

## CROP PRODUCTION

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### YIELD OF TOMATOES AND PEPPERS UNDER THE INFLUENCE OF *NOCARDIA VACCINII* IMV B-7405, *ACINETOBACTER CALCOACETICUS* IMV B-7241 AND *RHODOCOCCUS ERYTHROPOLIS* IMV AC-5017

Research article

#### Abstract

In this study both treatment of tomatoes and peppers with phytohormonal extracts obtained from cultural broth of *Nocardia vaccinii* IMV B-7405, *Acinetobacter calcoaceticus* IMV B-7241 and *Rhodococcus erythropolis* IMV Ac-5017 showed positive stimulation of growth and development of plants. Depending on the degree of dilution of the phytohormonal extracts, the total weight of tomatoes collected from the treated plants was on 11.3–145.0% higher than in the control variant. As for the peppers, the total weight of the fruits from treated plants exceeded control by 11.6–77.1%. The positive influence of extracellular exometabolites of surfactants producers on the yields of tomatoes and peppers is the basis for the development of the technology of obtaining microbial preparations for application in crop production using cheap industrial wastes.

**Keywords:** phytohormones, tomatoes, peppers, greenhouse experiments.

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### УРОЖАЙНОСТЬ ПОМИДОРОВ И ПЕРЦЕВ ПОД ВЛИЯНИЕМ ЭКЗОМЕТАБОЛИТОВ *NOCARDIA VACCINII* ИМВ В-7405, *ACINETOBACTER CALCOACETICUS* ИМВ В-7241 И *RHODOCOCCUS ERYTHROPOLIS* ИМВ АС-5017

Научная статья

#### Аннотация

В этом исследовании обработка томатов и перца фитогормональными экстрактами, полученными из культуральной жидкости *Nocardia vaccinii* ИМВ В-7405, *Acinetobacter calcoaceticus* ИМВ В-7241 и *Rhodococcus erythropolis* ИМВ Ас-5017, показала положительную стимуляцию роста и развития растений. В зависимости от степени разбавления фитогормональных экстрактов общая масса томатов, собранных с обработанных растений, была на 11,3–145,0% выше, чем в контрольном варианте. Что касается перца, то общая масса плодов обработанных растений превысила контроль на 11,6–77,1%. Положительное влияние внеклеточных экзосометаболитов производителей ПАВ на урожайность томатов и перца является основой для разработки технологии получения микробных препаратов для применения в растениеводстве с использованием дешевых промышленных отходов.

**Ключевые слова:** фитогормоны, помидоры, перец, тепличные опыты.

#### 1. Introduction

In the last decade, most of agriculturally oriented research have focused on the study of rhizospheric microorganisms. The association of plants with microorganisms that do not inhibit or even stimulate their development attracts the attention of scientists not only as the object of study with respect to the fundamentals of the coexistence and interaction of different organisms, but also because of their possible use in the practice of ecological production of agricultural products [1].

Plant growth promotion and development can be facilitated both directly and indirectly. Indirect plant growth promotion includes the prevention of the deleterious effects of phytopathogenic organisms. This can be achieved by the production of compounds with antibiotic activity. Direct plant growth promotion includes symbiotic and non-symbiotic PGPR (plant growth promoting rhizobacteria), which function through phosphate solubilization, production of plant hormones (such as auxins, cytokinins, gibberellins, ethylene and abscisic acid), siderophores and others compounds useful for plants [2].

The ability of rhizobacteria to synthesize phytohormones has long been considered a major factor in promoting plant growth. There have been many reports of significant improvements in growth and increased yields of agronomically important crops in response to inoculation by bacteria capable of synthesizing certain phytohormones [3], [4], [5]. However, most studies focus on individual phytohormones (indole-3-acetic acid, cytokinins, gibberellic acid 3). There are experiments that show that plant hormones control growth antagonistically or synergistically. In previous studies [6], we have shown that surfactants synthesized by *Nocardia vaccinii* IMV B-7405, *Acinetobacter calcoaceticus* IMV B-7241 and *Rhodococcus erythropolis* IMV Ac-5017, have antibacterial properties against some phytopathogenic bacteria, in particular the genera *Pseudomonas* and *Xanthomonas*. Later [7], [8] the ability to simultaneously synthesize surfactants and exometabolites with phytohormonal activity (auxins, cytokinins and gibberellins) was established.

