Integrated technology of the surfactants and phytohormones biosynthesis by *Nocardia vaccinii* IMV B-7405 for their use in crop production

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Abstract

Introduction. The strain *Nocardia vaccinii* IMV B-7405, which produces phytohormones and surfactants, is a promising agent for the development of integrated technologies to obtain complex preparation to be used in crop production.

Materials and methods. Cultivation of bacteria was carried out in a liquid medium with 2% refined or waste oil and 100–300 mg/l of tryptophan (the precursor of the phytohormone auxin biosynthesis) was added to the medium. The concentration of auxins was determined by the method of high-performance liquid chromatography, surfactants – by weight method. Antimicrobial activity of surfactant was analyzed by the indicator of minimum inhibitory concentration (MIC). Greenhouse experiments were carried out using tomato plants; the number of fruits and their weight were analyzed. Determination of the protective effect of surfactants against bacterial diseases of tomatoes was carried out by the method of separated leaves.

Results and discussion. It was established that in the presence of tryptophan in the culture medium of *N. vaccinii* IMV B-7405 the concentration of auxins was one to two orders of magnitude higher than without the biosynthesis precursor. The increase in auxins synthesis correlated with tryptophan transaminase activity – the key enzyme of auxins biosynthesis. Surfactants synthesized in the presence of tryptophan were characterized by higher antimicrobial activity against phytopathogenic bacteria: MICs were 2–4 times lower compared to those established for preparations formed without a precursor. Treatment of tomato leaves with solutions of surfactants synthesized by *N. vaccinii* IMV B-7405 in the presence of tryptophan contributed to the protection of leaves from phytopathogens damage. The exometabolites of *N. vaccinii* IMV B-7405 increased the productivity of tomatoes: the total weight increased by 82–91%, and the average fruit weight by 12–18%.

Conclusions. The complex preparation based on the exometabolites (the surfactants and phytohormones) of *N. vaccinii* IMV B-7405 can be used to control the number of phytopathogens and increased the productivity of tomatoes.

Introduction

At the present stage, the classical monobiotechnologies, the main postulate of which is "one producer – one carbon substrate – one target product", are being replaced by the so-called "integrated biotechnologies" (one producer – one or several carbon substrates – several target products) (Hori et al., 2011; Krishnan et al., 2019; Nitschke et al. 2011; Que et al., 2014; Zeng et al., 2013). The effectiveness of such technologies is obvious. First, there is the implementation of one technological process instead of several. Secondly, the scope of application of the target product, which contains a complex of metabolites with different properties, is much wider compared to mono bioformulations (Pirog et al., 2019a).

The available individual data on the surface-active substances (surfactants) biosynthesis as concomitant metabolites of phytohormones of auxin`s nature were summarized in the review (Pirog et al., 2019a). Note that the concentration of phytohormones synthesized by surfactant producers was low and didn`t exceed 5 mg/l. At the same time, microbial surfactants due to a complex of unique properties (the ability to reduce surface and interfacial tension, emulsify various substrates, destroy biofilms, and have antimicrobial and anti-adhesive activity) are multifunctional formulations and can be used in food technologies, pharmaceutical industry, agriculture, medicine and environmental technologies (Fenibo et al., 2019; Pirog et al., 2019a).

The ability of the surfactant producer *Nocardia vaccinii* IMV B-7405 to synthesize stimulating phytohormones such as auxins, cytokinins, and gibberellins was shown in the studies of Pirog et al. (2016). Since surfactants of *N. vaccinii* IMV B-7405 strain have a wide range of anti-adhesive and antimicrobial activity (Pirog et al., 2023), including phytopathogenic bacteria, the multifunctional formulation containing surfactant and phytohormones is promising for use in crop production. At the same time, the concentration of phytohormones synthesized by *N. vaccinii* IMV B-7405 was low (70-100 μg/l), which significantly reduced the effectiveness of using such formulation to stimulate plant growth.

In modern biotechnologies, one of the approaches to increasing the concentration of the target product can be the introduction of biosynthesis precursors. Thus, most researchers (Liu et al., 2019; McClerklin et al., 2018) determine the ability of microorganisms to synthesize auxins with the addition of tryptophan to the culture medium, which is a precursor to the synthesis of indole-3-acetic acid (IAA).

It was shown that the surfactant producer *N. vaccinii* IMV B-7405 synthesizes auxins under growth media conditions with various substrates without a precursor (Pirog et al., 2016), and, therefore, there are potential opportunities for increasing their synthesis.

However, it noted that both surfactants and phytohormones are secondary metabolites that are usually synthesized in the form of a complex of similar compounds, the composition and ratio of which may vary depending on the cultivation conditions (Pirog et al., 2019b). Therefore, there is no guarantee that surfactants synthesized in the presence of tryptophan in the culture medium will be characterized by high antimicrobial activity.

Therefore, the aim of this research was: (a) to define the possibility of auxin`s synthesis intensification due to introducing a precursor into the culture medium of *N. vaccinii* IMV B-7405; (b) to determine the antimicrobial activity of surfactants synthesized in tryptophan presence in the medium against phytopathogenic bacteria; (c) to check the effectiveness of a complex microbial bioformulations in vegetation conditions to stimulate the growth and increase the yield of tomatoes.

Materials and methods

Research objects

The main object of research was a strain of oil-oxidizing bacteria, *Nocardia vaccinii* IMV B-7405 from the Collection of Microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine (Pirog et al., 2019, 2023). By chemical nature, extracellular surfactants of *N. vaccinii* IMV B-7405 are a complex of glyco-, amino- and neutral lipids. Glycolipids are represented by trehalosomycolates.

This research used phytopathogenic bacteria from the Ukrainian Collection of Microorganisms (UCM): *Pseudomonas syringae* UCM B-1027, *Agrobacterium tumefaciens* UCM B-1000, *Xanthomonas vesicatoria* UCM B-1106, *Pectobacterium carotovorum* UCM B-1075. Also, strains from the Collection of the Department of Phytopathogenic Bacteria of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine: *Clavibacter michiganensis* subsp. *michiganensis* IMV B-10_2 and *Pseudomonas syringae* pv. IMV B-9167 were used.

