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ORIGINAL RESEARCH PAPER

SYNTHESIS OF GIBBERELLINS BY SURFACTANT PRODUCERS *NOCARDIA VACCINII* IMV B-7405, *ACINETOBACTER CALCOACETICUS* IMV B-7241 AND *RHODOCOCCUS ERYTHROPOLIS* IMV AC-5017

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Abstract: In this study, we established that the producers of surfactants *Nocardia vaccinii* IMV B-7405, *Acinetobacter calcoaceticus* IMV B-7241 and *Rhodococcus erythropolis* IMV Ac-5017 were able to synthesize phytohormones of gibberellic nature during cultivation on the traditional substrates (ethanol, hexadecane) and on the industrial waste sunflower oil and biodiesel production waste). The results of specific biotesting showed that the treatment of cucumber seedlings with phytohormonal extracts of all studied strains at 1:500 and 1:600 dilutions stimulated the extension of the cucumber hypocotyls as compared to the water control in values close to the treatment with gibberellic acid. The results of high-performance liquid chromatography showed *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 produce highly active forms of gibberellins GA_3 and GA_4. The level of its synthesis was nearly the same (6.0-10.0 $\mu g \cdot L^{-1}$) under cultivation of strains on every substrate. The exception was strain *N. vaccinii* IMV B-7405 which synthesized almost 47.0 $\mu g \cdot L^{-1}$ GA_3 and GA_4 while is growing on the waste oil from meat frying. The obtained results are the groundwork for the development of an economically profitable technology for the recycling of toxic wastes using *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017. Such technology will allow us to develop complex microbial preparations with various biological properties in a single process.

Keywords: *bacteria, GA_3, GA_4, phytohormones, specific biotesting, surface-active substances, toxic waste*

INTRODUCTION

Most researchers adhere to commonly accepted concept of "one producer – one product" while developing microbial biotechnologies and focusing their attention only on synthesis of the main target product. At the same time, the data about synthesis by microorganisms of several practically valuable metabolites, such as surface-active substances (surfactants) with polysaccharides, bacteriocins, polyhydroxyalkanoates or enzymes began to appear in the literature in the past few years [1 – 6]. The authors of these works note that the ability of strains to synthesize complexes of metabolites with various biological properties significantly expands the scope of their practical use.

We established the ability of *Nocardia vaccinii* IMV B-7405, *Acinetobacter calcoaceticus* IMV B-7241 and *Rhodococcus erythropolis* IMV Ac-5017 to synthesize exometabolites with phytohormonal activity (auxins and cytokinins) in the previous work [7]. We must note that synthesis of auxins and cytokinins depended on the nature of the source of carbon in the culture medium.

At the time of publication of the work [7], there were no data in the available literature on the ability of the surfactant producers to synthesize phytohormones. The production of indole-3-acetic acid by bacteria (mainly representatives of the genus *Rhodococcus*) isolated from soils contaminated with hydrocarbons and heavy metals was reported in 2016. But the authors were determining the surfactant synthesis ability by the emulsification index and reduction of surface tension, which was insignificant – no more than 60.0-65.0 $mN \cdot m^{-1}$ (comparing to 30.0–35.0 $mN \cdot m^{-1}$ by our producers of surfactants).

However, in recent years more and more publications have appeared about the microorganisms (mainly rhizobacteria) production of complexes of biologically active substances, in particular phytohormones and metabolites with antimicrobial, nematicidal, etc. activity [9 – 15]. Actinobacteria (in particular *Streptomyces*) [10 – 14], as well as the representatives of the genera *Bacillus* and *Paenibacillus* [9, 15], are the most active producers of complexes of such compounds with diverse biological activity.

The analysis of the literature data [16 – 19] showed that, usually, microorganisms form phytohormones of the three classes - auxins, cytokinins and gibberellins, and this is true for those who associates with the plants (rhizospheric, endophytic, nitrogen-fixing, phytopathogenic bacteria), and for those who do not participate in such interaction.

The aim of the present work is to study the ability to synthesize compounds of gibberellic nature by surfactant producers *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017.

MATERIALS AND METHODS

Object of research

The objects of research are *Nocardia vaccinii* K-8, *Acinetobacter calcoaceticus* K-4 and *Rhodococcus erythropolis* EK-1 strains, registered in Microorganisms Depositary of Institute of Microbiology and Virology, the National Academy of Sciences of Ukraine under the numbers IMV B-7405 (K-8), IMV B-7241 (K-4) and IMV Ac-5017(EK-1).

