Works on the creation of an automated plant maintenance system are conducted jointly with the laboratory for the introduction of tree plants of the Central Botanical Garden of the National Academy of Sciences of Belarus.

PRODUCTION OF THE COMPLEX MICROBIAL PREPARATION USING INDUSTRIAL WASTE

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In this study, we found that the producer of surfactants *Acinetobacter calcoaceticus* IMV B-7241 was able to synthesize phytohormones during cultivation on the industrial waste (fried sunflower oil and biodiesel production waste). The obtained results were used for the development of an economically profitable technology for the recycling of toxic wastes by *A. calcoaceticus* IMV B-7241. Such technology will allow to develop complex microbial preparations with various biological properties.

Keywords: industrial waste, phytohormones, surfactants, complex microbial preparations.

In the previous study the ability of *Acinetobacter calcoaceticus* IMV B-7241 to synthesize surface-active substances with anti-adhesive and antimicrobial properties was shown [1]. Widespread use of microbial surfactants is constrained by high costs on biosynthesis (materials, energy) and the isolation and purification of the final product. One way to reduce the cost of production is use less expensive substrates, including waste from other industries. New perspective area of biotechnology is to obtain and use complex microbial preparations with different properties, such as microbial surfactants with enzymes, bacteriocins, polysaccharides or phytohormones.

The aim of the present research is to study the possibility of synthesis of extracellular compounds with phytohormonal activity by surfactant producer *A. calcoaceticus* IMV B-7241 on industrial waste.

Bacteria were cultivated using a synthetic medium. Fried and refined sunflower oil (restaurant chain McDonald's, Kyiv), technical glycerol (Komsomolsk biofuel factory, Poltava region), and ethanol were used as the carbon sources. Substrate concentration was 2% (v/v).

Phytohormones of gibberellic nature were extracted from the supernatant culture liquid *A. calcoaceticus* IMV B-7241 after isolation of surfactants with mixture of chloroform and methanol in a ratio of 2:1 (Folch's mixture). Preliminary purification and concentration of the substances with gibberellic activity was performed by thin layer chromatography method. Qualitative and quantitative determinations of auxins and cytokinins were carried out using a scanning spectrodensitometer. Determination of gibberellins was carried out by high-performance liquid chromatography (HPLC).

Qualitative and quantitative composition of phytohormones in extracts of *A. calcoaceticus* IMV B-7241 is presented in Table 1.

Table 1

The synthesis of phytohormones under cultivation of *A. calcoaceticus* IMV B-7241 on different substrates

Carbon source in culture medium	Concentration (μg/L)			
	auxins	cytokinins	gibberellins	Total
Ethanol	104.2	3.5	9.28	116.98
Technical glycerol	122.0	363.9	7.36	**493.26**
Refined oil	39.6	75.1	8.0	122.7
Waste oil after frying meat	83.2	43.6	9.49	**136.29**

The data presented in Table 1 show that strain IMV B-7241 is able to synthesize all three classes of stimulating phytohormones on every studied substrate. Worth to mention that the total concentrations of phytohormones synthesized on technical glycerol and waste oil is higher. Given that the phytohormones show their stimulating effect in the extremely low concentrations (10^{-5}–10^{-12} mol/L), the rates of their synthesis by the producers of surfactants is acceptable for practical use in the plant growing.

The results obtained earlier and presented in this work are the groundwork for the development of the waste-free technology using *A. calcoaceticus* IMV B-7241 that will allow obtaining in one process the microbial prepa-

rations 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 cultural liquid – for further separation of the surfactants with anti-adhesive and antimicrobial properties (including against the phytopathogenic bacteria). 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 plants and increasing the yield.

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BIOSYNTHESIS OF BIOSURFACTANTS BY CULTIVATION OF *NOCARDIA VACCINII* IMV B-7405 ON TOXIC INDUSTRIAL WASTE

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The possibility of using mixture of technical glycerol (waste product of biodiesel production) and fried sunflower oil as a substrate for the synthesis of the extracellular surfactants by *Nocardia vaccinii* IMV B-7405 was investigated. The maximum concentration of surfactants synthesized by *N. vaccinii* IMV B-7405 (5,0 g/l), was reached in the medium with technical glycerol (3,25%, v/v) and fried oil (0,75%, v/v).

Keywords: Nocardia vaccinii IMV B-7405, surfactants, mixture of frying sunflower oil and crude glycerol.

Introduction. Previous studies have shown the possibility of using fried sunflower oil and technical glycerol (waste of biodiesel production) as a substrate for the synthesis of the extracellular surfactants by *Nocardia vaccinii* IMV B-7405 [1]. Cultivation on a mixture of these substrates allows not only to utilize toxic industrial waste, but also is one of the effective approaches to the intensification of the synthesis of biosurfactants [2]. Therefore, the purpose of this work is to establish the possibility of intensification of the synthesis of surfactants by *N. vaccinii* IMV B-7405 on a mixture of fried sunflower oil and technical glycerol.

Materials and methods. The strain *N. vacinii* IMV B-7405 was grown on the synthetic nutrient medium containing (g/L): $NaNO_3$ – 0.5; $MgSO_4 \cdot 7H_2O$– 0.1; $CaCl \cdot 2H_2O$ – 0.1; KH_2PO_4 – 0,1; $FeSO_4 \cdot 7H_2O$ – 0.01, yeast autolysate – 0.5%., v/v. Monosubstrates (technical glycerol and fried sunflower oil) at a concentration of 4%., v/v and a mixture of technical glycerol (1.0–3.25%., v/v) and fried oil (0.75 – 3.0%., v/v) was used. The culture in exponential growth phase, grown in a medium with technical glycerol, fried oil at a concentration of 0.5% v/v and a mixture of technical glycerol (0.25%, v/v) and fried oil (0.25%., v/v) were used as inoculum. Concentration of inoculum was 10%, v/v. Cultivation of the strain IMV B-7405 was carried out in flasks (750 ml) with 100 mL of medium in the shaker (320 rpm) at 30 ºC for 120 hours. The surfactants concentration was determined by gravimetrically after extraction from the supernatant of the culture liquid with a modified mixture of Folch (chloroform – methanol =2:1, pH 4.0−4.5 with addition of 1N HCl).

Results and Discussion. At the first stage of experiments we investigated the optimal method of preparation of inoculum. It was established that the highest concentration of surfactants (3.3 g/L) which were synthesized by the strain IMV B-7405, in a medium with mixture of fried sunflower oil (2%, v/v) and technical glycerol (2%, v/v), were observed when the technical glycerol and a mixture of technical glycerol and fried oil was used for inoculum preparation. In subsequent studies, the inoculum was grown on technical glycerol. It is known [2] that the efficiency of technologies of microbial product synthesis on mixed substrates depends both on the molar ratio of monosubstrates in the mixture and on their concentration. Therefore, at the next stage, the effect of various concentrations of technical glycerol and fried sunflower oil in the mixture on the synthesis of surfactant by the strain IMV B-7405 was investigated. Experiments have shown that increasing the concentration of monosubstrates in a mixture from 1% to 2.5% was accompanied by an increase of the concentration of surfactants from 2.4 to 3.6 g/L In the case of further increase in the concentrations of fried sunflower oil and technical glycerol, the biosurfactant synthesis rates decreased. In our opinion, this may be due to the low content of the nitrogen source in the media. On the third stage, we investigated the amount of surfactants synthesized by the IMV-B-7405 strain in the medium with different ratios of the concentrations of fried oil and technical glycerol in the culture medium. Experiments have shown that maximum concentration of surfactant (5.0 g/L) was observed when the strain IMV