Cultivation conditions

The strain *N. vaccinii* IMV B-7405 grown in a medium containing (g/l): $NaNO_3$ – 0.5–1.25; $MgSO_4 \cdot 7H_2O$ – 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). The source of carbon and energy is refined sunflower oil and oil after frying potato or meat, in a concentration of 2% (v/v).

Tryptophan was introduced into the medium as the 1% solution in the amount of 100, 200 or 300 mg/l at the beginning of the process or the end of the exponential phase of strain growth (at 48 h of cultivation).

Cultures in the exponential growth phase, grown on appropriate liquid media containing 0.5–1% (v/v) of the substrate used as inoculum. The amount of inoculum (10^4–10^5 cells/ml) was 5–10% of the volume of the nutrient medium. Cultivation of bacteria was carried out in flasks with a volume of 750 ml with 100 ml of medium on a shaker (320 rpm) at 28–30 ºC for 120 hours.

Release of extracellular auxins

Preparation of auxin extracts. Extracellular phytohormones auxins were isolated by triple extraction with organic solvents from the culture broth after surfactant extraction (Negretsky, 1988). Ethyl acetate, pH 3.0, was used as an organic solvent. The obtained extracts were evaporated under a vacuum at 40-45 °C. The dry sediment was dissolved in 80% ethanol and transferred to microtubes. The obtained extracts were stored at a temperature of – 24°C.

Determination of the qualitative and quantitative composition of auxins. Preliminary purification and concentration of extracts containing phytohormones (accumulative thin-layer chromatography) were carried out on plates with silica gel brand "Silufol UV_{254}" (Chemapol, Czech Republic) in a mixture of solvents, which were introduced sequentially: chloroform, 12.5% aqueous ammonia, and ethyl acetate: acetic acid (20:1).

The qualitative and quantitative composition of auxins was analysed by high-performance liquid chromatography (HPLC), using an Agilent 1200 liquid chromatograph (Agilent Technologies, USA) and an Agilent G1956B mass spectrometry (mass spectrometry – MS) detector. HPLC/MS analysis of auxin extracts was performed at the Center for Collective Use of Scientific Equipment at the Institute of Microbiology and Virology.

For comparison, standard synthetic phytohormones *Sigma* (Germany) and *Acros Organic* (Belgium) were used:

IAA – Indole-3-acetic acid, Indole-3-acetic acid (IOK);

ICal – Indole-3-carboxaldehyde, Indole-3-carboxaldehyde;

IC – Indole-3-carbinol, Indole-3-carbinol;

ICA – Indole-3-carboxylic acid, Indole-3-carboxylic acid;

IAA-hydr. – Indole-3-acetic acid hydrazide, Indole-3-acetic acid hydrazide;

IBut – Indole-3-butyric acid, Indole-3-butyric acid;

Methanol (A) and a 1% solution of acetic acid in water (B) were used as the mobile phase. The separation was carried out on a Zorbax SB-C18 chromatographic column (2.1 mm × 150 mm, 3 μm) (Agilent Technologies, USA), the flow rate through the column was 0.25 ml/min, the temperature of the thermostat was 30 °C, and the injection volume was 2 μl. Elution was carried out in the gradient mode: 0 min – A (30%): B (70%); 25 min – A (30%): B (70%); 35 min – A (100%): B (0%); 35 min - A (100 %): B (0 %).

Detection of compounds was carried out using a diode-matrix detector with signal registration at 254 and 280 nm and fixation of absorption spectra in the range of 191–700 nm. An Agilent G1956B mass spectrometric detector (Agilent Technologies, USA) was used to determine the molecular masses of the studied compounds. Ionization was carried out in the ESI and APCI modes with the fixation of positive ions in the SCAN mode in the range of 100–1200 m/z. Calibration was performed using standard auxin solutions. The amount of phytohormones was estimated in μg per 1 g of dry biomass of the producer.

Enzymatic analyses

Preparation of cell-free extracts. To obtain cell-free extracts, the culture liquid after cultivation of *N. vaccinii* IMV B-7405 in a liquid mineral medium with used oil after frying potatoes was centrifuged (4000 g, 15 min, 4 °C). The cell sediment was washed twice from the residues of the medium with 0.05 M K-phosphate buffer (pH 7.0), centrifuging (4000 g, 15 min, 4 °C). Washed cells were suspended in 0.05 M K-phosphate buffer (pH 7.0) and destroyed by ultrasound (22 kHz) 3 times for 60 seconds at 4 °C on the UZDN-1 apparatus. The disintegrated biomass was centrifuged (12,000 g, 30 min, 4 °C), the sediment was discarded, and the supernatant was used as a cell-free extract.

Tryptophan transaminase activity. The activity of tryptophan transaminase (EC 2.6.1.27, other names: L-phenylalanine-2-oxoglutarate aminotransferase; tryptophan aminotransferase; 5-hydroxytryptophan-ketoglutaric transaminase; hydroxytryptophan aminotransferase; tryptophan aminotransferase; L-tryptophan transaminase) was determined by the formation of indole-pyruvate from L- tryptophan and 2-oxoglutarate, which was analysed spectrophotometric at 330 nm (Collier and Kohlhaw, 1972).

Determination of the concentration of surface-active substances and preparation of surfactant solutions

The amount of extracellular surfactants was determined by the weight method after their extraction from the supernatant of the culture liquid.

Surfactants were obtained from a supernatant via extraction with a chloroform–methanol mixture at a ratio of 2:1 (Folch mixture). Cultural liquid obtained after cultivation of *N. vaccinii* IMV B-7405 in the medium containing used oil after frying meat or potato as a carbon source with 300 mg/L tryptophan added at the beginning of growth was used.