Medium composition and conditions of cultivation

N. vaccinii IMV B-7405 was grown in the liquid mineral medium (g·L^{-1} distilled water): $NaNO_3$ – 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, *p*H 6.8–7.0.
Strain *A. calcoaceticus* IMV B-7241 was cultivated in the liquid medium (g·L^{-1} distilled water): $(NH_2)_2CO_3$ – 0.35, $MgSO_4 \cdot 7H_2O$ – 0.1, NaCl – 1.0, Na_2HPO_4 – 0.6, KH_2PO_4 – 0.14, *p*H 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.
Strain *R. erythropolis* IMV Ac-5017 was grown in the liquid mineral medium (g·L^{-1} distilled water): $NaNO_3$ – 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, *p*H 6.8–7.0.
The refined and fried sunflower oil (McDonald's Restaurant Network, Kiev, Ukraine), technical glycerol (Komsomolsk biofuel plant, Poltava region, Ukraine), *n*-hexadecane and ethanol were used as the carbon and energy sources in concentration of 2.0 % v/v.
The culture in the exponential phase was used as inoculum and added in concentration of 5 - 10 % of nutritive medium volume. The concentration of the corresponding carbon source in the medium for the inoculum obtainment was 0.5 % v/v.
The cultivation was carried out in 750 mL flasks, containing 100 mL of medium, on the shaker (320 rpm) at 28 – 30 ºC during 7 days.

Determination of indexes of growth and biosurfactant synthesis

The biomass was determined by the gravimetrical method. The double treatment of the culture broth with hexane or petrol ether was carried out to remove the residual *n*-hexadecane or oil respectively before the cell sedimentation (5000 g, 30 min). The necessity of this operation was caused by the precipitation of *n*-hexadecane or oil with cells that overestimate the biomass level.
The amount of exocellular surfactant was defined by weighing after it had been extracted from the culture broth supernatant with the mixture of chloroform and methanol (2 : 1). To obtain the supernatant, the culture broth was centrifuged at 5000 g for 20 minutes. Five milliliters of 1 N HCl were added to a 100 mL cylindrical separating funnel with 25 mL supernatant; the funnel was closed with the lap stopper and shaken for 3 minutes; then, a 15 mL mixture of chloroform and methanol (2 : 1) was added and stirred (extraction of lipids) for 5 minutes. The mixture obtained after the extraction was kept in the funnel until the separation of phases, then the lower fraction was poured (organic extract 1) and the water phase was re-extracted. At the repeated extraction, 5 mL of 1 N HCl solution and 15 mL of the mix of chloroform and methanol (2 : 1) were added to the water phase for extraction of lipids, which took 5 minutes. After the separation of the phases, the lower fraction was poured off and organic extract 2 was obtained. At the third step, 25 mL of chloroform and methanol (2 : 1) were added to the water phase and extraction was carried out as above. As a result, organic extract 3 was obtained. Extracts one to three were merged and evaporated in the IR-1M2 rotor evaporator (OJSC Khimlaborpribor, Russia) at 50 °C and at an absolute pressure of 0.4 atm until reaching constant mass.

Obtaining of extracts with phytohormonal activity (phytohormonal extracts)

After cultivation of the strains *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017, the biomass was separated by centrifugation (5000 g) for 25 min. Residuals of sunflower oil and hexadecane were extracted from the culture broth using petroleum ether or hexane (ratio 1 : 1).
Gibberellic extracts were obtained from the supernatant of the culture broth by extraction with ethyl acetate at *p*H 2.5. The extracts were evaporated to dryness under vacuum and redissolved in 80 % ethanol. The obtained extracts were stored at -24 °C. In the article, these preparations are called phytohormonal extracts.

Determination of gibberellic activity

To determine gibberellic activity we used hypocotyls of seedlings of cucumber grade Nezhinskiye by technique Brian's and Lemming's bioassay in the modification Ahnistykova [21]. After germination of cucumber seeds during 3-4 days (temperature 27 °C) hypocotyls with length 2.0 ± 0.2 cm were selected. Hypocotyls were placed in Petri dishes with aliquots of aqueous solutions of the phytohormonal extracts at dilutions 1 : 400, 1 : 500 and 1 : 600. After incubation for 1 - 2 days at 27 °C elongation of hypocotyls was measured and it was compared to controls (treatment with distilled water and a solution gibberellic acid (GA_3) at a concentration of 10^{-5} M).

