on

the medium composition and the mass transfer coefficient. Later we found that the qualitative composition of neutral, glyco- and phospholipids produced by *A. calcoaceticus* IMV B-7241 on ethanol depended both on pH of cultivation medium and the nature of the titrating agent [10]. Using NaOH to maintain pH within the optimal range (6.0–7.0) accompanied by a decrease lipid spectrum compared to titration of KOH solution. However, in the aforementioned studies [9, 10] we had not studied the influence of producers' cultivation conditions on the biological properties of produced surfactants.

Also, the literature contains fragmentary reports that some microorganisms under certain cultivation conditions can produce not only the surfactants, but other metabolites as well (enzymes, bacteriocins, polysaccharides, etc.) [11–14]. It is rather likely that the properties of such complexes of surfactants and other metabolites can differ from the properties of pure surfactants.

With this in mind, the aim of our current work was to research the antimicrobial against phytopathogenic bacteria properties surfactants synthesized under different cultivation conditions of *N. vaccinii* IMV B-7405.

Materials and Methods

Study object. The main study object was the strain *Nocardia vaccinii* K-8, registered in the Depository of microorganisms of the Zabolotny Institute of Microbiology and Virology of NAS of Ukraine under the number IMV B-7405.

By the chemical nature, the extracellular surfactants of IMV B-7405 strain are a complex of neutral, glyco- and aminolipids [8].

We used phytopathogenic bacteria from the Ukrainian Collection of Microorganisms (UCM): *Pectobacterium carotovorum* UCM B-1095, *Pseudomonas syringae* pv. *atrofaciens* UCM B-1015, *P. syringae* pv. *coronafaciens* – UCM B-1154, *Xanthomonas campestris* pv. *campestris* UCM B-1049.

We also used the following pathogens from the collection of the Department of pathogenic bacteria of the Zabolotny Institute of Microbiology and Virology of NAS of Ukraine: *Pseudomonas corrugate* 9070, *Xanthomonas vesicatoria* 7790.

The phytopathogen strains were kindly given by our colleagues in the Department of phytopathogenic bacteria of the Zabolotny Institute of Microbiology and Virology of NAS of Ukraine.

Composition of medium and cultivation conditions. Strain *N. vaccinii* IMV B-7405 was grown in synthetical medium (g/L): $NaNO_3$ — 0.5, $MgSO_4 \cdot 7H_2O$ – 0.1, $CaCl_2 \cdot 2H_2O$ — 0.1, KH_2PO_4 — 0.1, $FeSO_4 \cdot 7H_2O$ — 0.01, yeast autolysate – 0.5% (v/v). As a source of carbon and energy we added glycerol at 1.5% (v/v) and refined sunflower oil "Oleyna" (Dnipropetrovsk oil extraction plant), waste oil after frying potatoes and meat (the McDonald's network of fast food restaurants, Kyiv). The content of oil-containing substrates in the medium was 2% (v/v).

As inoculum we used the cultures in the exponential growth phase, grown in the media of the described compositions and containing 1–2% (v/v) of the substrates. The inoculation material (10^4–10^5 cells/ml) amounted to 5–10% of the volume of the cultivation medium. The bacteria were cultivated in flasks of 750 ml with 100 ml of medium on the shaker (320 rpm) at 28–30 °C during 5 and 7 days, respectively.

Surfactant extraction. From the cultural liquid supernatant containing the surfactant (preparation 1), via extraction with a chloroform–methanol mixture at a ratio of 2:1 (Folch mixture) we isolated surfactant (preparation 2). The water phase remained after the surfactants extraction was termed as preparation 3.

To obtain supernatant, cultural liquid was centrifuged (5000 g) for 25 min. We purified the cultural liquid from the residual sunflower oil by extracting it three times with petroleum ether at the ratio of 1:1, as described in [15].

Preparations 1–3 were obtained as described in our previous reports [6, 16]. The surfactants concentration in preparations 1 and 2 was established by the weight technique after extraction with a Folch mixture.