Grown cells were centrifuged (5000 g) for 45 min, and the supernatant was further treated. For this purpose, 50 mL of supernatant was placed in a 200 mL cylindrical separating funnel, 50 mL of the Folch mixture was added, and the funnel was closed with a stopper and vortexed (lipids were extracted) for 5 min. The mixture obtained after the extraction procedure was left in a separating funnel for phase separation; after that, the lower fraction was poured out (organic extract 1), and the water phase was re-extracted as described above. After the phase separation, the lower fraction was poured out and organic extract 2 was obtained. In the third stage, 50 mL of the Folch mixture was added to the water phase, extraction was performed, and organic extract 3 was obtained.

In the studies, solutions of surface-active substances with different concentrations were used as preparations. For this, the dry residue was diluted by sterile tap water to the original volume. All the preparations were sterilized at 112°C for 30 min.

Determination of the surfactant antimicrobial activity

The antimicrobial activity of surface-active substances was determined according to the indicator minimum inhibitory concentration (MIC). Determination of MIC was carried out by the method of two-fold serial dilutions in meat-peptone broth (MPB). Under sterile conditions, 1 ml of the medium was introduced into 10 test tubes, 1 ml of a surfactant solution of a certain concentration was added to the first, after which it was mixed, 1 ml was taken and transferred to the next test tube. Similarly, dilution was carried out for the next nine test tubes. 1 ml was taken from the last test tube. Thus, the final volume in each tube was 1 ml (MPB and surfactant solution), and the concentration of surfactant in each subsequent tube was reduced by 2 times. As a control, 1 ml of MPB without the addition of a surfactant solution was used. Next, 0.1 ml of test culture suspension (10^5–10^6 CFU/ml) was added to each test tube and mixed. The test tubes were incubated for 24 hours at 28-30 °C.

The results were assessed visually by the turbidity of the medium: (+) – test tubes in which the turbidity of the medium was observed (growth of the test culture), (−) – there was no turbidity (no growth). The minimum inhibitory concentration of the surfactant solution was determined as the concentration of surfactant in the last test tube where growth was absent.

Determination of the exometabolites effect on the growth and yield of tomatoes

The experiment was conducted in greenhouses from April to September. Tomatoes plant variety Salad (*Solanum lycopersicum* L.) was used as a test crop. The crop was harvested in the period from July to September. Before planting in the soil, the root system of tomato seedlings was kept for two hours in the supernatant or culture liquid of *N. vaccinii* IMV B-7405 (dilution 1:200 and 1:400). Seedlings kept for two hours in tap water were used as a control. There were three plants in each variant. During the experiment, the amount of tomato fruits and their weight were analysed.

Determination of the protective effect of surface-active substances of *N. vaccinii* IMV B-7405 against bacterial diseases of tomatoes

The determination was carried out by the method of detached leaves. *X. vesicatoria* IMV B-9098 and *C. michiganensis* subsp. *michiganensis* IMV B-102 were used as test cultures of phytopathogens, which cause necrosis on the plant leaves.

2 months after setting up the vegetative experiment, the plants in the flowering phase were treated with a surfactant solution with a concentration of 20 μg/ ml. A day later, leaves were collected from the surfactant-treated and untreated plants. The leaves were placed in Petri dishes on sterile cotton swabs moistened with 5 ml of sterile tap water. After that, the leaves were sprayed with a suspension of phytopathogenic bacteria (10^7-10^8 CFU/ml), and grown on MPA for 48 hours. Leaves treated with sterile tap water were used as a control. Closed Petri dishes were incubated at room temperature under natural light for 7 days and the course of the disease was observed. The degree of development of tomato bacterial disease was estimated as a percentage ratio of the area of the leaf affected by the disease to the area of the entire leaf.

Statistical processing

All experiments were repeated three times; the number of determined parameters was from 3 to 5. The experimental data were statistically processed according to Lakin (Pirog et al., 2016). Differences between mean parameters were considered significant at $p < 0.05$.

Results and discussion

Effect of precursors on auxins biosynthesis by *N. vaccinii* IMV B-7405

Previous studies have shown that the synthesis of auxin metabolites depended on the nature of the carbon source in the culture medium of *N. vaccinii* IMV B-7405 (Pirog et al., 2016).

The data given in Table 1 testify those regardless of the time of tryptophan introduction into the culture medium of strain *N. vaccinii* IMV B-7405 with both refined and spent oil, a significant increase in auxin synthesis was observed compared to indicators on the medium without this precursor.

Among the synthesized auxins, IAA prevailed, whose precursor is tryptophan. Note that the highest concentration of auxins was achieved when 300 mg/l of tryptophan was added to the medium with both substrates.

It is known (Pidgorsky et al., 2010) that most of the precursors are involved in the processes of the biosynthesis of secondary metabolites at the end of the exponential or the beginning of the stationary phases of growth. This was observed under the conditions of growing *N. vaccinii* IMV B-7405 on refined oil: the introduction of tryptophan at the end of the exponential phase of growth was accompanied by an increase in the concentration of synthesized auxins by 1.8-41.3 times.

At the same time, other regularities were observed during the cultivation of *N. vaccinii* IMV B-7405 in a medium with spent oil: for most variants, the highest concentration of auxins was observed when tryptophan was added at the beginning of the cultivation process. We assume that such results may be due to the quality of the waste oil used as a substrate, in particular, the presence of components in its composition that can somehow affect the biosynthesis of phytohormones. Our further research will be devoted to clarifying these issues.

The data given in Table 1, testify that the concentration of synthesized auxins increased with an increase in the concentration of the precursor in the culture medium of *N. vaccinii* IMV B-7405. A further increase in tryptophan may be accompanied by an intensification of auxin synthesis. However, at this stage, to create an effective microbial

preparation with growth-stimulating properties, this is not necessary, since at the achieved concentration of auxins (3000−5000 µg/l, see Table 1), the culture liquid of *N. vaccinii* IMV B-7405 to process seeds or the root system of plant seedlings must be diluted at least 200 times.

