https://doi.org/10.15407/microbiolj84.06.050

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EFFECT OF TRYPTOPHANE ON SYNTHESIS OF CERTAIN EXOMETABOLITES BY BACTERIA OF GENUS *ACINETOBACTER*, *NOCARDIA*, AND *RHODOCOCCUS* AND THEIR PROPERTIES

The efficiency of integrated microbial biotechnologies for obtaining several practically valuable metabolites in one technological process is determined both by the maximum concentration of these substances and their properties. This is especially true for secondary metabolites, the composition and properties of which vary depending on the cultivation conditions of the producer. ***Aim****. To research the effect of tryptophan (a precursor of auxin biosynthesis) in the culture media on the synthesis of certain exometabolites by Rhodococcus erythropolis IMV Ac-5017, Acinetobacter calcoaceticus IMV B-7241, and Nocardia vaccinii IMV B-7405 as well as their properties.* ***Methods****. R. erythropolis IMV Ac-5017, A. calcoaceticus IMV B-724, and N. vaccinii IMV B-7405 were cultivated in a medium containing refined and waste sunflower oil, biodiesel waste, or ethanol as a carbon source. The concentration of tryptophan in the medium was 300 mg/L. Surfactants were extracted from the supernatant of the cultural liquid with a modified Folch mixture. Phytohormones were isolated from the supernatant by sequential extraction with organic solvents after surfactant extraction. Thin-layer chromatography was used for preliminary purification and concentration of phytohormones. Qualitative and quantitative determination of auxins was performed using high-performance liquid chromatography. The antimicrobial activity of surfactants was analysed by the minimum inhibitory concentration. The activity of enzymes of surface-active glyco- and aminolipids biosynthesis (phosphoenolpyruvate synthetase, phosphoenolcarboxykinase, and NADP$^+$-dependent glutamate dehydrogenase) was determined spectrophotometrically during the oxidation of NADH or NADP.* ***Results****. It was found that the presence of tryptophan in the culture medium of the strains under study did not affect the number of synthesized surfactants, which was 1.80–1.90, 1.55–1.75, and 1.50–1.65 g/L, respectively. At the same time, cultivation of R. erythropolis IMV Ac-5017, A. calcoaceticus IMV B-724, and N. vaccinii IMV B-7405 in the media with tryptophan*

Citation: Pirog T.P., Piatetska D.V., Zhdanyuk V.I., Leonova N.O., Shevchuk T.A. Effect of Tryptophane on Synthesis of Certain Exometabolites by Bacteria of Genus *Acinetobacter*, *Nocardia*, and *Rhodococcus* and Their Properties. *Microbiological journal.* 2022 (6). P. 50—61. https://doi.org/10.15407/microbiolj84.06.050

increased the number of phytohormones: it was higher than the amount of phytohormones synthesized during cultivation without a precursor. The introduction of tryptophan into the culture medium of the strains was accompanied by the formation of surfactants. These compounds showed 2–4 times higher antimicrobial activity against the phytopathogenic bacteria (Agrobacterium tumefaciens UCM B-1000, Pseudomonas syringae UCM B-1027^T, Xanthomonas vesicatoria UCM B-1106, Pectobacterium carotovorum UCM B-1075^T, Clavibacter michiganensis IMV B-10$_2$ and Pseudomonas syringae pv. tomato IMV B-9167) than compounds synthesized on a medium without a precursor. The antimicrobial activity of surfactants synthesized by A. calcoaceticus IMV B-7241 in the presence of tryptophan either did not change compared to that for surfactants obtained without tryptophan, or increased slightly. Data on the activity of surfactant biosynthesis enzymes correlated with the indicators of their antimicrobial activity. In the presence of tryptophan in the culture medium of N. vaccinii IMV B-7405 and R. erythropolis IMV Ac-5017, NADP$^+$-dependent glutamate dehydrogenase activity in the cells of these strains (a key enzyme for biosynthesis of aminolipids responsible for antimicrobial activity) increased almost by 1.4 times compared to that on a tryptophan-free medium. ***Conclusions****. As a result of this work, it was found that the presence of tryptophan in the culture medium of researched strains did not affect the number of surfactants. The antimicrobial activity of surfactants against phytopathogenic bacteria either increased or remained unchanged compared to that established for surfactants synthesized without a precursor of auxin biosynthesis. The obtained data testify to the high efficiency of the potential use of surfactants complex preparations and phytohormones in crop production to stimulate the growth of plants and biocontrol of phytopathogenic bacteria.*

Keywords: *surfactants, phytohormones, biosynthesis precursor, enzyme activity, antimicrobial activity.*

A new direction of biotechnology, which is intensively developing in recent years, is the creation of so-called integrated biotechnology, in which simultaneously with the target product, other practically valuable concomitant metabolites are synthesized [1–4]. Such technologies allow one process to be carried out instead of several ones, which is clearly cost-efficient. In addition, if exo- and endometabolites are available among the target products, such production will be characterized by practical waste-freeness [5].