Antimicrobial properties of the preparations. In the initial suspension of one-day-old phytopathogenic test cultures grown in an agar medium (wort and meat peptone agars at a ratio of 1:1) at 30 °C, the number of alive cells was assessed by the Koch method (colony-forming units, CFU/ml). Then, 1.5 ml of the test culture suspension was placed in test tubes, and 1.5 ml of preparations 1–3 were added and kept for 1 h at 30 °C; after that, the number of living cells was assessed. The survival of phytopathogenic bacteria was determined as a ratio of the number of cells in the variants treated with preparations 1–3 to the number of cells in the initial suspension and was expressed in percentage points.

Determination of the minimum inhibitory concentration (MIC) of the surfactants was carried out according to the procedure described in [16, 17]. To determine MIC we used the method of two serial dilutions in Gromyko liquid medium (meat-peptone and wort, 1:1). In aseptic conditions, the medium was added to ten test tubes at 1 ml. In the first test tube, we added 1 ml of sterile solution of the surfactant (preparation 2) a certain concentration (usually 0.4 mg/ml), mixed it, 1 ml was taken and placed into the next tube.

Similarly, we carried out the dilution in the next nine test tubes. From the last test tube, we pipetted out 1 ml. Therefore, the final volume in every test tube was 1 ml (Gromyko medium and the surfactant solution), but the concentration of the surfactant was twice reduced at every step. As a control we used 1 ml of Gromyko medium without preparation 2. Next, into every test tube 0.1 ml of the test culture suspension (10^5–10^6 CFU/ml) was added and the contents were mixed. The test tubes were incubated for 24 hours at 30 °C. The results were evaluated visually by the clouding of the medium: (+) — test tubes in which the medium became cloudy (the test culture grew), (–) — no clouding occurred (no growth). The minimum inhibitory concentration of surfactant was determined as the mean of the surfactant concentrations in the last test tube where the growth was not observed, and the next one, where it was.

All the experiments were thrice replicated; the number of parallel determinations in the experiments was 3 to 5. The statistical treatment of the experimental data was carried out as described previously [6, 16]. The differences between the means were considered significant at $P < 0.05$.

The specifics of determination and calculation of the bacterial survival and the minimum inhibitory concentration are such that in the tables and the figures, we cannot separately present the control data since they were used in the formulae to calculate the parameters. While all data in the tables and on figures are statistically significant and should be tagged with an asterisk (*), for ease of reading we carried the (*) to the Survival column (Tables 1 and 2), MIC column (Table 3), and to the ordinate axis (Fig. 1 and 2, Survival parameter) as characterizing all the shown data. In the tables and figures legends we also stated the results from control (the number of test culture cells before the addition of surfactant preparations).

Results and Discussion

Currently, fighting the diseases of agricultural plants is seen as substantial contribution towards the solution of global food production problems [18]. Application of biological agents to control the spread of the phytopathogens is an important condition of the stable development of agricultural systems [19]. The literature on microbial surfactants contains some data on inhibiting mostly phytopathogenic fungi [5]. However, bacterial infections of plants problem remain so far an urgent problem, as well [20]. And yet there are few data on the capability of microbial surfactants to inhibit the growth of phytopathogenic bacteria. It is known that surfactants produced by rhizospheric isolates of *Pseudomonas* and *Bacillus* showed antimicrobial action on the soft rot pathogens *Pectobacterium* and *Dickeya* spp. [21]. The authors of [22] showed the possibility of using the cells of *Bacillus subtilis* 6051, as well as their metabolites (surfactin) to fight the phytopathogenic bacteria of *Pseudomonas syringae* pv. *tomato* DC3000, which infects the roots of Arabidopsis. Iturin and surfactin (lipopeptides of *B. subtilis* OG) at the concentration of 5 mg/ml were revealed to have antimicrobial effect towards *Xanthomonas campestris* and *Xanthomonas axonopodis* [23]. The preparations we studied recently, surfactants of *N. vaccinii* IMV B-7405, synthesized in the medium with purified glycerol, were quite efficient against the phytopathogenic bacteria *X. campestris* pv. *campestris* UCM B-1049 in lower concentrations (0.85 mg/ml) [6].