Table 1

Synthesis of auxins under growth conditions of *N. vaccinii* IMV B-7405 in an environment with oil-containing substrates and tryptophan

Oil as a substrate	Trp, mg/l	The moment of introduction of tryptophan (growth phase)	Concentration of auxins, µg/l				
			IAA	ICA	ICal	IAA-hydr.	Total amount
Refined oil	Without tryptophan		64.9	6.3	2.1	3.6	76.9
	100	Lag phase	29.0	11.6	17.7	–	58.3
		The end of the exponential	40.6	14.4	84.9	–	139.9
	200	Lag phase	348.6	71.7	11.3	–	431.6
		The end of the exponential	854.0	501.5	–	–	1355.5
	300	Lag phase	331.4	90.0	–	–	421.4
		End of the exponential	1986,7	1157.1	–	–	3143.7
Waste oil	0	Lag phase	4.5	1.8	–	6.9	13.2
	100	Lag phase	1258.9	472.6	–	–	1731.5
		The end of the exponential	874.3	292.1	–	–	1185.7
	200	Lag phase	2331.2	470.6	–	–	2801.8
		The end of the exponential	2166.4	725.8	12.7	–	2910.8
	300	Lag phase	4666.7	1139.2	–	–	5806.0
		The end of the exponential	1538.8	719.73	–	–	2258.6

Note. Oil after frying potatoes was used as a substrate. IAA – indole-3-acetic acid; ICA – indole-3-carboxylic acid; ICal – indole-3-carboxaldehyde; IAA-hydr. – indole-3-acetic acid hydrazide. "–" – not found. When determining the concentration of auxins, the error did not exceed 5%.

Activity of tryptophan transaminase under different conditions of cultivation of *N. vaccinii* IMV B-7405

To confirm that exogenous tryptophan is involved in the biosynthesis of auxins, the activity of tryptophan transaminase was analysed – one of the key enzymes in the synthesis of indole-3-acetic acid, which catalyses the reaction of the formation of indole-3-pyruvic acid from tryptophan and 2-oxoglutarate. As evidenced by the data shown in Figure 1, in the presence of tryptophan in the culture medium of the *N. vaccinii* IMV B-7405, a 2.4-5.7-fold increase in the activity of this enzyme was observed compared to the cultivation of this strain without a precursor.

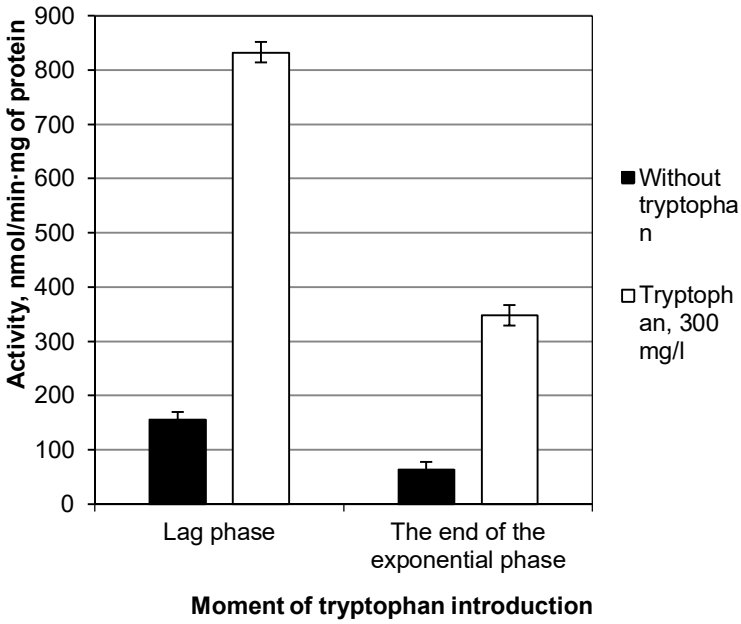

Figure 1. The influence of tryptophan on the activity of tryptophan transaminase in *N. vaccinii* IMV B-7405 cells grown on refined oil

Antimicrobial activity of surfactant *N. vaccinii* IMV B-7405 against phytopathogenic bacteria

The data given in Table 2 showed that surfactants synthesized in the presence of tryptophan were characterized by high antimicrobial activity against phytopathogenic bacteria: the MICs of such surfactants were 2-4 times lower compared to the indicators established for surfactants formed in an environment without a precursor.

Table 2

Antimicrobial activity of surfactants synthesized by *N. vaccinii* IMV B-7405 in the presence of tryptophan

Growth substrate	Tryptophan, mg/l	Minimum inhibitory concentrations (μg/ml) relative to phytopathogenic bacteria					
		UCM B-1000	UCM B-1027	UCM B-1106	UCM B-1075	IMV B-10_2	IMV B-9167
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

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

Influence of surfactants on tomato bacterial pathogens

The following experiments showed that pretreatment of tomato leaves with solutions of surfactants synthesized by *N. vaccinii* IMV B-7405 in the presence of tryptophan contributed to the protection of leaves from damage by phytopathogens (Figures 2 and 3). Thus, within 7 days, no symptoms of the disease were detected on the treated leaf infected with phytopathogens (Figure 2a, Figure 3a). The treatment proved to be effective protection against *C. michiganensis* subsp. *michiganensis* IMV B-102 and *X. vesicatoria* IMV B-9098 strains.

At the same time, the degree of damage by phytopathogens to untreated leaves with solutions of surface-active substances ranged from 8 to 50% (Figure 2b, Figure 3b). Such results testify to the high efficiency of the antimicrobial effect of surface-active substances against bacterial diseases of tomatoes.