In 2019, we published a review [6], which summarized the available at that time literature data on the production of several practically valuable metabolites in one technological process. In particular, microbial technologies of co-synthesis of exopolysaccharides, amino acids, polyhydroxyalkanoates, and surfactants with other metabolites were considered. In this review, we focused on the ability of the *Rhodococcus erythropolis* IMV Ac-5017, *Acinetobacter calcoaceticus* IMV B-7241, and *Nocardia vaccinii* IMV B-7405 strains to synthesize phytohormones (auxins, cytokinins, gibberellins) simultaneously with surfactants that have antimicrobial activity. Currently, there are few reports about the ability of microbial surfactant producers to synthesize phytohormones, and the available literature data indicate only the synthesis of auxins by surfactant producers, mostly indole-3-acetic acid [6].

Later [7, 8], we found the possibility of increasing the number of synthesized auxins by two or three orders of magnitude in the case of low concentration of tryptophan (precursor of their biosynthesis) introduced in the culture medium of strains *R. erythropolis* IMV Ac-5017 and *A. calcoaceticus* IMV B-7241 not only with ethanol but also with industrial waste (biodiesel production waste or waste oil). The obtained results are the basis for increasing the efficiency of the use of complex preparations with growth-stimulating and antimicrobial properties against phytopathogenic bacteria in crop production.

However, the disadvantage of microbial surfactants is the dependence of their properties on the growing conditions because surfactants are secondary metabolites and they are synthesized as complexes of similar compounds, the composition and ratio of which can vary under different conditions. Therefore, there is no guarantee that surfactants synthesized under the cultivation conditions that enhance auxin synthesis will be characterized by the biological activity required for practical use.

In this regard, **the aim** of the work was to research the effect of tryptophan (a precursor of auxin biosynthesis) in the culture medium of *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241, and *N. vaccinii* IMV B-7405 on the synthesis of certain exometabolites and their properties.

Materials and methods. The main objects of research were strains *R. erythropolis* EC-1, *A. calcoaceticus* K-4, and *N. vaccinii* K-8 [9] isolated from oil-contaminated soil samples and registered in the Depository of Microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine (ZIMV) under the numbers IMV Ac-5017, IMV B-7241, and IMV B-7405, respectively.

Phytopathogenic bacteria *Agrobacterium tumefaciens* UCM B-1000, *Pseudomonas syringae* UCM B-1027^T, *Xanthomonas vesicatoria* UCM B-1106, *Pectobacterium carotovorum* UCM B-1075^T, *Clavibacter michiganensis* IMV B-10$_2$, and *Pseudomonas syringae* pv. *tomato* IMV B-9167, provided by the Department of Phytopathogenic Bacteria of the ZIMV of NAS of Ukraine, were used as test cultures to determine the antimicrobial activity of surfactants.

Strain *R. erythropolis* IMV Ac-5017 was grown in the liquid mineral medium (g/L): $NaNO_3$ — 1.3, NaCl — 1.0, $Na_2HPO_4 \cdot 12H_2O$ — 0.6, KH_2PO_4 — 0.14, $MgSO_4 \cdot 7H_2O$ — 0.1, $FeSO_4 \cdot 7H_2O$ — 0.001, pH 6.8—7.0. Ethanol, as well as waste sunflower oil, was used as the carbon and energy source at a concentration of 2% v/v.

Strain *N. vaccinii* IMB B-7405 was grown in the liquid medium containing (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; $FeSO_4 \cdot 7H_2O$ — 0.001; yeast autolysate — 0.5% (v/v). Refined and waste sunflower oils were used as carbon and energy sources at a concentration of 2% v/v.

Strain *A. calcoaceticus* IMV B-7241 was cultivated in the liquid medium (g/L): $(NH_2)_2CO$ — 0.35, $MgSO_4 \cdot 7H_2O$ — 0.1, NaCl — 1.0, Na_2HPO_4 — 0.6, KH_2PO_4 — 0.14, pH 6.8—7.0. Yeast autolysate — 0.5% v/v and solution of trace elements — 0.1% v/v were also added to the medium. Trace elements' solution contained (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 — 0.5. Crude glycerol (waste of biodiesel production, Poltava region, Ukraine) and ethanol were used as carbon and energy sources at a concentration of 2.0% v/v.