The ability to simultaneously synthesize of surfactants and plant hormones under conditions of growth on different substrates [7], [8], [9] provides the basis for developing a waste-free technology for the complex microbial preparations with various biological properties. Also we can conduct researches for integrating such technology into the practice of agricultural production.

Thus, we have tested the effect of culture broth *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 on the growth and development of tomatoes and barley [10]. For barley the increase of yield on 33.3-83.3% compared to control (treatment with water) has been observed. At the same time for the most of tomatoes treatment options the weight of fruits was less than control, although the number of fruits was higher. Obtained result we associate to the fact that the culture broth of the strains contained too high concentration of plant hormones, which did not show optimal effect on tomatoes.

In connection with the above, the aim of this work is to study the effect of various concentrations of phytohormones synthesized by *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 on the growth and yield of tomatoes and peppers.

## 2. Materials and methods

### 2.1. Object of research

*Nocardia vaccinii* K-8 strain, *Acinetobacter calcoaceticus* K-4 strain and *Rhodococcus erythropolis* EK-1 strain, registered in Microorganisms Depository of Institute of Microbiology and Virology, the National Academy of Sciences of Ukraine under the numbers IMV B-7405, IMV B-7241 and IMV Ac-5017 respectively.

### 2.2. Medium composition and conditions of cultivation

*N. vaccinii* IMV B-7405 were grown in the liquid mineral medium (g/L distilled water):  $\text{NaNO}_3 - 0.5$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O} - 0.1$ ,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O} - 0.1$ ,  $\text{KH}_2\text{PO}_4 - 0.1$ ,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O} - 0.01$ , yeast autolysate - 0.5 % v/v, pH 6.8–7.0.

Strain *A. calcoaceticus* IMV B-7241 was cultivated in the liquid medium (g/L distilled water):  $(\text{NH}_2)_2\text{CO} - 0.35$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O} - 0.1$ ,  $\text{NaCl} - 1.0$ ,  $\text{Na}_2\text{HPO}_4 - 0.6$ ,  $\text{KH}_2\text{PO}_4 - 0.14$ , 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):  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O} - 1.1$ ,  $\text{MnSO}_4 \cdot \text{H}_2\text{O} - 0.6$ ,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O} - 0.1$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O} - 0.004$ ,  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O} - 0.03$ ,  $\text{H}_3\text{BO}_3 - 0.006$ ,  $\text{KI} - 0.0001$ ,  $\text{EDTA} - 0.5$ .

Strain *R. erythropolis* IMV Ac-5017 was grown in the liquid mineral medium (g/L distilled water):  $\text{NaNO}_3 - 1.3$ ,  $\text{NaCl} - 1.0$ ,  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O} - 0.6$ ,  $\text{KH}_2\text{PO}_4 - 0.14$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O} - 0.1$ ,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O} - 0.001$ , pH 6.8–7.0.

The refined sunflower oil was used as the carbon and energy source in concentration of 2.0 % v/v.

The culture in the exponential phase was used as the 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 °C during 7 days.

### 2.3. 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 were extracted from the culture broth using petroleum ether (ratio 1:1).

Phytohormonal extracts were obtained from the supernatant of culture broth (CL). Gibberellin and auxin extracts were obtained from the supernatant of the culture broth by three times extraction with ethyl acetate (ratio 1:1) at pH 2.5, and cytokinin extracts were obtained by three times extraction with butanol (ratio 1:1) at pH 8.0. The extracts were evaporated to dryness under vacuum and redissolved in 80% ethanol. The obtained extracts were stored at -24 °C.

### 2.4. Determination of the influence of exometabolites on the test cultures of plants

Greenhouse experiments were carried out at the greenhouses of the D.K. Zabolotny Institute of Microbiology and Virology of National Academy of Sciences of Ukraine.

Greenhouse experiments with tomatoes. The experiments were conducted in greenhouses during 6 months from April to September. As a test culture, tomatoes of the Hrusha variety were used. The yield was harvested during 3 months from July to

September. Before planting in the soil, the root system of tomato seedlings was kept for two hours in solutions of phytohormonal extracts in the following variants: 1) the extract of *N. vaccinii* IMV B-7405 in dilutions 1:5000 and 1:10000; 2) the extract of *A. calcoaceticus* IMV B-7241 in dilutions 1:3000 and 1:5000. Seedlings, which were kept for an hour in tap water, were used as a control. There were three plants in each variant. During the experiment, the number of tomatoes and their weight were analyzed.