In our present work for study the dependence of the antimicrobial properties of the surfactants on the cultivation conditions of IMB B-7405 strain we used preparations with the concentrations of 0.4 mg/ml.

The influence of the nature of the carbon source in the cultivation medium of N. vaccinii IMV B–7405 on the antimicrobial properties of the surfactants. The data presented in Table 1 are evidence that preparations 2 (solutions of surfactants synthesized on all studied growth substrates) proved to be more efficient antimicrobial agents than the corresponding preparations 1 (the cultural liquid supernatant). Thus, after treating test cultures of the phytopathogenic bacteria belonging to the genera *Pseudomonas, Xanthomonas* and *Pectobacterium* with preparation 2, their survival was 20–75, 38–71 and 44–85%, respectively. The most

significant antimicrobial effect against almost all of the studied bacteria, with the exception of *X. campestris* pv. *campestris* UCM B-1049, was found in preparations 2, synthesized on waste sunflower oil after potatoes frying (Table 1). These results indicate that using industrial waste (fried sunflower oil) allows not only to reduce cost of *N. vaccinii* IMV B-7405surfactant biosynthesis but also to obtain the final product with high antimicrobial properties. Besides, the data in Table 1 show that the antimicrobial properties of the surfactants of IMV B-7405 strain depend on the nature of carbon source in the medium, and also on the type of test culture. So, for example, the survival of *X. campestris* pv. *campestris* UCM B-1049 was 38–42% after treating the cells with preparations 1 and 2, obtained after cultivation of *N. vaccinii* IMV B-7405 on glycerol, and was 1.4–2.4 times lower than in the presence of surfactants obtained on oil-containing substrates. Meanwhile, after treating the suspension of *P. syringae* pv. coronafaciens UCM B-1154 and *P. corotovorum* UCM B-1095 with preparations of surfactants synthesized on glycerol, cell survival remained quite high (75–97%) (Table 1).

All studied preparations 3 (water phase) practically did not influence on survival of *Pseudomonas* bacteria, but did show antimicrobial effect against *X. campestris* pv. *campestris* UCM B-1049 (Fig. 1). Preparations, obtained after cultivating *N. vaccinii* IMV B-7405 on oil-containing substrates, turned out to be more efficient antimicrobial agents (the survival of cells of UCM B-1049 strain was 70–82%) compared to those obtained on glycerol (growth stimulation) (Fig. 1).

The treatment of *Pectobacterium carotovorum* UCM B-1095 suspension with preparation 3, obtained after growing IMV B-7405 strain on waste oil after potatoes frying, was accompanied by the dying 25% of cells, while in the presence of other preparations 3 — only 3–5%.

In our previous work [6] we established that after treatment with preparation 3, synthesized under *N. vaccinii* IMV B-7405 cultivation on glycerol, the survival of phytopathogenic bacteria belonging to the genera *Pseudomonas, Xanthomonas* and *Pectobacterium* was not exceeded 0.2–1%. After surfactant extraction from the cultural liquid supernatant, the liquid did not have surface-active properties, and this led us to conclude that in preparations 3, surfactants are absent. In our study [6] we supposed that *N. vaccinii* IMV B-7405 synthesized other antimicrobial metabolites, beyond surfactants, for example, antibiotics, as do many of bacteria in the genus *Nocardia.*

The data presented here differ from our previous findings [6] and are evidence of the absent or insignificant antimicrobial action of preparation 3. The differences in the

Table 1. **Influence of the nature of carbon source in the medium of *N. vaccinii* IMV B-7405 cultivation on the antimicrobial properties of the surfactants**