Qualitative and quantitative determination of gibberellins

Purification and concentration of the phytohormonal extracts were carried out on silicagel plates of the mark Silufol UV254 (Chemapol, Czech Republic) in a mixture of solvents used sequentially: chloroform, 12.5 % aqueous ammonia, ethyl acetate : acetic acid (20 : 1).
The qualitative and quantitative composition of gibberellins was analyzed by high performance liquid chromatography (HPLC) using the Agilent 1200 liquid chromatograph (Agilent Technologies, USA) and the Agilent G1956B mass spectrometry (MS) detector. The separation was carried out on analytical column Zorbax SB-C18 (2.1 mm × 150 mm, 3 μm; Agilent Technologies, USA), the flow rate of the mobile phase was 0.35 $mL \cdot min^{-1}$. The volumes of injections were 3 μL. Columns were kept at 30 °C. Elution was carried out in gradient mode in acetonitrile (A) - 0.1 % / water + formic acid (B): 20 % A for 5 minutes, then the content of A was gradually changed from 20 to 80 % for 10 minutes, followed by an increase of A to 100 % for 0.5 minutes. This ratio was kept for the next 8.5 minutes. Standard solutions of gibberellins GA_3, GA_4 and GA_7 (Sigma-Aldrich, Germany) were used for identification.
Detection of gibberellins was carried out using a diode-matrix detector with a signal recording at a wavelength of 198 and 210 nm. Mass spectrometric analysis was performed with the recording of positive and negative ions in the ratio m/z in the range 190 - 400 nm. The fluorescence detector was used in the extinction mode at 210 nm, and in the emission mode at 410 nm.

Analysis of the molecular weight of gibberellins

The molecular mass was determined using a single quadrupole mass spectrometric detector. Ionization was carried out in the mode of electrostatic spraying (ESI) with the formation of negative ions. Ion detection was performed in SCAN and SIM mode (selected ion monitoring) in the range of 200 ÷ 500 m/z. GA_3, GA_4 and GA_7 were registered by comparing the retention time, the values of the molecular weights of the ions, the spectral characteristics of the obtained peaks. The quantitative content of GA_3, GA_4 and GA_7 was determined by the external calibration method using the SIM mode by the 345, 331 and 329 m/z ions.
HPLC/MS analysis of gibberellic extracts of *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 was made at the Center for collective use at the D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine.
All the experiments were repeated three times, and the number of parallel measurements in each experiment made up 3 – 5. The statistical processing of the experimental data was carried out in accordance with the algorithm described in [22]. Differences of mean indicators were deemed as reliable at the significance level $p < 0.05$. All the used reagents were analytical grade.

RESULTS AND DISCUSSION

Synthesis of surfactants by *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 on different substrates

The data of Table 1 confirm that the concentration of the surfactants synthesized by studied strains depended on nature of carbon source in cultivation medium. The highest synthesis rates were observed in case of using waste sunflower oil (2.3-3.5 $g \cdot L^{-1}$) or technical glycerol (4.2 $g \cdot L^{-1}$) as a substrate. The concentration of surfactants, synthesized on the traditional substrates (ethanol, hexadecane, refined oil) was lower (1.5-2.0 $g \cdot L^{-1}$).

Table 1. *The effect of nature of carbon source in culture medium of N. vaccinii IMV B-7405, A. calcoaceticus IMV B-7241 and R. erythropolis IMV Ac-5017 on synthesis of surfactants*

Strain	Carbon source in culture medium	Concentration of surfactant, [$g \cdot L^{-1}$]
N. vaccinii IMV B-7405	Refined oil	1.8±0.09
	Waste oil after frying potatoes	2.3±0.11
	Waste oil after frying meat	2.6±0.13
A. calcoaceticus IMV B-7241	Ethanol	1.5±0.08
	Technical glycerol	4.2±0.21
	Refined oil	2.0±0.10
	Waste oil after frying meat	3.5±0.17
R. erythropolis IMV Ac-5017	Ethanol	1.8±0.14
	n-Hexadecane	1.5±0.08
	Refined oil	1.8±0.09
	Waste oil after frying meat	2.3±0.11

Gibberellin-specific phytotesting

One of the approaches for studying the ability of gibberellin synthesis by bacteria is the phytotesting which gives us the opportunity to evaluate the biological effect of the phytohormonal extracts. Therefore, at the next stage, bioassay was performed using cucumber seedlings which are a typical test culture in gibberellin-specific phytotests.