Greenhouse experiments with peppers. The experiments were conducted in greenhouses during 6 months from April to September. As a test culture, tomatoes of the Rotunda variety were used. The yield was harvested during 3 months from July to September. Before planting in the soil, the root system of tomato seedlings was kept for two hours in solutions of phytohormonal extracts in the following variants: 1) the extract of *R. erythropolis* IMV Ac-5017 in dilutions 1:1000 and 1:2000; 2) the extract of *N. vaccinii* IMV B-7405 in dilution 1:10000; 3) the extract of *A. calcoaceticus* IMV B-7241 in dilution 1:3000. Seedlings, which were kept for an hour in tap water, were used as a control. There were three plants in each variant. During the experiment, the number of peppers and their weight were analyzed.

## 2.4. Statistical analysis

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 [18]. Differences of mean indicators were deemed as reliable at the significance level  $p < 0.05$ .

## 3. Results and discussion

In the present research, the tomatoes root system was treated with the solutions of phytohormones and not with a culture broth, since the main aim of the experiment was to find an effective concentration of phytohormones for the stimulating effect on plants. In the future, this will help to determine the optimal dilution for preparations based on the culture broth *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017. The choice of dilutions of phytohormonal extracts was based on the results of a previous study published in [10], according to which the increase of the tomato yield was shown only under treatment with IMV Ac-5017 strain. This is due to the fact that the culture broth *R. erythropolis* IMV Ac-5017 contained up to 25 times lower concentration of phytohormones than *N. vaccinii* IMV B-7405 and *A. calcoaceticus* IMV B-7241 (Table 1). Accordingly, for strains IMV B-7405 and IMV B-7241, it is necessary to find a concentration of phytohormones that will be at the level of the effective concentration of the strain IMV Ac-5017.

Table 1 – Synthesis of phytohormones under cultivation of *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 on refined oil

Strain	Auxins, µg/L	Gibberellins, µg/L	Cytokinins, µg/L	Total concentration, µg/L
<i>N. vaccinii</i> IMV B-7405	770.4	5.96	348.0	1124.36
<i>A. calcoaceticus</i> IMV B-7241	39.6	8.0	75.1	122.7
<i>R. erythropolis</i> IMV Ac-5017	19.4	7.8	17.1	44.3

### 3.1. Yield of tomatoes of Hrusha variety under the treatment with exometabolites of strains IMV B-7405 and IMV B-7241

During the experiment, the effect on the growth and development of tomatoes of phytohormones of only two strains, which in the previous work did not show positive results due to the high concentration of phytohormones, was determined.

The experiments have shown that the treatment of tomato seedling of Hrusha variety with exometabolites of *N. vaccinii* IMV B-7405 and *A. calcoaceticus* IMV B-7241 strains before planting into the soil stimulated the growth of seedlings, increased the number of fruits and accelerated their maturation (Table 2, Figure 1).

Table 2 – Indicators of tomatoes yield under the treatment with phytohormonal extracts, obtained after cultivating *N. vaccinii* IMV B-7405 and *A. calcoaceticus* IMV B-7241 on refined oil

Treatment	Dilution of phytohormonal extract	No. of fruit	Average fruit weight (g)	Increase of total weight compared to control, %
Control (water)	–	17	25.2	–
<i>N. vaccinii</i> IMV B-7405	1:5000	19	25.3	11.3
	1:10000	25	28.2	63.9
<i>A. calcoaceticus</i> IMV B-7241	1:3000	41	25.7	145.0
	1:5000	28	25.0	62.8

These data demonstrate that the number of fruits on plants treated with phytohormonal extracts (regardless of their dilution degree) was higher than after treatment with water. It should be noted that in most experimental variants the average fruit weight almost did not differ from control (25.0–25.7 g). Slightly higher was the average fruit weight (28.2 g) on plants treated with

10000 times diluted phytohormonal extract of *N. vaccinii* IMV B-7405. Depending on the degree of dilution of the phytohormonal extracts, the total weight of fruits collected from the treated plants was on 11.3-145.0% higher than in the control variant. We can conclude that the optimal dilution for *N. vaccinii* IMV B-7405 was 1:10000 (+63.9%), and for *A. calcoaceticus* IMV B-7241 it was 1:3000 (+145.0%), because they showed the best stimulation of the tomatoes growth.