Carbon source in the medium	Surfactant preparations	Survival*,%					
		P. syringae pv. *coronafaciens* UCM B-1154	*P. syringae* pv. *atrofaciens* UCM B-1015	*P. corrugate* 9070	*X. vesicatoria* 7790	*X. campestris* pv. *campestris* UCM B-1049	*P. carotovorum* UCM B-1095
Waste oil after frying potatoes	1	22.8	82.8	84.2	79.0	92.0	63.0
	2	20.1	53.2	47.5	61.5	50.0	43.5
Waste oil after frying meat	1	74.4	90.5	90.1	83.0	83.1	89.1
	2	60.3	69.0	74.7	71.0	54.7	65.2
Refined sunflower oil	1	60.1	90.0	88.3	90.0	89.3	71.7
	2	56.4	63.4	63.8	69.0	62.8	63.0
Glycerol	1	97.0	91.3	71.7	86.0	41.7	91.3
	2	74.9	74.0	53.2	63.2	37.7	84.8

Notes. N. vaccinii IMV B-7405 was cultivated for 5 days. Tables 1 and 2: surfactant concentration in preparations 1 (cultural liquid supernatant) and 2 (surfactant solution) was 0.4 mg/ml; exposition time 1 hour; when determining the cell survival the error did not exceed 5%; the amount of cells of the strains UCM B-1154, UCM B-8291, 9070, 7790, UCM B-1049, and UCM B-1095 before the addition of the surfactant preparations was in the range of $4.5–5.0 \cdot 10^5$ CFU/ml (control); here and hereafter * stands for $P < 0,05$ relative to control.

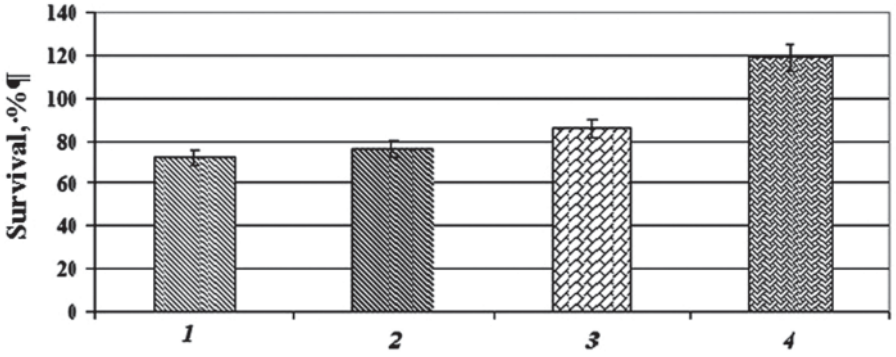

Fig. 1. **The effect of preparations 3, obtained after cultivating *N. vaccinii* IMV B-7405 on oil-containing substrates (*1–3*) and glycerol (*4*) on the survival of *X. campestris* pv. *campestris* UCM B-1049:**
1 — waste oil after potatoes frying; *2* — waste oil after frying meat; *3* — refined sunflower oil. Duration of cultivation was 5 days. Number of the cells of *X. campestris* pv. *campestris* UCM B-1049 before the addition of the preparation was $4{,}5 \cdot 10^5$ CFU/ml (100% — control)

biological properties of *N. vaccinii* IMV B-7405 metabolites can be explained by different cultivation conditions of the producer: while in [6] the glycerol content in the medium for inoculum obtaining was 0.5% , here we added it at the concentration of 1% (v/v). Quite likely, the physiological state of the inoculation material used in the studies was different, too. The data suggest that not only the nature of the carbon source in the medium cultivation of surfactant producers, but the concentration of the substrate and the method of inoculum preparation could influence on the biological properties of the synthesized extracellular metabolites.

Unfortunately, the literature almost totally lacks data on the effect of producer cultivation conditions on the properties of synthesized surfactants. Study [24] notes that on dextrose, saccharose or glycerol *Bacillus amylofaciens* AR2 produced a mixture of lipopeptides (surfactin, iturin, fengicin), while on maltose, lactose and sorbitol — only iturin. The maximal antifungal activity against micromycetes of the genera *Fusarium, Cladosporium, Alternaria etc*. was shown for surfactants produced on saccharose and dextrose.