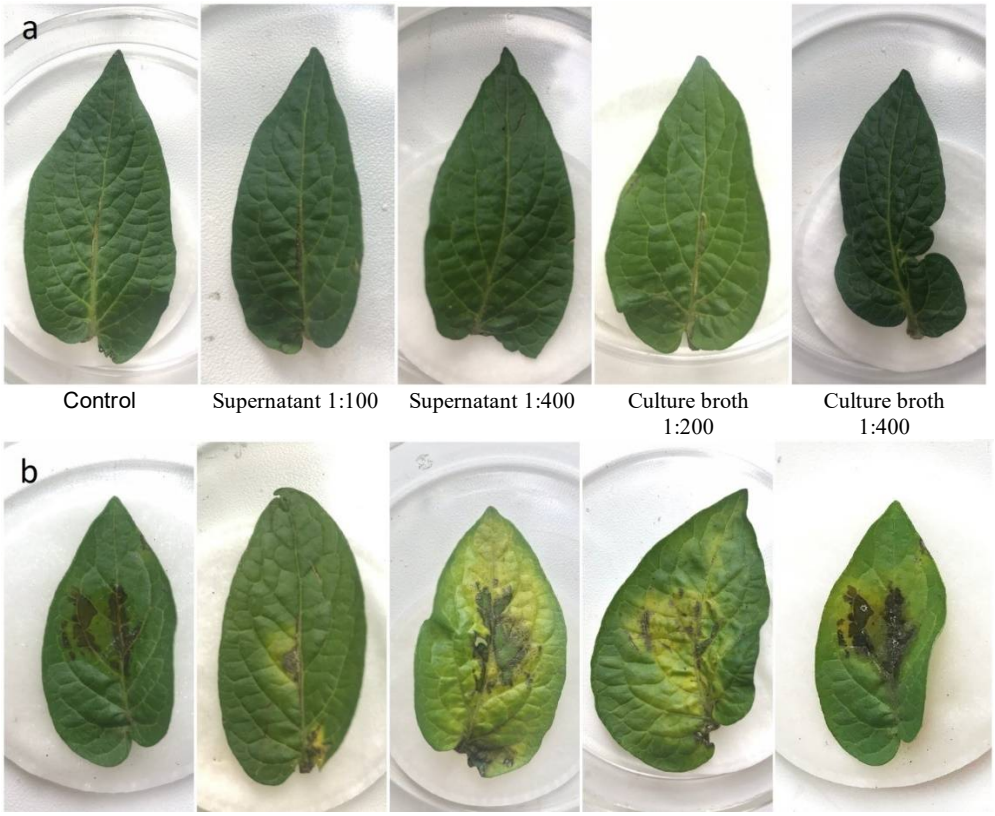

Figure 2. Inoculation of tomato leaves with strain *Xanthomonas vesicatoria* IMV B-9098 pre-treated with surfactant (a) and without pre-treatment (b)

Figures 2 and 3: leaves were taken from tomatoes, the root system of seedlings of which before planting in the soil was treated with water (control), diluted 200 and 400 times with the supernatant and culture liquid after growing *N. vaccinii* IMV B-7405 on oil spent after frying potatoes according to the presence of tryptophan (300 mg/l)

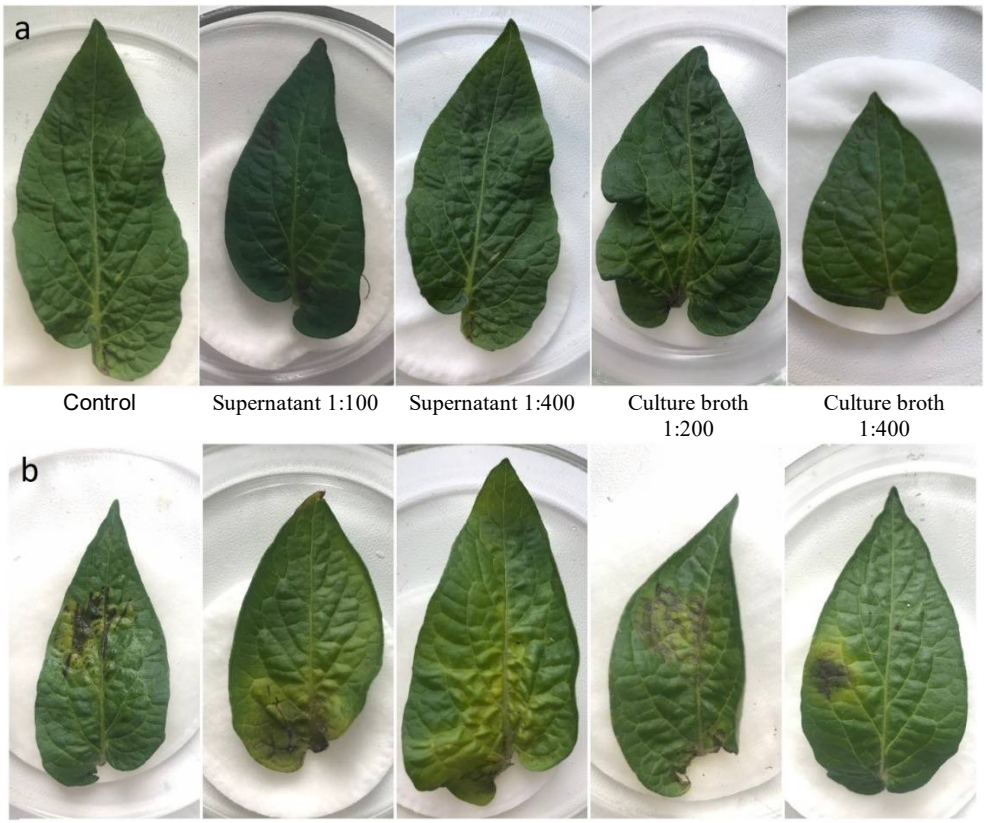

Figure 3. The effect of surfactants pre-treatment of tomato leaves (a) and its absence (b) on development of *Clavibacter michiganensis* subsp. *michiganensis* IMV B-102

Effects of *N. vaccinii* IMV B-7405 exometabolites on the yield of tomatoes

Data on the yield of tomatoes are given in Table 3. These data prove that for all variants of plant treatment with exometabolites of *N. vaccinii* IMV B-7405, an increase in yield indicators was observed. The greatest increase in both the total weight of tomatoes and the average weight of the fruit (82-91 and 12-18%, respectively) was observed in the case of using culture liquid and supernatant in a dilution of 1:400. In the case of a decrease in the degree of dilution of the culture liquid and supernatant, the yield indicators decreased slightly. The obtained data can be explained by the fact that the optimal concentration of phytohormones in the working solution was achieved at a dilution of 1:400.