It is known that all three classes of phytohormones take part in the process of growth and development of plants, and each of them plays a role at a particular stage of the plant development. That is why the best stimulating effect is observed for the combined influence of all phytohormones. In the literature data there are studies about impact of phytohormone complexes [11], [12] and individual groups on the development of the plants [13]. For example, it was determined [11] that bacterial endophyte *Sphingomonas* sp. LK11 due to the ability to synthesize indole-3-acetic acid (IAA) and gibberellins (in particular, GA<sub>4</sub>), stimulated the growth of tomatoes (*Tephrosia apollinea*). In plants treated with a culture broth of the strain the shoot length was 68.6% higher than control (treatment with distilled water), and the dry weight of the plant was 48.9% higher. In the work [12], it was determined that 12 PGPR-strains, which belong to the genera *Pseudomonas*, *Bacillus* and *Azospirillum*, synthesize both IAA and wide range of cytokinins (trans-zeatin, cis-zeatin, zeatin-riboside and dihydrozeatin riboside). The effect of exometabolites of strains in the treatment of wheat seeds was manifested in the increase in height of the shoot (up to 30%), the spike length (up to +33%) and the weight of 100 seeds (up to 39%).

Analysis of 88 endophyte strains isolated from tomato seeds (*Lycopersicon esculentum* Mill.) showed that 22 of them belong to *Bacillus subtilis* and have properties, inherent to growth-promoting bacteria. Thus, in particular, the synthesis of IAA was at the level of 1.08–5.49 mg/L. Treatment of tomato seeds with one of the strains, *B. subtilis* HYT-12-1, stimulated the increase of the root length by 10.7%, the shoot length by 5.1%, the fresh root weight by 50.0%, and the fresh shoot weight by 57.7% [13].

It should be noted that in the works [11], [12], [13] the authors did not analyze the number of fruits and their weight, as in our studies. However, there are data in the literature on the influence of plant growth-promoting rhizobacteria (PGPR) on the fruit weight. So, in the work [4] pre-sowing inoculation of the tomato seeds with different strains of *Pseudomonas* bacteria, capable of phosphate solubilization and auxin formation, positively influenced the weight of fruits, collected during the experiment for 11 weeks. The total fruit weight of the plants treated with bacteria exceeded control (treatment with distilled water) by 9–14%. However, it should be noted that the treatment with one of the strains, *P. fluorescens* Migula G, resulted in a 1% decrease in fruit weight compared to control.

Other researchers have shown [14] that pre-sowing treatment with PGPR strains *P. putida*, *P. fluorescens*, *Serratia marcescens*, *Bacillus amyloliquefaciens*, *B. subtilis* and *B. cereus* increased the shoot weight, the height of the tomato plants and their yield. The treatment with *S. marcescens* strain gave the best effect on the yield, due to which the number of fruits exceeded control by 128.0% and weight by 129.4%. For other strains, these indicators were in the range of 57.0–85.8% and 85.2–103.1% respectively. Only the treatment with *B. amyloliquefaciens* had a negative effect – though the number of fruits exceeded control by 85.0%, the weight was lower by 37.8%.

Babu et al. [5] demonstrated the positive effect of treatment with all five unidentified studied PGPR strains, which are capable of synthesizing IAA. Due to the treatment the weight of tomatoes exceeded control by 51.3–116.0%. Moreover, the authors noted the earlier formation of flowers on plants treated with bacteria. The same effect was observed during the study of the influence of exometabolites *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 on tomatoes and peppers.

Therefore, literature data confirm the possibility of using the bacteria that are capable of synthesizing growth-promoting metabolites in crop production to increase yields. In most cases, researchers observed an increase in both the number of fruits and their weight. Our further studies will focus on the intensification of the synthesis of individual phytohormones and the selection of optimal ratios of plant hormone classes for the best stimulating effect.

### 3.2. Yield of peppers of Rotunda variety under the treatment with exometabolites of strains IMV B-7405, IMV B-7241 and IMV Ac-5017

Rotunda peppers, which, as well as tomatoes, belong to the Solanaceae family, were chosen as the second test culture.