Another study [2] established that *Bacillus subtilis* SK.DU.4 produces two antimicrobial peptides (bacteriocin-like peptide and iturin-like lipopeptide), for which the maximal synthesis and antimicrobial properties are found if the strain is grown in the medium containing glucose, meat extract, yeast extract and pepton, optimal pH 7.2. The complex of surfactants and bacteriocin had much higher antibacterial and antifungal activity against the bacteria of the genera *Bacillus, Staphylococcus, Listeria, Micrococcus* and fungi *Asperigillus terreus* Lab isolate, than separate peptides [2]. The report [2] is the first to show the ability of the bacteria of the genus *Bacillus* to simultaneously synthesize bacteriocin and surfactants. Recently, there was a information about *Lactobacillus casei* MRTL3 synthesizing both surfactants and bacteriocin [13]. The authors of [2, 13] note that the ability of the strains to produce a complex of biologically active metabolites significantly widens the range of their practical applications, in particular as antimicrobial agents.

Strain *B. subtilis* B38 synthesizes a complex of three bacillomycin D-like lipopeptides (a_1, a_2 and a_3), with antimicrobial properties against pathogenic species *Candida albicans* [25]. It was established that the fatty acid chain length in the lipopeptides influences on their antifungal properties. The most efficient antimicrobial agent was lipopeptide a_3, containing 16 carbon atoms in the acyl radical: minimum inhibitory concentration for different pathogenic species of *C. albicans* was 7.38–59.07 mM, while the MIC of lipopeptides a_1 and a_2 (14 and 15 carbon atoms in the fatty acid chain) — 60.68–970.87 and 60.68–485.43 mM, respectively.

B. subtilis CMB32 also synthesizes a complex of lipopeptides (iturin A, fengicin and surfactin A), inhibiting the growth of anthracnose infectious agent *Colletotrichum gloeosporioides* and other phytopathogenic fungi (*Botrytis cinerea* KACC 40573, *Fusarium oxysporum* KACC 40037, *Rhizoctonia solani* AG-2-2 (IIIB) KACC 40151, *Phytophthora capsici* KACC

40157, *Fusarium solani* KACC 40037, *F. solani* KCTC 6328) [26]. The fractions of iturin A and fengicin had the antifungal properties, while surfactin A showed a synergistic effect, strengthening the antimicrobial properties of iturin A. Unfortunately, the authors of [25, 26] did not study the effect of *B. subtilis* B38 cultivation conditions on the composition and antimicrobial properties of the lipopeptide complex.

Quite probably, the difference in the action on phytopathogenic bacteria of surfactants produced by *N. vaccinii* IMV B-7405 on glycerol, refined and waste sunflower oil (Table 1) is based on the changes in the qualitative and quantitative composition of the surfactant complex depending on the nature of the carbon source. We shall dedicate our following studies to clarify these regularities.

The dependence of antimicrobial properties of the surfactants on the cultivation duration of N. vaccinii IMV B–7405. The antimicrobial properties of the surfactants synthesized by the strain IMV B-7405 on various carbon substrates after 5 and 7 days of cultivation are represented in Table 2. Regardless of the nature of the oil-containing substance (refined or waste oil) and the degree of surfactant purification (supernatant, surfactant solution), increasing the duration of *N. vaccinii* IMV B–7405 cultivation to seven days was accompanied by production of the surfactants with more prominent antimicrobial properties against the phytopathogenic bacteria compared to the surfactants obtained if the producer was cultivated for only five days.

Regardless of cultivation duration, the surfactant solutions turned out to be more efficient antimicrobial agents compared to the corresponding supernatants (Table 2).

Fig. 2 shows the effects of preparations 3 (water phases after surfactant extraction for the five- and seven-days-long cultivation periods) on the survival of *P. syringae* pv. *coronafaciens* UCM B-1154 cells.