Cultures in the exponential growth phase grown on the corresponding liquid media containing 0.5—1% (v/v) of substrate were used as inoculum. The amount of inoculum (10^4—10^5 cells/mL) was 5—10% of the nutrient medium volume. Bacteria were cultured in 750 ml flasks with 100 ml of the medium on a shaker (320 rpm) at 28—30 °C for 120 h.

The number of extracellular surfactants was determined using the Bly and Dyer method modified by us [10] after extraction with a mixture of chloroform and methanol (2:1) from the supernatant of the culture liquid. To obtain the supernatant, the culture liquid was centrifuged at 5000 g for 20 min.

Given that the strains under study synthesize a complex of polar and nonpolar lipids, whereas the known Bly and Dyer method [10], used to isolate surfactants, allows isolating mainly nonpolar lipids, we modified the classical system of solvents (Folch mixture) by adding 1 M HCl, which allows us to fully isolate both polar and non-polar lipids.

In a cylindrical separating funnel with a volume of 100 mL, 25 mL of supernatant was placed, and 1 N HCl solution was added to achieve pH 4.0—4.5 (about 5 mL). The funnel was closed with a ground stopper and shaken for 3 min, then 15 mL of chloroform and methanol (2:1) was added and shaken (lipid extraction) for 5 min. The mixture obtained after extraction was left in a separatory funnel for phase separation, after which the lower fraction was drained (organic extract 1), and the aqueous phase was subjected to re-extraction. Upon re-extraction, 1 N HCl solution was added to the aqueous phase to achieve a pH of 4.0—4.5

(about 5 mL), then 15 mL of a mixture of chloroform and methanol (2:1) was added, and the lipids were extracted for 5 min. After phase separation, the lower fraction was poured to obtain organic extract 2. In the third step, 25 mL of a mixture of chloroform and methanol (2:1) was added to the aqueous phase, and extraction was performed as described above to obtain organic extract 3. Extracts 1—3 were combined and evaporated on a rotary evaporator IR-1M2 at 50 °C and absolute pressure of 0.4 atm to constant weight.

Phytohormones were determined in the supernatant, which was obtained by the culture liquid centrifugation (5000 g) for 25 min. Residues of sunflower oil from the culture broth were removed by triple extraction with petroleum ether or hexane (1:1).

Extracellular phytohormones auxins were isolated by the method of redistribution of phytohormones in two phases of solvents that do not mix with each other, as described earlier [7, 8]. Ethyl acetate, pH 3.0, was used as an organic solvent. The obtained extracts were evaporated in a vacuum at 40—45 °C. The dry residue was redissolved in 80% ethanol and transferred to microtubes. The obtained extracts were stored at —24 °C.

Preliminary purification and concentration of phytohormonal extracts (accumulative thin-layer chromatography) were performed on plates with silicagel «Silufol UV_{254}» (*Chemapol*, Czech Republic) in a mixture of solvents, introduced sequentially: chloroform, 12.5% aqueous ammonia, and ethyl acetate: acetic acid (20:1).

The qualitative and quantitative compositions of auxins were analyzed by high-performance liquid chromatography (HPLC) using an Agilent 1200 liquid chromatograph (*Agilent Technologies*, USA) and a mass spectrometry (MS) detector Agilent G1956B. HPLC/MS analysis of auxin extracts of *N. vaccinii* IMV B-7405 was performed at the Center for Collective Use at the ZIMV of NAS of Ukraine as described in previous works [7, 8].

The antimicrobial activity of surfactants was analyzed by the minimum inhibitory concentration (MIC). Determination of MIC was performed by the method of double serial dilutions in meat-peptone broth for bacteria and liquid wort for fungi as described previously [11]. The results were assessed visually by the turbidity of the medium: (+) — tubes in which turbidity of the medium was observed (growth of the test culture), (—) — tubes with no turbidity (no growth). The minimum inhibitory concentration of the surfactant solution was defined as the surfactant concentration in the last tube where there was no growth.

To obtain cell-free extracts, the culture broth gained after cultivation of all researched strains in the liquid mineral medium with crude glycerol was centrifuged (4000 g, 15 min, 4 °C). The cell pellet was washed twice from the residual medium with 0.05 M K^+ phosphate buffer (pH 7.0) by centrifuging (4000 g, 15 min, 4 °C). The washed cells were resuspended in 0.05 M K+ phosphate buffer (pH 7.0) and destroyed by ultrasound (22 kHz) 3 times for 60 s at 4 °C on a UZDN-1 apparatus. The disintegrated cells were centrifuged (12000 g, 30 min, 4 °C), the precipitate was discarded and the supernatant was used as a cell-free extract.