The results of the experiment demonstrate that in all studied variants of treatment the number of fruits or their weight were increased compared to control, and also the flowering and ripening of the fruits accelerated (Table 3).

Table 3 – The effect of phytohormonal extracts obtained after cultivating *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 on refined oil, on yield of peppers

Treatment	Dilution of phytohormonal extract	No. of fruit	Average fruit weight (g)	Increase of total weight compared to control, %
Control (water)	–	8	32.25	–
<i>R. erythropolis</i> IMV Ac-5017	1:1000	10	28.8	+11.6
	1:2000	8	32.9	+2.0
<i>N. vaccinii</i> IMV B-7405	1:10000	10	45.7	+77.1
<i>A. calcoaceticus</i> IMV B-7241	1:3000	12	33.83	+57.3

The data in the table show that the treatment with strains *N. vaccinii* IMV B-7405 in dilution of 1:10000 and *A. calcoaceticus* IMV B-7241 in dilution of 1:3000 had the best stimulation of the growth and development of peppers, both by the weight and the number. For strain IMV B-7405 the number of fruits exceeded the control by 25.0%, and the total weight by 77.1%, as for the strain IMV B-7241 these figures increased by 50.0% and 57.3% respectively. Such results coincide with those obtained in

the experiment with tomatoes, indicating that such concentration of phytohormones is optimal for stimulating plant growth. As for the *R. erythropolis* IMV Ac-5017 strain, we couldn't determine which of the dilutions is more optimal for treatment, since the total weight of the fruits in both variants practically coincides with that obtained under the treatment with water. These results require further discussion.

There are examples of stimulation of pepper plants in the literature by bacteria isolated from the rhizosphere of peppers and others.

Isolated from the rhizosphere of pepper, *Serratia nematodiphila* PEJ1011, produces GA<sub>4</sub> in the amount of 8650 ng/L [15]. The inoculation of pepper plants with *S. nematodiphila* PEJ1011 significantly increased plant growth rates compared to control. In the experimental conditions the length of the root and shoot increased by 55.8% and 12.9% respectively. Strains *Bacillus cereus* MJ-1, *B. macroides* CJ-29 and *B. pumilus* CJ-69 isolated from the rhizosphere of red pepper form biologically active gibberellins GA<sub>1</sub>, GA<sub>3</sub>, GA<sub>4</sub> and GA<sub>7</sub> at a concentration 17–87 ng/L [16]. Plant height increased by 11.3–13.5% compared to control, and the fresh plant weight increased by 21.2–30.3%. The authors note that the stimulation of plant growth is caused by the synthesis of gibberellins, since no other phytohormones were detected in the culture broth of the studied strains.

In another work [17], 13 of nitrogen-fixing bacteria strains belonging to different genera were analyzed for their growth-promoting properties. All strains were able to synthesize IAA, salicylic acid and nitrogen fixation. Three strains were selected for experiments with red pepper in the greenhouse. The most significant effect among them was the strain *Pseudomonas* sp. RFNB3, which contributed to the plant height extension by 26% and dry weight by 28% compared to the control.

In the available literature, we have not been able to find data demonstrating the stimulating effect of PGPR bacteria on the number and weight of fruits, which makes it difficult to make a comparison. However, there is an obvious link between the treatment with bacteria that form extracellular phytohormones and the stimulation of plant growth and development, which is consistent with our research.

#### 4. Conclusion

The results obtained in this work give us grounds for the development of preparations based on the culture broth of *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017, since we were able to find the optimal concentration of phytohormones for the manifestation of stimulating effect on tomatoes and peppers. The results obtained earlier [6], [7], [8], [9], [10] and the results presented in this article are the basis for the development of a waste-free technology 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. Thus, in the preparation of surfactants, the precipitated cells can be used to purify water from oil; obtained culture supernatant – for further isolation of surfactants with anti-adhesive and antimicrobial properties (including against phytopathogenic bacteria). And the aqueous phase, which remains after the extraction of surfactants and contains phytohormones of auxin, cytokinin and gibberellin nature, will be used in agriculture to stimulate plant growth.

#### Conflict of Interest

None declared.

#### Конфликт интересов

Не указан.

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