As for preparations 1 and 2, increasing the duration of *N. vaccinii* IMV B–7405 cultivation was followed by a rise in the antimicrobial activity of preparations 3. The levels of surfactants synthesized by the strain IMV B-7405 during seven days on all oil-containing substrates were 20–25% higher, than the concentration obtained after five days. The data, presented in Table 2 and on

Table 2. **Effect of cultivation duration of *N. vaccinii* IMV B-7405 on antimicrobial preparations of the surfactants**

Carbon source in the medium	Surfactant preparations	Duration of cultivation, days	Survival*,%					
			P. syringae pv. *coronafaciens* UCM B-1154	*P. syringae* pv. *atrofaciens* UCM B-1015	*P. corrugate* 9070	*X. vesicatoria* 7790	*X. campes-tris* pv. *campes-tris* UCM B-1049	*P. carotovorum* UCM B-1095
Waste oil after frying potatoes	1	5	22.8	82.8	84.2	79.0	92.0	63.0
		7	11.9	69.9	67.5	67.0	54.5	57.5
	2	5	20.1	53.2	47.5	61.5	50.0	43.5
		7	5.0	40.5	39.7	51.0	31.0	32.3
Waste oil after frying meat	1	5	74.4	90.5	90.1	83.0	83.1	89.1
		7	28.2	72.4	77.3	69.0	67.9	73.9
	2	5	60.3	69.2	74.7	71.0	54.7	65.2
		7	20.3	57.0	59.1	49.0	42.4	57.5
Refined sunflower oil	1	5	60.1	90.0	88.3	90.0	89.3	71.7
		7	49.5	80.0	75.6	82.5	79.1	54.3
	2	5	56.4	63.4	63.8	69.0	62.8	63.0
		7	44.2	51,4	50.8	43.1	54.2	43.7

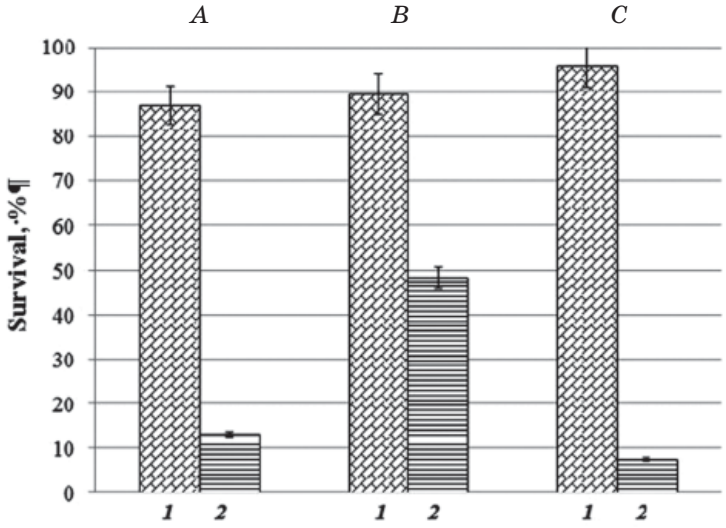

Fig. 2. **Dependence of antimicrobial properties of preparations 3 against *P. syringae* pv. *coronafaciens* UCM B-1154 on cultivation duration of *N. vaccinii* IMV B-7405 on oil-containing substrates:**
A — waste oil after potatoes frying; *B* — waste oil after meat frying; *C* — refined sunflower oil. Cultivation duration (days): *1* — 5; *2* — 7. Number of *P. syringae* pv. *coronafaciens* УКМ B-1154 cells before addition of the preparation $4{,}9 \cdot 10^5$ CFU/ml (100% — control)

Fig. 2, can be evidence that prolongation of the process leading to increasing production of both surfactants and non-surface-active metabolites with pronounced antimicrobial properties.

Determination of the minimum inhibitory concentration of N. vaccinii IMV B-7405 surfactants. A criterion of activity of a preparation with antimicrobial properties is MIC – the minimum concentration of the preparation which induces total inhibition of the test culture growth, visible with the naked eye [17]. This is an independent parameter, which can be used to compare the efficiency of many antimicrobial agents at once. Compared to other analysis techniques of the same preparations, MIC has a number of advantages: simplicity and speed of the analysis, the possibility to simultaneously evaluate several test cultures and varying concentrations of the preparations, the possibility to compare the efficiency of different preparations or preparations of different degrees of purification [17].