It should be noted that the yield indicators of tomatoes after treatment with supernatant and culture liquid were almost the same, but the use of culture liquid in crop production is more expedient from an economic point of view, as it makes it possible to exclude the biomass separation stage from the technological process.

Table 3

Effects of *N. vaccinii* strain IMV B-7405 exometabolites on the yield of tomatoes

Variant of treatment of the tomato root system	Total weight, % of control	Average fruit weight, % of control
Culture liquid supernatant (1:200)	147	106
Culture liquid supernatant (1:400)	**191**	**118**
Culture liquid (1:200)	128	103
Culture fluid (1:400)	**182**	**112**

Note. Control (100%) – treatment of the seedling root system with water; control variants were not treated with surfactant solutions. To obtain the culture fluid (supernatant), *N. vaccinii* IMV B-7405 was grown on oil used after frying potatoes in the presence of 300 mg/l tryptophan. Dilutions were chosen based on the effective action of phytohormones.

In this research, the choice of substrates for growing *N. vaccinii* IMV B-7405 was determined by the following reasons. First, under the conditions of growth on refined oil, the strain of *N. vaccinii* IMV B-7405 synthesized the highest amount of auxins (770.4 μg/ l) compared to that on other substrates (Pirog et al., 2016). Secondly, a complex microbial preparation should be characterized by high antimicrobial activity against phytopathogenic bacteria, and earlier (Pirog et al., 2023) it was established that such properties are inherent in surface-active substances synthesized in the process of cultivating *N. vaccinii* IMV B-7405 on refined and spent after frying potato oil. Thirdly, used oil is a toxic waste, the emissions of which are not regulated in Ukraine, and its use as a substrate will make it possible to simultaneously dispose of hazardous waste and reduce the cost of the target product for crop production.

The first reports about the ability of South African producers to synthesize phytohormones appeared in 2008 (Buensanteai et al., 2008). (The ability of surfactant producers' *R. erythropolis* IMV As-5017, *Acinetobacter calcoaceticus* IMV B-7241 and *N. vaccinii* IMV B-7405 to synthesize phytohormones auxin nature was shown in study (Pirog et al., 2016). Later, there was a report on the formation of indolyl-3-acetic acid by bacteria isolated from soils contaminated with hydrocarbons and heavy metals (Pacwa-Płociniczak et al., 2016). In 2018-2019, several works (Jayakumar et al., 2019; Sabaté et al., 2018; Wu et al., 2018) were published, in which the ability of producers of surface-active lipopeptides and rhamnolipids to synthesize auxins was established. However, in those works, the authors did not try to improve the synthesis of IAA. Thus, our work is the first to report the intensification of auxin synthesis by surfactant producers in the presence of tryptophan.

Note that the literature contains data on the tryptophan effect on the IAA microorganisms' synthesis that don't synthesize surfactants (Dasri et al., 2014; Gopalakrishnan et al., 2015; Kumari et al., 2018; Lebrazi et al., 2020; Nutaratat et al., 2017). Kumari et al. (2018) found that the IAA concentration synthesized by *Bacillus subtilis* DR2 (associated with *Eragrostis cynosuroides*) increased almost 1.2 times (up to 168.1 μg/ml) in the presence of 1.2 g/l tryptophan in a medium with tryptone. In 2020, Lebrazi et al. (2020) showed that the rhizosphere strain *Rhizobium* sp. formed 116.42 μg/ml IAA when 2 g/l tryptophan was added to the medium, which is 1.3 times more than without the precursor. The synthesis of IAA by the unidentified strain DPY-05 increased almost 27 times (up to 67.18 μg/ml) in the presence of 0.5 g/l tryptophan (Dasri et al., 2014). *Enterobacter* sp. DMKU-RP206 strain, isolated from the surface of rice leaves, synthesized on a medium with

lactose and 11 g/l tryptophan up to 5.56 g/l IAA, which is 13.4 times more than without tryptophan (Nutaratat et al., 2017).

It is worth noting that the described producers (Dasri et al., 2014; Kumari et al., 2018; Lebrazi et al., 2020; Nutaratat et al., 2017) are in a certain interaction with plants – as endophytes, phyllosphere or as associated microorganisms. This leads to the formation of higher phytohormones concentrations by these strains to ensure a beneficial interaction with plants. At the same time, the *N. vaccinii* IMV B-7405 studied by us belongs to free-living soil bacteria, for which the synthesis of compounds that stimulate plant growth is not characteristic at all.

However, there are reports about the ability to synthesize phytohormones by free-living bacteria that don't directly participate in the vital activity of plants (McClerklin et al., 2018; Myo et al., 2019). Thus, Myo et al. (2019) established that the neomycin producer *Streptomyces fradiae* NKZ-259 synthesized 4.876 mg/l of IAA on a medium without tryptophan, and in its presence (2 g/l) the level of synthesis increased 20 times (up to 82.363 mg/l).

Data (Dasri et al., 2014; Nutaratat et al., 2017; Kumari et al., 2018; Myo et al., 2019; Lebrazi et al., 2020) indicate that the introduction of precursors is effective in increasing the synthesis of phytohormones. However, in these works, high concentrations of tryptophan (2-11 g/l) were introduced into rich nutrient media with tryptone (Kumari et al., 2018), mannitol (Lebrazi et al., 2020), peptone (Dasri et al., 2014), lactose (Nutaratat et al., 2017), starch (Myo et al., 2019), and the degree of intensification was not exceeded 20 times. Our studies have shown the possibility of intensifying the synthesis of IAA more than 400 times on the medium with spent oil (if only 0.3 g/l of tryptophan, see Table 2). There is currently no such information in the literature.