Table 2. *The effect of phytohormonal extracts of IMV B-7405, IMV B-7241 and IMV Ac-5017 on prolongation of hypocotyls of cucumbers grade Nezhinskiye*

Strain	**Carbon source in culture medium**	**Length of hypocotyls (cm) after treatment by**			
		phytohormonal extracts in dilution		**water (control)**	**gibberellic acid (control)**
		1 : 500	**1 : 600**		
N. vaccinii IMV B-7405	Refined oil	8.1 ± 0.1	8.4 ± 0.1	7.5 ± 0.1	8.3 ± 0.2
	Waste oil after frying potatoes	8.2 ± 0.1	8.1 ± 0.1	7.5 ± 0.1	8.4 ± 0.2
	Waste oil after frying meat	8.2 ± 0.1	8.5 ± 0.2	7.6 ± 0.1	8.5 ± 0.2
A. calcoaceticus IMV B-7241	Ethanol	4.7 ± 0.1	4.8 ± 0.1	4.2 ± 0.1	4.6 ± 0.2
	Technical glycerol	5.0 ± 0.1	5.3 ± 0.1	4.7± 0.1	5.8 ± 0.3
	Refined oil	11.1 ± 0.2	11.3 ± 0.2	10.2 ± 0.1	11.4 ± 0.3
	Waste oil after frying meat	9.5 ± 0.1	9.1 ± 0.1	8.7 ± 0.1	9.8 ± 0.2
R. erythropolis IMV Ac-5017	Ethanol	5.6 ± 0.2	6.0 ± 0.3	4.0 ± 0.1	5.9 ± 0.2
	n-Hexadecane	5.7 ± 0.1	5.8 ± 0.1	5.4 ± 0.1	6.0 ± 0.1
	Refined oil	6.5 ± 0.1	6.8 ± 0.2	5.7 ± 0.1	6.6 ± 0.2
	Waste oil after frying meat	7.2 ± 0.2	6.9 ± 0.1	5.8 ± 0.2	6.6 ± 0.2

Our studies have shown that in case of treatment of seedlings with phytohormonal extracts of strains *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 the stimulation of prolongation of hypocotyls was observed comparing with using water or gibberellic acid solution for treatment (Table 2).

Data presented in Table 2 show that, regardless of the dilution of the extracts (500 or 600 times), the results of treatment were almost the same. However, with further 800 and 1000 times dilution the stimulation of hypocotyl prolongation wasn't observed. It should be noted that the effectiveness of the phytohormonal extracts depended on the nature of carbon source in cultivation medium of the surfactant producing strains.

Results obtained in the case of treatment of cucumber seedlings with the phytohormonal extracts in dilution 1 : 500, 1 : 600 are close to values obtained in the case of treatment with gibberellic acid. This allows us to assume that the extracts contain compounds of gibberellic nature.

The synthesis of gibberellins by *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 on different substrates

Qualitative and quantitative composition of gibberellins in phytohormonal extracts of *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 are presented in Table 3.

These data show that all studied strains are able to synthesize gibberellins A_3 and A_4, but the level of synthesis was different and depended on the nature of carbon source in the cultivation medium.