Data for the MIC of the preparations 2 (surfactant solutions), produced in varying conditions of *N. vaccinii* IMV B–7405 cultivation, against phytopathogenic bacteria, are shown in Table 3. The results are evidence that the most efficient antimicrobial agents are surfactants, synthesized when the strain IMV B–7405 was cultivated in the medium with waste oil after potatoes frying, for seven days: the MIC for the phytopathogenic bacteria under study, was 7–20 μg/ml.

The MIC of surfactants synthesized for seven days on all oil-containing substrates, was lower (sometimes, by an order of magnitude) than surfactants obtained during five days of *N. vaccinii* IMV B–7405 cultivation (Table 3).

The literature contains but few studies where the authors determined the MIC of microbial surfactants against phytopathogenic bacteria. For example, in [22] it was shown that the MIC of surfactin, produced by *B. subtilis* 6051, against *P. syringae* pv. *tomato* DC3000, was 25 μg/ml. In [27] the researchers presented data for MIC of microbial glycolipids (rhamnolipids, sophorolipids, mannosyl-erythritol lipids) against a number of pathogenic bacteria (*Enterobacter aerogenes, Klebsiella pneumoniae, Staphylococcus aureus, Proteus vulgaris, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens*) and fungi (*Chaetonium globosum, Candida albicans, Cryptococcus terreus*), including phytopathogens. Thus, MIC of microbial rhamnolipids against the phytopathogenic fungi *Fusarium solani, Penicillium funiculosum, Alternaria* was 16–75 μg/ml, sophorolipids against *Glomerella cingulata* – 50 μg/ml [27].

Hence, the results show that substituting traditional substrates for *N. vaccinii* IMV B-7405 cultivation (purified glycerol, refined sunflower oil) with waste (fried)

Table 3. **Minimum inhibitory concentration of preparations 2 (surfactant solutions), synthesized under varying cultivation conditions of *N. vaccinii* IMV B-7405**

Carbon source in the medium	Cultivation duration, days	MIC*, μg/ml					
		P. syringae pv. *coronafaciens* UCM B-1154	*P. syringae* pv. *atrofaciens* UCM B- 1015	*P. corrugate* 9070	*X. vesicatoria* 7790	*X. campestris* pv. *campestris* UCM B-1049	*P. carotovorum* UCM B-1095
Waste oil after frying potatoes	5	100	14	110	100	16	52
	7	7	8	20	7	8	15
Waste oil after frying meat	5	360	85	93	360	52	90
	7	38	40	55	38	16	16
Refined sunflower	5	21	25	156	21	21	85
	7	12	16	100	12	13	30
Glycerin	5	21	80	38	21	19	75

Note: while determining MIC, the error did not exceed 5%. The control was the concentration of surfactants in the last test tube where the growth of test cultures was observed.

oil allowed not only cutting the costs of the biosynthesis process, but also strengthening the antimicrobial action of the synthesized surfactants against phytopathogenic bacteria belonging to the genera *Xantomonas, Pectobacterium* and *Pseudomonas*. We determined the dependency of antimicrobial properties of the surfactants not only on the degree of purification and type of the test cultures, but also on the nature of the carbon source and duration of the cultivation. The results confirm the necessity of studying the influence of the cultivation conditions of the producers of microbial surfactants on the biological properties of the compounds.