The activity of key enzymes of surface-active glyco- and aminolipids biosynthesis was analyzed as described previously [11]. The activity of phosphoenolpyruvate (PEP)-synthetase (EC 2.7.9.2) was determined by the rate of pyruvate formation, which was analyzed by oxidation of NADH at 340 nm in a conjugated reaction with lactate dehydrogenase; PEP-carboxykinase (EC 4.1.1.49) — in the formation of oxidation of NADH, and glutamate dehydrogenase (EC 1.4.1.4) — by the formation of glutamate during the oxidation of NADPH at 340 nm.

The enzyme activity was expressed in nmol of the reaction product obtained in 1 min per 1 mg of protein. The protein content in cell-free extracts was determined by Bradford. Enzyme activity was analyzed at 28—30 °C, the optimal temperatures for the growth of researched strains.

All experiments were performed in 3 replicates, and the number of parallel determinations in the experiments ranged from 3 to 5. Statistical processing of experimental data was performed as described previously [11]. Differences in the averages were considered significant at a significance level of $p<0.05$.

Results. Table 1 shows the synthesis of surfactants and auxins during the cultivation of all strains on media with different carbon substrates and tryptophan.

These data suggest that the presence of a precursor of auxin biosynthesis in the culture medium with different growth substrates does not affect the concentration of surfactants synthesized by all studied strains. Thus, regardless of the nature of the carbon source and the presence of tryptophan in the culture medium of *A. calcoaceticus* IMV B-7241 and *N. vaccinii* IMV B-7405, the concentration of surfactants was 1.55–1.75 and 1.50–1.65 g/L, respectively. Similar patterns were observed during the cultivation of *R. erythropolis* IMV Ac-5017 on ethanol: the surfactant concentration was in the range of 1.50–1.54 g/L. Note that when using waste oil as a substrate, a slight increase (up to 1.80–1.90 g/L) was observed in the concentration of surfactants synthesized by *R. erythropolis* IMV Ac-5017 strain compared to that on ethanol medium (Table 1). At the same time, in the process of growing *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241, and *N. vaccinii* IMV B-7405 in media with tryptophan, the phytohormones' amount formed was orders of magnitude higher than synthesized during cultivation without a precursor of their biosynthesis.

Table 1. **Synthesis of surfactants and auxins by *Acinetobacter calcoaceticus* IMV B-7241, *Nocardia vaccinii* IMV B-7405, and *Rhodococcus erythropolis* IMV Ac-5017 in the presence of tryptophan**

Producer strain	Growth substrate	Tryptophan content, mg/L	Concentration of	
			surfactants, g/L	auxins, μg/L
Acinetobacter calcoaceticus IMV B-7241	Ethanol	0	1.55±0.08	220.4
		300	1.60±0.08	2262
	Biodiesel waste	0	1.65±0.08	175.4
		300	1.75±0.09	4851
Nocardia vaccinii IMV B-7405	Refined oil	0	1.50±0.07	76.9
		300	1.65±0.08	3144
	Waste oil	0	1.55±0.08	13.2
		300	1.60±0.08	2259
Rhodococcus erythropolis IMV Ac-5017	Ethanol	0	1.50±0.07	135.9
		300	1.54±0.07	5634
	Waste oil	0	1.80±0.09	9.9
		300	1.90±0.09	2389

In the next stage, the effect of surfactants synthesized in a complex with phytohormones on phytopathogenic bacteria was researched (Table 2). The introduction of tryptophan into the culture medium of *N. vaccinii* IMV B-7405 was accompanied by the formation of surfactants that showed higher antimicrobial activity against most of the studied phytopathogenic bacteria than surfactants synthesized on an unprecedented medium (minimum inhibitory concentrations were 0.7–45 and 2.8–90 μg/mL, respectively). The same patterns were observed for surfactants of *R. erythropolis* IMV Ac-5017: antimicrobial activity of surfactants formed in the presence of tryptophan was 2–4 times higher than that for surfactants synthesized without a precursor of auxin biosynthesis. It should be noted that the MIC values for the studied test cultures, determined for surfactants *N. vaccinii* IMV B-7405 and *R. erythropolis* IMV Ac-5017, were almost the same.

A slightly different pattern was observed for surfactants synthesized by *A. calcoaceticus* IMV B-7241. First, the surfactants of this strain were characterized by several times lower antimicrobial activity against phytopathogenic bacteria than surfactants of *N. vaccinii* IMV B-7405 and *R. erythropolis* IMV Ac-5017. Second, the minimum inhibitory concentrations for some surfactants test cultures synthesized in the presence of tryptophan either didn't change compared

with those established for preparations obtained without a precursor of auxin biosynthesis, or decreased slightly (from 90 to 45 μg/mL) (Table 2).

The last stage of research was devoted to the analysis of the activity of key enzymes of biosynthesis of surface-active amino- ($NADP^+$-dependent glutamate dehydrogenase) and glycolipids (PEP-carboxykinase and PEP-synthetase) in all strains grown in media with and without tryptophan (Table 3).