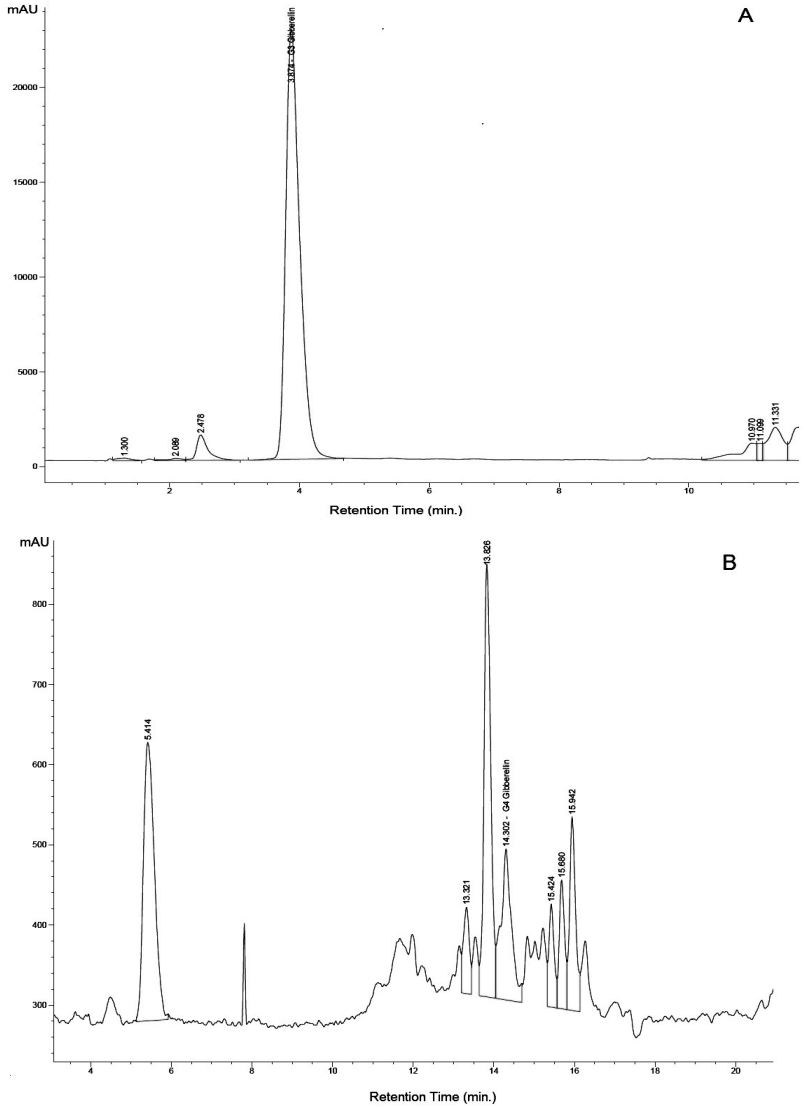

Figure 1. *HPLC/MS analysis of metabolites of gibberellic nature extracted from the culture liquid supernatant of N. vaccinii IMV B-7405 under cultivation on the waste oil after frying potatoes. Shown are (A) GA_3 analysis and (B) GA_4 analysis*

The highest concentration of GA_3 and gibberellin-synthesizing ability was observed for strain *N. vaccinii* IMV B-7405 under cultivation on the waste oil after frying potatoes or meat (Table 3, Figure 1). For other strains the rates of synthesis were an order of

magnitude lower. Under cultivation of *A. calcoaceticus* IMV B-7241 on the technical glycerol and *R. erythropolis* IMV Ac-5017 on the hexadecane the synthesis of gibberellic acid was almost not observed.

The data presented in Table 3 show that regardless of nature of carbon source in cultivation medium every strain had almost the same level of GA_4 synthesis: the concentration of GA_4 was in the range 5.63 -7.36 $\mu g \cdot L^{-1}$, and gibberellin-synthesizing ability was within 3.77 - 8.38 μg GA_4/g of biomass.

Experiments have shown that all strains synthesized trace amounts of GA_7 in the studied conditions of cultivation.

Thus, the HPLC data are consistent with the phytotests data and indicate the ability of surfactants producing strains *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 to synthesize GA_3, GA_4 and GA_7.

To date, the ability to synthesize phytohormones of gibberellic nature is established for a wide range of living organisms. Plants, bacteria, fungi and yeasts synthesize over 130 different forms of gibberellins [23]. However, only some of them demonstrate the high biological activity - its GA_1, GA_4, GA_4 and GA_7, and the other gibberellins are physiologically inactive and are the precursors of the biosynthesis of other forms.

Gibberellins are synthesized by many micromycetes [24 – 27], and not only by representatives of the genus *Fusarium* (*Gibberella*), which are the industrial producers of the gibberellic acid [28]. The synthesis of gibberellins by the endophytic fungi of the genus *Penicillium* is one of the mechanisms that allow the plants survive under salt stress [26]. As for the connection between pathogenicity and phytohormonal activity, the research of growth stimulating and pathogenic strains of *Fusarium culmorum* showed that the latter synthesized 4 times less gibberellin [27].

