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INDUCTION OF AUXINS SYNTHESIS BY *RHODOCOCCUS ERYTHROPOLIS* IMV AC-5017 WITH THE ADDITION OF TRYPTOPHAN TO THE CULTIVATION MEDIUM

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The ability of surfactant producers to synthesize phytohormones expands the scope of their practical application and provides prospects for the development of microbial preparations with growth-stimulating properties. The possibility to intensify the phytohormone-stimulants synthesis by bacterial strains increases the efficiency of such preparations. ***Aim****. The aim is to research the possibility of extracellular auxin synthesis induction in the presence of tryptophan in the cultivation medium of surfactant producer Rhodococcus erythropolis IMV Ac-5017 and establish the optimal concentration of tryptophan and time of introduction into the medium to ensure maximum synthesis of auxins.* ***Methods****. Biochemical, microbiological, biotechnological. Cultivation was performed in the liquid mineral medium using ethanol and waste sunflower oil as substrates. Tryptophan was added to the medium as a 1 % solution in an amount of 200 or 300 mg/l at the beginning of the cultivation process or at the end of the exponential growth phase. Phytohormones were isolated by triple extraction with organic solvents from the culture broth supernatant after surfactant extraction. Preliminary purification and concentration of phytohormones was performed by thin layer chromatography. Qualitative and quantitative determination of auxins was performed using high performance liquid chromatography.* ***Results****. It was found that regardless of the concentration and time of tryptophan introduction to the culture medium of R. erythropolis IMV Ac-5017 with both substrates, a significant increase (by two to three orders of magnitude) was observed in the amount of synthesized auxins compared to tryptophan-free medium. The highest concentration of auxins (5552–5634 μg/l) was achieved by adding 300 mg/l of tryptophan into the culture medium of R. erythropolis IMV Ac-5017 with ethanol, while without the precursor their amount was only 143 μg/l. In contrast to the cultivation of the strain on culture medium with ethanol, where the synthesis of auxins did not depend on the time of tryptophan introduction, R. erythropolis IMV Ac-5017 formed the maximum amount of auxins when 300 mg/l tryptophan was added to the culture medium with waste oil at the end of the exponential growth phase (2398 μg/l compared to 9.8 μg/l on the medium without tryptophan). As auxin compounds were identified: indole-3-acetic acid, indole-3-carboxylic acid and indole-3-butyric acid. However, the highest amount of indole-3-acetic acid was synthesized, the precursor of which is tryptophan. The synthesis of this auxin (the most common plant auxin) in the presence of 300 mg/l of tryptophan increased more than 40 times on ethanol medium and more than 700 times on medium with waste oil. Induction of auxin synthesis by strain R. erythropolis IMV Ac-5017 correlated with the activity of tryptophan transaminase: when cultured on ethanol without tryptophan, it was 138 nmol·min^{-1}·mg^{-1} of protein, while cultured in the presence of precursor it was increased by 5.2 times (up to 714 nmol·min^{-1}·mg^{-1} of protein). The obtained results suggest that indole-3-acetic acid biosynthesis by the strain IMV Ac-5017 occurs due to the formation of indole-3-pyruvate.* ***Conclusions****. Thus, it was established the possibility of increasing by two or three orders the amount of synthesized auxins in the case of low concentrations of tryptophan introducing to the culture medium of R. erythropolis IMV Ac-5017 not only with ethanol but also with industrial waste (waste oil). The obtained results can be considered as promising for use of exometabolites of R. erythropolis IMV Ac-5017 with growth-stimulating properties in crop production.*

Keywords: surfactants producer Rhodococcus erythropolis IMV Ac-5017, extracellular auxins, tryptophan, tryptophan transaminase, induction of auxins synthesis.

Phytohormones are chemical messengers that are involved in regulating the metabolism of higher plants at certain physiological (10^{-6} M) concentrations. Phytohormones are usually divided into five classes – auxins, abscisic acid, cytokinins, gibberellins and ethylene – together with their precursors and synthesized analogs [1, 2].