Data on the surfactant biosynthesis enzymes activity in the studied strains given in Table 3 correlate with the indicators of antimicrobial activity of the surfactants synthesized by them enzymes (Table 2). Thus, in the presence of a precursor of auxin biosynthesis in the culture medium of *N. vaccinii* IMV B-7405 and *R. erythropolis* IMV Ac-5017, $NADP^+$-dependent glutamate dehydrogenase activity in cells of these strains (a key enzyme of aminolipid biosynthesis) increased by almost 1.4 times compared to that on a tryptophan-free medium (Table 3).

During the cultivation of *N. vaccinii* IMV B-7405 and *R. erythropolis* IMV Ac-5017, surfactants with higher antimicrobial activity were synthesized in a tryptophan medium (Table 2). Note that the presence of a precursor in the culture medium of these strains did not affect the activity of both key enzymes of surfactant glycolipids biosynthesis.

At the same time, in the case of tryptophan introduction into the culture medium of *A. calcoaceticus* IMV B-7241, the activity of $NADP^+$-dependent glutamate dehydrogenase did not change, whereas the activity of PEP-carboxykinase increased by almost 2 times compared to that without tryptophan (Table 3). These indicators of key enzymes activity of surface-active amino- and glycolipids biosynthesis in *A. calcoaceticus* IMV B-7241 cells grown in the presence of tryptophan and without it explain almost the same antimicrobial activity of surfactants synthesized under such conditions of this strain cultivation (Table 2).

Table 2. **Antimicrobial activity of surfactants synthesized by *Acinetobacter calcoaceticus* IMV B-7241, *Nocardia vaccinii* IMV B-7405, and *Rhodococcus erythropolis* IMV Ac-5017 in the presence of tryptophan**

Producer strain	Growth substrate	Tryptophan, mg/L	Minimum inhibitory concentrations (μg/mL) relative to					
			Agrobacterium tumefaciens UCM B-1000	*Pseudomonas syringae* UCM B-1027^T	*Xanthomonas vesicatoria* UCM B-1106	*Pectobacterium carotovorum* UCM B-1075^T	*Clavibacter michiganensis* IMV B-10$_2$	*Pseudomonas syringae* pv. *tomato* IMV B-9167
Acinetobacter calcoaceticus IMV B-7241	Ethanol	0	90	180	90	90	90	90
		300	90	180	90	45	90	45
	Biodiesel waste	0	90	180	90	180	180	180
		300	90	180	90	90	90	90
Nocardia vaccinii IMV B-7405	Refined oil	0	5.6	5.6	22.5	5.6	90	90
		300	1.4	1.4	11.3	1.4	22.5	45
	Waste oil	0	90	22.5	2.8	22.5	2.8	90
		300	45	5.6	0.7	1.4	1.4	22.5
Rhodococcus erythropolis IMV Ac-5017	Ethanol	0	11.3	90	90	5.6	2.8	2.8
		300	5.6	45	45	2.8	1.4	1.4
	Waste oil	0	11.3	90	2.8	2.8	2.8	90
		300	5.6	45	1.4	1.4	1.4	22.5

Note: when determining the minimum inhibitory concentrations, the error did not exceed 5%.

Discussion. The choice of test cultures of phytopathogenic microorganisms to determine the antimicrobial activity of surfactants *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241, and *N. vaccinii* IMV B-7405 was due to the following reasons. **First**, to date, the vast majority of scientific studies on the effects of microbial surfactants on phytopathogens have been related to their antifungal activity [12–17], and there are significantly fewer reports on the antibacterial activity of these microbial synthesis products [18–25]. However, today's problem is the fight against bacteriosis of crops, which we focused on in the review [26]. **Second**, in our studies, it was found that the treatment of the root system of tomato seedlings with *N. vaccinii* IMV B-7405 exometabolites increased the total fruit weight by 30–90% relative to control (water treatment). **Third**, in Ukraine tomatoes are affected by the bacterial cancer *Clavibacter michiganensis* subsp. *michiganensis*, bacterial spot *Pseudomonas syringae* pv. *tomato*, black bacterial spot *Xanthomonas vesicatoria*, and strains of *Pseudomonas syringae* (spot of different plant species), *Pectobacterium carotovorum* (rot of different plant species), and *Agrobacterium tumefaciens* (galls of different species), which are polyphagous and common both in Ukraine and in the world [27].