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ВПЛИВ УМОВ КУЛЬТИВУВАННЯ НА АНТИМІКРОБНІ ВЛАСТИВОСТІ ПОВЕРХНЕВО-АКТИВНИХ РЕЧОВИН *Nocardia vaccinii* IMV B-7405

Т. П. Пирог[1, 2]
К. В. Панасюк[1]
Л. В. Никитюк[1]
Г. О. Іутинська[2]

[1]Національний університет харчових технологій, Київ, Україна
[2]Інститут мікробіології і вірусології ім. Д. К. Заболотного НАН України, Київ

E-mail: tapirog@nuft.edu.ua

Метою роботи було дослідження антимікробної дії поверхнево-активних речовин, синтезованих за різних умов культивування *Nocardia vaccinii* IMB B–7405, щодо фітопатогенних бактерій родів *Pseudomonas, Xanthomonas* і *Pectobacterium*. Їхні антимікробні властивості визначали у суспензійній культурі за методом Коха, а також за показником мінімальної інгібуючої концентрації. Поверхнево-активні речовини екстрагували із супернатанта культуральної рідини сумішшю хлороформу і метанолу (2:1). Встановлено, що антимікробні властивості залежали від природи джерела вуглецю в середовищі (рафінована, а також відпрацьована після смаження картоплі та м'яса соняшникова олія, гліцерол), тривалості культивування (5 і 7 діб), ступеня очищення (супернатант культуральної рідинии, розчин поверхнево-активних речовин) і типу тест-культур. Максимальну антимікробну дію виявляли розчини поверхнево-активних речовин, синтезованих на відпрацьованій олії після смаження картоплі (зниження виживання фітопатогенних бактерій на 50–95%), а також утворюваних упродовж 7 діб культивування штаму IMB B–7405 на всіх досліджуваних субстратах (мінімальна інгібуюча концентрація 7–40 мкг/мл, що в кілька разів нижче, ніж у речовин, синтезованих упродовж 5 діб). Результати є перспективними для розроблення екологічно безпечних біопрепаратів для контролю чисельності фітопатогенних бактерій.

Ключові слова: *Nocardia vaccinii* IMB B-7405, поверхнево-активні речовини.

ВЛИЯНИЕ УСЛОВИЙ КУЛЬТИВИРОВАНИЯ НА АНТИМИКРОБНІЕ СВОЙСТВА ПОВЕРХНОСТНО-АКТИВНЫХ ВЕЩЕСТВ *Nocardia vaccinii* IMB B-7405

Т. П. Пирог[1, 2]
Е. В. Панасюк[1]
Л. В. Никитюк[1]
Г. А. Иутинская[2]

[1]Национальный университет пищевых технологий, Киев, Украина
[2]Институт микробиологии и вирусологии им. Д. К. Заболотного НАН Украины, Киев

E-mail: *tapirog@nuft.edu.ua*

Целью работы было исследование антимикробного действия поверхностно-активных веществ, синтезированных *Nocardia vaccinii* IMB B-7405 в различных условиях культивирования, по отношению к фитопатогенным бактериям родов *Pseudomonas, Xanthomonas* и *Pectobacterium*. Их антимикробные свойства определяли в суспензионной культуре по методу Коха, а также по показателю минимальной ингибирующей концентрации. Поверхностно-активные вещества экстрагировали из супернатанта культуральной жидкости смесью хлороформа и метанола (2:1). Установлено, что антимикробные свойства зависели от природы источника углерода в среде (рафинированное, а также отработанное после жарки картофеля и мяса подсолнечное масло, глицерол), длительности культивирования (5 и 7 сут), степени очистки (супернатант культуральной жидкости, раствор поверхностно-активных веществ) и типа тест-культур. Максимальное антимикробное действие проявляли растворы поверхностно-активных веществ, синтезированных на отработанном после жарки картофеля масле (снижение выживаемости фитопатогенных бактерий на 50–95%), а также образуемых в течение 7 сут культивирования штамма IMB B–7405 на всех исследуемых субстратах (минимальная ингибирующая концентрация 7–40 мкг/мл, что в несколько раз ниже, чем у веществ, синтезируемых в течение 5 сут). Результаты являются перспективными для разработки экологически безопасных биопрепаратов для контроля численности фитопатогенных бактерий.

Ключевые слова: *Nocardia vaccinii* IMB B-7405, поверхностно-активные вещества.