Data (Gopalakrishnan et al., 2015) show that the auxins synthesis intensification in the presence of tryptophan was caused by the fact that in microorganisms this amino acid is a precursor to the biosynthesis of IAA. The conversion of tryptophan to IAA can be carried out in three ways: through indole-3-pyruvic acid and indole-3-acetaldehyde, tryptamine or indole-3-acetamide.

The results obtained (Figure 1) allow us to assume that the biosynthesis of IAA in *N. vaccinii* IMV B-7405 occurs through the formation of indole-3-pyruvate.

It is known that the criterion of the antimicrobial effect of bioformulations is the MIC – the lowest concentration that causes complete inhibition of the test culture growth visible to the naked eye (Lotfabad et al., 2013). Determination of the MIC makes it possible to simultaneously compare the effectiveness of different antimicrobial formulations.

The choice of test cultures to determine the antimicrobial activity of *N. vaccinii* IMV B-7405 surfactant was determined by the fact that the bacteria *A. tumefaciens*, *P. syringae*, *X. vesicatoria*, *P. carotovorum*, *C. michiganensis*, *P. syringae* pv. *tomato* are known phytopathogens that affect tomato plant cultures (Mansfield et al., 2012). Among these cultures, three cause diseases of tomatoes specifically on the territory of Ukraine (*X. vesicatoria*, *C. michiganensis*, *P. syringae* pv. *tomato*), and three are polyphagous (*A. tumefaciens*, *P. syringae*, *P. carotovorum*), that is except for tomatoes also affect other plants.

The results of our research showed that the addition of tryptophan to the culture medium of *N. vaccinii* IMV B-7405 was accompanied by the synthesis of surfactants, the antimicrobial activity of which not only didn't decrease but also higher than that of surface-active substances obtained without precursor of auxin synthesis. Thus, in the presence of tryptophan, both an increase in the synthesis of auxins (see Tables 1, 2) and an increase in the antimicrobial activity of surfactants synthesized in a complex with phytohormones were observed.

Currently, lipopeptides are the most studied surfactants with antimicrobial action. However there are few works in the literature that determined the MIC of lipopeptides against phytopathogenic bacteria (Abdallah et al., 2018; Bais et al., 2004; Chopra et al., 2020; Etchegaray et al., 2008; Fan et al., 2017; Luo et al., 2015; Mansfield et al., 2012; Phae et al., 1990; Zeriouh et al., 2011). Thus, the MIC of *B. subtilis* 6051 surfactin against *P. syringae* pv. *tomato* DC3000 was 25 μg/ml (Bais et al., 2004). The *B. subtilis* 9407 surfactant complex, the main component of which is surfactin A C13-C16, had an antimicrobial effect on *Acidovorax citrulli* MH21, *P. syringae* pv. *tomato* DC3000, *Khanthomonas campestris* pv. *campestris* Xcc 8004, *Pectobacterium carotovorum* subsp. *carotovorum* Ecc 09, *Pectobacterium atrosepticum* SCRI1043 (zones of growth inhibition 10–18 mm) (Fan et al., 2017). Iturin, synthesized by *B. subtilis* NB22, showed antimicrobial activity against *Xanthomonas oryzae* and *Pseudomonas lachrymans* (MIC 3.13−12.5 μg/ml) (Phae et al., 1990). Iturin, formed by *B. subtilis* OG inhibited the growth of phytopathogenic bacteria *Xanthomonas axonopodis* pv. *citri* and X. *campestris* pv. *campestris* (MIC 10-50 μmol/l) (Etchegaray et al., 2008).

In 2020, Medeot et al. reported that *B. amyloliquefaciens* MEP218 synthesizes fengycin with antibacterial activity against *Xanthomonas axonopodis* pv. *vesicatoria* activity, which distinguishes it from other fengycins, which are mainly antifungal.

In addition to lipopeptides, rhamnolipids and sophorolipids show antibacterial activity. Thus, it was established that the MIC for *Erwinia carotovora* pv. *carotovora*, *R. solanacearum* and *X. campestris* pv. *vesicatoria*, rhamnolipids of *P. aeruginosa* B5, were >50 μg/ml (Kim et al., 2000). Leite et al. (2016) established that under the influence of 15 μl of supernatant (rhamnolipid concentration 0.57 g/l), obtained after cultivation of *P. aeruginosa* P1R16 in olive oil, the growth retardation zone of *R. solanacearum* 1226 was 22 mm. Finally, Chopra et al. (2020) showed that the MIC for *X. campestris* dirhamnolipid synthesized by *P. aeruginosa* RTE4 on glucose (2%) was 5 mg/ml.

Schofield et al. (2013) reported that sophorolipid derivatives (with different degrees of lactonization and acetylation, as well as different acyl chain lengths) and combinations of sophorolipid derivatives showed antibacterial activity against *Acidovorax carotovorum*, *Erwinia amylovora*, *Pseudomonas cichorii*, *P. syringae*, *P. carotovorum, R. solanacearum* and *X. campestris*. Minimum inhibitory concentrations ranged from 2.5 to 10 mg/ml for individual sophorolipid derivatives and from 0.009 to 10 mg/ml for combinations of these surfactants.

The MIC of *N. vaccinii* IMV B-7405 about most of the phytopathogenic bacteria studied by us was in the range of 1.41-22.5 μg/ml. Such indicators are lower than in the above-mentioned articles (Kim et al., 2000; Bais et al. 2004; Schofield et al., 2013; Fan et al., 2017; Chopra et al., 2020), which indicates the high antimicrobial activity of surface-active substances *N. vaccinii* IMV B-7405 against phytopathogenic bacteria. The advantage of the *N. vaccinii* IMV B-7405 strain compared to those described in these works is the ability to simultaneously synthesize surfactants with high antimicrobial activity and phytohormones.