Our results have shown that the introduction of tryptophan into the culture medium of strains *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241, and *N. vaccinii* IMV B-7405 was not accompanied by a decrease in the synthesis of surfactants (Table 1) and their antimicrobial activity against phytopathogenic bacteria (Table 2). Moreover, surfactants synthesized by *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV B-7405 in the presence of tryptophan showed higher antibacterial activity than surfactants formed on a medium without a precursor of auxin synthesis. In our opinion, this can be explained by the involvement of exogenous tryptophan in the formation of not only auxins but also surface-active aminolipids, as evidenced by the increase in the activity of $NADP^+$-dependent glutamate dehydrogenases (key enzymes of aminolipid biosynthesis, responsible for antimicrobial activity) in cells of *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV B-7405. Note that by the chemical nature, *R. erythropolis* IMV Ac-5017 surfactants are a complex of glyco- (trehalose mono- and dimycolates), neutral, phosphor- and aminolipids. Glyco- (trehalose mono- and dimycolates, trehalose mono- and diacelates) and aminolipids are contained in the surfactants of *A. calcoaceticus* IMV B-7241. *N. vaccinii* IMV B-7405 synthesizes a complex of

Table 3. **Effect of tryptophan in the culture medium of *Acinetobacter calcoaceticus* IMV B-7241, *Nocardia vaccinii* IMV B-7405, and *Rhodococcus erythropolis* IMV Ac-5017 on the enzymes of surfactant biosynthesis activity**

Producer strain	Growth substrate	Tryptophan content, mg/L	Activity, $nmol \cdot min^{-1} \cdot mg^{-1}$ protein		
			$NADP^+$-glutamate dehydrogenase	PEP-synthetase	PEP-carboxykinase
Acinetobacter calcoaceticus IMV B-7241	Biodiesel waste	0	529 ± 26	11780 ± 580	278 ± 13
		300	543 ± 27	10500 ± 515	543 ± 27
Nocardia vaccinii IMV B-7405	Waste oil	0	850 ± 42	10940 ± 540	1700 ± 85
		300	1220 ± 61	11350 ± 567	1800 ± 90
Rhodococcus erythropolis IMV Ac-5017	Waste oil	0	588 ± 29	4000 ± 200	236 ± 11
		300	805 ± 40	4023 ± 200	240 ± 12

neutral, glyco- and aminolipids. Neutral lipids are represented by mycolic and n-alkanoic acids; glycolipids are represented by trehalose diacelates and trehalose mycolates [28]. In our opinion, the different antimicrobial activity of surfactants synthesized by studied strains may be due to the difference in the content of aminolipids in their composition.

Our data differ from those established in [29] on the effect of exogenous indole on the formation of biomass and aminolipids of *Bacillus amyloliquefaciens* Pc3. Indole is formed in bacterial cells as a by-product of tryptophan biosynthesis from phosphoenolpyruvate and erythroso-4-phosphate, and indole can be further converted to tryptophan by tryptophan synthase. Profiles of intracellular metabolites required for the synthesis of *B. amyloliquefaciens* Pc3 aminolipids, determined on the basis of GC/MS-metabolomics, showed a decrease in the asparagines amount, aspartic acid, glutamine, glutamic acid, threonine, valine, isoleucine, hexadecanoic and octadecanoic acid in cells grown in the presence of indole. The introduction of indole into the culture medium of the Pc3 strain was accompanied by a change in the direction of metabolism towards biomass formation and a decrease in the synthesis of aminolipids due to the lack of amino acid and fatty acid precursors.

Comparison of antimicrobial activity against phytopathogenic bacteria of surfactants synthesized by *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241, and *N. vaccinii* IMV B-7405 in the presence of tryptophan showed that the MICs of the studied surfactants are comparable with those established for the world-famous aminolipids [18–21] and glycolipids [22–25] and even exceed some of them.

In [18], it was found that the MIC of *Bacillus subtilis* 32a lipopeptides against phytopathogenic bacteria *Agrobacterium tumefaciens* C58, *Agrobacterium tumefaciens* B6, *Erwinia amylovora*, *Pseudomonas savastanoi* ranged 2–156 μg/mL.

Cao et al. [19] showed that the MIC of lipopeptides synthesized by *Bacillus velezensis* Y6 and *B. velezensis* F7 strains relative to *Ralstonia solanacearum* were 100 and 500 μg/mL, respectively. The researchers also found that the antimicrobial activity of the lipopeptide complex of strains Y6 and F7 increased due to the synergism of different lipopeptides synthesized by these two strains and was 75 μg/mL.