Many bacteria also synthesize gibberellins, moreover as phytopathogenic and associated with plants [18, 19, 29] and freely existing [30]. In the 80's of the 20th century was carried out the study of 10 wild and mutant (*nod* , *fix*) strains of *Rhizobium phaseoli* that showed that incapable of nodulation and nitrogen fixation mutants also synthesized gibberellins, and therefore, nitrogen-fixing ability is not related to phytohormonal activity [31].

Despite the fact that GA_4 and GA_7 are not inferior to GA_3 on biological activity, the industrial production for them is not developed. This is due to the following reasons: they are synthesized in extremely low concentrations, which causes high price of the preparations; as a rule, they are synthesized as a mixture of GA_4 and GA_7, in which the ratio GA_4/GA_7 varies greatly; excretion of individual preparations GA_4 and GA_7 from the mixture is complicated because of their very close polarity [32].

The ability to synthesize GA_4 and GA_7 has been identified as in microorganisms associated with plants - rhizospheric [33], endophytic [34], and in freely existing [35].

In 2017, a strain *Bacillus aryabhattai* SRB02 was isolated from soybean rhizosphere, which contributed to the better heat resistance of soybean plants. It produced the entire spectrum of biologically active gibberellins, among which prevailed GA_4 - 31 $ng \cdot L^{-1}$, and the level of synthesis of others (GA_1 - 1 $ng \cdot L^{-1}$, GA_3 - 6 $ng \cdot L^{-1}$, GA_7 - 12 $ng \cdot L^{-1}$) was lower [32].

Bacterial endophyte *Sphingomonas* sp. LK11 due to the ability to synthesize indol-3-acetic acid and gibberellins stimulates the growth of tomatoes (*Tephrosia apollinea*). In the culture broth of *Sphingomonas* sp. LK11, the presence of physiologically active GA_4 in the amount of 2970 $ng \cdot L^{-1}$, as well as inactive GA_9 (980 $ng \cdot L^{-1}$) and GA_{20}

(2410 ng·L^{-1}), was detected [34].

Strain *Leifsonia soli* sp. SE134 in the terms of experiment promoted the growth of cucumbers, tomatoes and young radishes, by increasing shoot length, plant fresh weight and chlorophyll in the leaves as compared with control. The wide spectrum of gibberellins, including GA_4 (15.8 ng·L^{-1}) and GA_7 (5.4 ng·L^{-1}), was identified in the culture broth of *L. soli* sp. SE134 [35].

Despite the fact that microorganisms of various physiological groups are able to synthesize gibberellins [23 – 35], in the available literature we could not find the information on the synthesis of gibberellins by the producers of surfactants.

Our research showed that producers of surfactants *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 synthesize gibberellins GA_3, GA_4 and GA_7 under cultivation on the different substrates, including the industrial waste. It is worth mentioning that the level of gibberellin synthesis practically independent of the nature of the carbon source in the cultivation medium of the studied strains. An exception has appeared to be strain *N. vaccinii* IMV B-7405, which synthesized on the orders of magnitude higher amounts of GA_3 on the waste oil than on the refined oil. In our opinion it's because in the waste oil may be compounds, which are activators of enzymes of the gibberellin synthesis. Our further research will be devoted to clarifying this issue.

In Table 4 is presented generalized data on synthesis of the phytohormones of the three main classes - auxins, cytokinins and gibberellins, by the studied producers of surfactants.

Table 4. *Influence of cultivation conditions of N. vaccinii IMV B-7405, A. calcoaceticus IMV B-7241 and R. erythropolis IMV Ac-5017 on synthesis of phytohormones*

Carbon source in culture medium	**Strain**	**Concentration [µg·L^{-1}]**			
		auxins [2]	**cytokinins [2]**	**gibberellins**	**Total**
Ethanol	IMV B-7241	104.2	3.5	9.28	116.98
	IMV Ac-5017	84.3	–	8.26	92.56
Technical glycerol	IMV B-7241	122.0	363.9	7.36	493.26
	IMV B-7405	139.9	–	–	139.9
n-Hexadecane	IMV Ac-5017	44.8	21.4	5.73	71.93
Refined oil	IMV B-7241	39.6	75.1	8.0	122.7
	IMV B-7405	770.4	348.0	5.96	1124.36
	IMV Ac-5017	19.4	17.1	7.8	44.3
Waste oil after frying meat	IMV B-7241	83.2	43.6	9.49	**136.29**
	IMV B-7405	23.3	53.9	46.8	**124.0**
	IMV Ac-5017	91.3	37.8	5.83	**134.93**
Waste oil after frying potatoes	IMV B-7405	84.7	15.9	18.16	**118.76**