It should be noted that in recent years, reports on the antimicrobial activity of surfactants synthesized in a complex with phytohormones began to appear in the literature (Chen et al., 2021; Chlebek et al., 2020). Thus, the endophyte *Pseudomonas fluorescens* BRZ63 isolated from rapeseed roots produced rhamnolipids, IAA (59.62 μg/ml), siderophores, and salicylic acid (Chlebek et al., 2020). Under the influence of *Pseudomonas fluorescens* BRZ63 metabolites, the degree of growth inhibition of the phytopathogenic fungi *Rhizoctonia solani* W70, *Colletotrichum dematium* K, *Sclerotinia sclerotiorum* K2291 and *Fusarium avenaceum* ranged from 37 to 62%.

Bacillus atrophaeus B44 synthesizes lipopeptides and gibberellins at the same time

(Chen et al., 2021). Lipopeptides of strain B44 showed antimicrobial activity against *R. solani* (growth inhibition zone 16 mm). In contrast to the *N. vaccinii* IMV B-7405 which synthesizes phytohormones and surfactants and is characterized by high antibacterial activity against phytopathogens the surfactants described in (Chen et al., 2021; Chlebek et al., 2020) have only an antifungal effect.

To confirm the possibility of using surfactant *N. vaccinii* IMV B-7405 to control the number of phytopathogens during infection in vivo, *X. vesicatoria* IMV B-9098 and *C. michiganensis* subsp. *michiganensis* IMV B-102 were chosen as test cultures. During their interaction with plants, these pathogens affect, first, the leaves, in connection with which it is possible to conduct a visual assessment of the intensity of the course of the disease.

The results of our research showed the absence of infection for 7 days on a leaf previously treated with surfactant (see Figures 2 and 3). So it was assumed that the treatment of tomato plants with the *N. vaccinii* IMV B-7405 strain will help protect them from damage by phytopathogens.

In the literature (Bolivar-Anillo et al., 2021; Ghadamgahi et al., 2022; Tomar et al., 2014) there is similar information about the use of similar model systems to study the ability of surfactant and phytohormone producers to reduce damage by phytopathogens to the leaves of crops. However, in these works, only the antifungal activity of surfactants was researched. At the same time, in the last two decades, the protection of vegetable crops from bacterial diseases is one of the urgent problems due to their high prevalence and harmfulness. Bacterial diseases cause great economic losses to agriculture, affecting almost all cultivated plant species.

It was established that the pre-treatment of potato leaves with surfactant-containing supernatant of *P. aeruginosa* 1 or a suspension of surfactant-producing cells made it possible to reduce by almost 100% damage to leaves by the phytopathogenic fungus *Phytophtora infestans* (Tomar et al., 2014). Preliminary inoculation of bean leaves with a suspension of *B. subtilis* (lipopeptide producers and phytohormones) led only to the appearance of weak symptoms of *Botrytis cinerea* B05.10 (Bolivar-Anillo et al., 2021). Treatment of potato and strawberry leaves with a *P. aeruginosa* FG106 suspension (producer of rhamnolipids and auxins) made it possible to reduce the infection zone of *B. cinerea*, *P. infestans* and *Phytophthora colocasiae* from 1.6-2.1 cm^2 in control variants (treatment with phosphate-salt buffer) up to 0.1-0.2 cm^2 (Ghadamgahi et al., 2022).

At the last stage, the effect of exometabolites of *N. vaccinii* IMV B-7405 on the yield of tomatoes was studied. For the plants treatment the culture liquid and supernatant after cultivation of the strain on waste oil in the presence of 300 g/l tryptophan was used, since under such conditions the maximum level of auxins was achieved (see Table 2) and high antimicrobial activity of surfactants against tomato bacterial pathogens.

It is known that phytohormones have a positive effect on the tomatoes growth and development (Ahirwar et al., 2015; Almaghrabi et al., 2013; Babu et al., 2015). It was shown that the pre-sowing treatment of tomatoes with PGPR (plant growth-promoting rhizobacteria) strains *P. putida*, *P. fluorescens*, *Serratia marcescens*, *Bacillus amyloliquefaciens*, *B. subtilis* and *B. cereus* increased stem weight, plant height and their yield (Almaghrabi et al., 2013). Thus, after treatment with the *S. marcescens*, the number of fruits increased by 6 pcs/plant, and their total weight increased by 180.3 g. Babu et al. (2015) found that after seed treatment with five unidentified PGPR strains capable of IAA synthesis, the weight of tomatoes exceeded the control by 51.3–116.0%. In addition, the authors noted an earlier formation of flowers on plants treated with bacteria. Note that the same effect was observed during the study of the *N. vaccinii* IMV B-7405 exometabolites influence on tomatoes. Ahirwar et al. (2015) showed that the treatment of seeds with a culture liquid after growing the

Pseudomonas fluorescence SS5 strain isolated from the rhizosphere of tomatoes, capable of IAA synthesis, was accompanied by an increase in the number of fruits by 57% and the total weight of tomatoes by 28% compared to the control.

Conclusion

Therefore, in the presence of low concentrations of tryptophan in the medium with both refined and spent sunflower oil, the surfactant producer *N. vaccinii* IMV B-7405 synthesizes one to two orders higher amounts of auxins than without the precursor of phytohormone biosynthesis. The activity of tryptophan transaminase confirms that tryptophan is involved in the metabolism of *N. vaccinii* IMV B-7405 strain through the indole-3-pyruvate pathway of auxin biosynthesis. In addition, surfactants synthesized in a medium with tryptophan were characterized by higher antimicrobial activity of surfactants against phytopathogenic bacteria compared to those obtained during cultivation without the precursor of auxin synthesis. The ability of surfactant *N. vaccinii* IMV B-7405 to biocontrol the number of phytopathogenic bacteria was manifested not only in *in vitro* studies but also in vivo when conducting research with detached leaves (detached leaf assay). Treatment of seeds of tomato plants with a cultural liquid contributed to the increase in yield and increase in fruit weight. Thus, in the future, the bioformulations based on the exometabolites of *N. vaccinii* IMV B-7405 can be used to control the number of phytopathogens due to the ability to synthesize surfactants and to stimulate plant growth due to the formation of auxins.

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