Khanh et al. [20] found that the MIC of pelgipeptins (PGP) synthesized by *Paenibacillus elgii* JCK-5075 ranged from 3.13 μg/mL to 100 μg/mL against 14 species of plant pathogens. The most sensitive (MIC 3.13–6.25 μg/mL) to the action of PGP were *A. tumefaciens*, *Xanthomonas arboricola* pv. *pruni*, *Xanthomonas vesicatoria*, *Xanthomonas axonopodis* pv. *citri* and *Xanthomonas oryzae* pv. *oryzae*, and the most resistant were *P. carotovorum* subsp. *carotovorum* and *C. michiganensis* subsp. *michiganensis* (MIC 25 and 100 μg/mL, respectively).

In 2020, scientists in China [21] showed that the MIC for *R. solanacearum* aminolipids synthesized by *B. velezensis* FJAT-46737 was 500 μg/mL.

The MIC of *B. amyloliquefaciens* Ar10 glycolipids against pathogens of plant bacteriosis *P. carotovorum* II16, *E. amylovora*, and *Pseudomonas aeruginosa* ATCC 27853 ranged from 1 to 50 μg/mL [24]. MIC values of surface-active rhamnolipids for phytopathogenic bacteria were slightly higher. Thus, in [22] it was found that the MIC of rhamnolipids synthesized by *P. aeruginosa* B5 against phytopathogens *Erwinia carotovora* pv. *carotovora*, *R. solanacearum*, and *Xanthomonas campestris* pv. *vesicatoria*, which affect most agricultural plants, were >50 μg/mL. Sanchez et al. [23] showed that the cultivation of *P. syringae* pv. *tomato* DC3000 and *P. syringae* pv. *tomato* AvrRPM1 in the presence of 0.2 and 1 mg/mL rhamnolipids did not inhibit the growth of phytopathogenic bacteria. In 2020 [25], it was shown that the MIC for *X. campestris* diramnolipids synthesized by *P. aeruginosa* RTE4 on glucose (2%) was 5 mg/mL.

In addition to the high antimicrobial activity of surfactants against phytopathogenic bacteria (Table 2), studied strains *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241 and *N. vaccinii* IMV B-7405 have another significant advantage compared to producers of amino- and glycolipids described in [18—25], namely the ability to simultaneously synthesize both surfactants and phytohormones with a stimulating effect. In the review [6], we have presented the limited available information about the co-synthesis of microbial surfactants and phytohormones, in particular, auxins. In recent years, there have been several publications on the formation of surfactants and auxins (mainly indole-3-acetic acid) by plant-associated bacteria, in particular members of the genera *Bacillus* and *Pseudomonas* [30—33].

Note that only in one research [33], the authors established antimicrobial activity of synthesized surfactants against phytopathogenic bacteria. Thus, in the presence of a surfactant-containing supernatant, inhibition of the growth of *C. michiganensis* subsp. *michiganensis* PVCT156.1.1 and *Xanthomonas euvesicatoria* pv. *perforans* NCPPB432 was observed, but no indicators of antimicrobial activity are given in the article. Simultaneously with the surfactants, the presence of which was determined by the qualitative reaction, the strain *P. aeruginosa* FG106 isolated from the rhizosphere of healthy tomatoes formed indole-3-acetic acid (211 μg/mL). The surfactants described in [30—32] were characterized exclusively by the antifungal activity.

In 2021, a paper reporting on the ability of *Bacillus atrophaeus* B44 to simultaneously synthesize an aminolipid complex (422.6–446.9 mg/L) with antifungal activity and biologically active gibberellin GA_3 (7.7–23.1 mg/L) was published [34]. As shown, the qualitative composition of the aminolipid complex and the level of gibberellin synthesis depend on the composition of the nutrient medium (the presence of certain salts and trace elements).

It should be noted that at present we have not been able to find information in the available literature on the simultaneous synthesis of surfactants with antibacterial activity against phytopathogens and phytohormone complex (auxins, cytokinins, and biologically active gibberellins), as established for our studied strains *R. erythropolis* Ac-5017, *A. calcoaceticus* IMV B-7241, and *N. vaccinii* IMV B-7405.

Therefore, as a result of this work, it was found that the presence of tryptophan in the culture medium of *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241, and *N. vaccinii* IMV B-7405 does not affect the level of synthesis of surfactants, and antimicrobial activity of synthesized surfactants against phytopathogenic bacteria either remained unchanged or increased by 2—4 times compared with that established for surfactants synthesized without a precursor of auxin biosynthesis. The obtained data testify to the high efficiency of the potential use of surfactants complex preparations and phytohormones in crop production to stimulate the plant growth and biocontrol of phytopathogenic bacteria.