Despite the fact that under the cultivation on such substrates as refined oil and glycerol, the total concentration of the phytohormones (500-1100 µg·L^{-1}) is the highest, their use for obtaining microbial preparations is limited due to the high cost. That's why the synthesis of phytohormones on the waste oil deserves attention: for all strains, the total concentration was in the range of 120-140 µg·L^{-1}. This level of synthesis was lower compared with the microorganisms, which are traditionally used as the growth

stimulators of plant (they synthesize from 600 to 40000 $\mu g \cdot L^{-1}$ phytohormones) [18, 19, 21]. But, given that the phytohormones show their stimulating effect in the extremely low concentrations ($10^{-5} – 10^{-12}$ $mol \cdot L^{-1}$), the rates of their synthesis by the producers of surfactants is acceptable for practical use in the plant growing.

Our previous research has shown that the surfactants of *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 are the preparations of multifunctional purpose. So, they intensify the processes of decomposition of oil in water and soil, they have the antimicrobial activity, including against phytopathogenic bacteria; and also the surfactants of studying strains are characterized with the anti-adhesive properties and the ability to destroy biofilms [36 – 40].

Compared with the other microorganisms that are able to synthesize a complex of biologically active substances, in particular, phytohormones and metabolites with antimicrobial, nematicidal etc. activity [9 – 15], the complex preparations of strains *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 have the following advantages.

First, these preparations have much wider range of properties (besides the antimicrobial and growth stimulating activities, also the destruction of oil, the anti-adhesive properties and the ability to destroy biofilms). Due to that they can be used not only in the plant growing for growth stimulation and control of pests number, but also in the environmental technologies for the environmental purification, in food, in pharmaceutical industry and medicine as the antimicrobial and antiadhesive agents.

Second, according to the literature data the antimicrobial metabolites synthesized simultaneously with the phytohormones are mostly characterized by an antifungal (rarely – nematicidal) activity. If it is antibacterial activity, then such products of microbial metabolism are antibiotics. Thus, streptomycetes synthesize geldanamycin [11], avermectins [13], blasticidin S, kasugamycin, oligomycin A, paramycin and pyrroles [14], and therefore, their use may be accompanied by the emergence of resistant forms of microorganisms. The mechanism of antimicrobial activity of surfactants is to ruin the integrity of the cytoplasmic membrane of the test cultures, which leads to the loss of cell viability [41]. In this case, the appearance of the microorganisms resistant to the microbial surfactants is unlikely.

Third, the microorganisms synthesize phytohormones on the rich nutrient mediums, which contain glucose, sucrose, dextrose, glucuronic acid, peptone, tryptone, mannitol as the source of carbon, and also tryptophan is being added exogenously to the medium for auxin formation, as it is the precursor of biosynthesis [42, 43]. Our research have shown the possibility of formation of the phytohormones of auxin, cytokinin [7] and gibberellin nature on the cheap mediums using toxic industrial waste (in particular, the waste oil and the technical glycerol as the waste of biodiesel production) as the substrates, as well as the possibility of the auxin synthesis without adding tryptophan to the medium [7].

Thus, the implementation of technologies based on *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 will allow us not only to dispose the toxic industrial waste, but also to obtain the complex microbial preparations of wide range of application in the one process.

CONCLUSIONS

The results obtained earlier and presented in this article are the basis for the development of the waste-free technology using *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 that will allow obtaining in a single process the microbial preparations with the various biological properties. Thus, when receiving surfactants, the precipitated cells can be used to purify water from oil; the obtained supernatant of the culture broth - for further separation of the surfactants with anti-adhesive and antimicrobial properties (including against the phytopathogenic bacteria). Since the aqueous phase, which remains after extraction of the surfactants, contains the phytohormones of auxin, cytokinin and gibberellic nature, it can be used to stimulate the growth of the microorganisms and plants.

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