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Received 22.05.2022

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ВПЛИВ ТРИПТОФАНУ НА СИНТЕЗ ДЕЯКИХ ЕКЗОМЕТАБОЛІТІВ БАКТЕРІЯМИ РОДІВ *ACINETOBACTER*, *NOCARDIA*, *RHODOCOCCUS* ТА ЇХНІ ВЛАСТИВОСТІ

Ефективність інтегрованих мікробних біотехнологій, що дають змогу отримати в одному технологічному процесі кілька метаболітів, визначається не тільки їхньою максимальною концентрацією, а й наявністю необхідних для практичного використання властивостей цих цільових продуктів. Особливо це стосується вторинних метаболітів, склад і властивості яких змінюються залежно від умов культивування продуцента. **Мета.** Дослідити вплив триптофану (попередник біосинтезу ауксинів) у середовищі культивування на синтез деяких екзометаболітів *Rhodococcus erythropolis* IMB Ac-5017, *Acinetobacter calcoaceticus* IMB B-7241 і *Nocardia vaccinii* IMB B-7405 та їхні властивості. **Методи**. *R. erythropolis* IMB Ac-5017, *A. calcoaceticus* IMB B-7241 і *N. vaccinii* IMB B-7405 вирощували у середовищах, що містили як джерело вуглецю рафіновану або відпрацьовану соняшникову олію, відходи виробництва біодизелю, етанол. Концентрація триптофану

у середовищі становила 300 мг/л. Поверхнево-активні речовини (ПАР) екстрагували з супернатанту культуральної рідини модифікованою сумішшю Фолча. Фітогормони виділяли шляхом послідовної екстракції органічними розчинниками із супернатанту культуральної рідини після екстракції поверхнево-активних речовин. Попереднє очищення і концентрування фітогормонів здійснювали методом тонкошарової хроматографії. Якісне та кількісне визначення ауксинів проводили за допомогою високоефективної рідинної хроматографії. Антимікробну активність ПАР аналізували за показником мінімальної інгібуючої концентрації. Активність ферментів біосинтезу поверхнево-активних гліко- та аміноліпідів (фосфоенолпіруватсинтетази, фосфоенолкарбоксикінази, НАДФ$^+$-залежної глутаматдегідрогенази) визначали спектрофотометрично під час окиснення НАДН або НАДФН при 340 нм. **Результати.** Встановлено, що наявність триптофану у середовищі вирощування досліджуваних штамів практично не впливала на концентрацію синтезованих ПАР, яка становила відповідно 1,80–1,90, 1,55–1,75 і 1,50–1,65 г/л незалежно від умов культивування штамів. У той же час у процесі вирощування *R. erythropolis* IMB Ac-5017, *A. calcoaceticus* IMB B-7241 і *N. vaccinii* IMB B-7405 у середовищах з триптофаном кількість утворених фітогормонів була на порядки вищою порівняно з біосинтезом їх без попередника. Внесення триптофану у середовище культивування досліджуваних штамів супроводжувалося утворенням ПАР, які проявляли вищу у 2–4 рази антимікробну активність щодо досліджуваних фітопатогенних бактерій (*Agrobacterium tumefaciens* УКМ B-1000, *Pseudomonas syringae* УКМ B-1027^T, *Xanthomonas vesicatoria* УКМ B-1106, *Pectobacterium carotovorum* УКМ B-1075^T, *Clavibacter michiganensis* IMB B-10$_2$ і *Pseudomonas syringae* pv. *tomato* IMB B-9167), ніж синтезовані на середовищі без попередника. Антимікробна активність ПАР, синтезованих *A. calcoaceticus* IMB B-7241 за наявності триптофану або не змінювалася порівняно з такою для препаратів, одержаних без попередника біосинтезу ауксинів, або незначно підвищувалася. Дані щодо активності ферментів біосинтезу ПАР корелювали з показниками їхньої антимікробної активності. За наявності попередників біосинтезу ауксинів у середовищі культивування *N. vaccinii* IMB B-7405 і *R. erythropolis* IMB Ac-5017, активність НАДФ$^+$-залежної глутаматдегідрогенази (ключового ферменту біосинтезу аміноліпідів, відповідальних за антимікробну активність ПАР комплексу) в клітинах цих штамів підвищувалася майже в 1,4 рази порівняно з такою на середовищі без триптофану. **Висновки**. У результаті проведеної роботи встановлено, що наявність триптофану у середовищі культивування досліджуваних штамів не впливала на рівень синтезу ПАР, а їхня антимікробна щодо фітопатогенних бактерій активність або підвищувалася, або залишалася без змін порівняно з встановленою для препаратів, синтезованих без попередника біосинтезу ауксинів. Одержані дані свідчать про високу ефективність потенційного використання комплексних препаратів поверхнево-активних речовин і фітогормонів у рослинництві для стимуляції росту сільськогосподарських культур і біоконтролю чисельності фітопатогенних бактерій.

Ключові слова: *поверхнево-активні речовини, фітогормони, попередник біосинтезу, активність ферментів, антимікробна активність***.**