Auxin phytohormones are involved in plant growth and development [3]. This is an important compound that regulates plant growth and development [4].

Previous studies have established the ability of the surfactants producer *Rhodococcus erythropolis* IMV Ac-5017 to synthesize stimulatory phytohormones – auxins, cytokinins and gibberellins [5, 6]. In our opinion, such results may be promising for the development of metabolic preparations with growth-stimulating properties for possible use in plant production. Subsequent studies [7] confirmed that the supernatant of *R. erythropolis* IMV Ac-5017 culture broth has a positive effect on the growth and development of tomato and barley plants.

Since the mid-90's of the twentieth century scientists' interest in microbial phytohormones has increased and their active research has begun [8, 9]. During the last 10 years, many works have been published on the regulation of the synthesis of phytohormones, in particular, auxins, by changing the conditions of microorganisms' cultivation [10, 11] or by introducing precursors of biosynthesis [22–32].

In our published review [12], we focused on the fact that most soil microorganisms, both associated and non-associated with plants, synthesize phytohormones of the auxin nature in the presence of exogenous tryptophan in the culture medium, which is the precursor of indole-3-acetic acid (IAA) synthesis. Moreover, the researchers added tryptophan into the medium at the beginning of the cultivation process and usually at a sufficiently high concentration (up to 10 g/l). We note that phytohormones are secondary metabolites, the formation of which begins in the stationary phase of growth, so it seems more logical to add a precursor at this stage of the process. In addition, the concentration of precursors used for the intensification of synthesis in microbial biotechnology, as a rule, is 0.1–0.2 % of the carbon source content in the culture medium [13].

It should be noted that in [5] we found that *R. erythropolis* IMV Ac-5017 synthesizes small amounts of auxins under growth conditions on medium with different substrates without a precursor, and therefore there are potential opportunities for enhancing of their synthesis.

In connection with the above, the aim of this work is to investigate the possibility of extracellular auxin synthesis induction in the presence of tryptophan in the cultivation medium of surfactant producer *R. erythropolis* IMV Ac-5017 and establish the optimal concentration of tryptophan and time of introduction into the medium to ensure maximum synthesis of auxins.

Materials and methods. The object of the research is *Rhodococcus erythropolis* K-9 strain, registered in Microorganisms Depositary of D.K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine with the number IMV Ac-5017.

Strain *R. erythropolis* IMV Ac-5017 was grown in the liquid mineral medium (g/l 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, pH 6.8–7.0. Waste oil after frying meat and ethanol were used as the carbon and energy sources in concentration of 2.0 % (v/v).

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

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 1.0 % v/v. The cultivation was carried out in 750 ml flasks, containing 100 ml of medium, on the shaker (320 rpm) at 28–30º C during 7 days.

Extracellular auxins were isolated in the supernatant by the method as described previously [14].

The qualitative and quantitative content 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 (MS) detector. HPLC/MS analysis of auxin extracts of *R. erythropolis* IMV Ac-5017 was performed at the Laboratory of Biological Polymer Compounds, D.K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine (Center for Collective Use).

In the research were used the synthetic standards of phytohormones produced by companies *Sigma–Aldrich* (Germany) and *Acros Organic* (Belgium): IAA – Indole-3-acetic acid; ICal – Indole-3-carboxaldehyde; IC – Indole-3-carbinol; ICA –

Indole-3-carboxylic acid; IAA-hydr. – Indole-3-acetic acid hydrazide; IBut – Indole-3-butyric acid.

Methanol (A) and 1 % acetic acid solution in water (B) were used as the mobile phase. Separation was performed on a Zorbax SB-C18 chromatographic column (2.1 mm×150 mm, 3 μm) (*Agilent Technologies*, USA), column flow rate 0.25 ml/min, thermostat temperature 30° C, injection volume 2 μl. Elution was performed in 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 %).

Compound detection was performed using a diode array detector with signal recording at 254 and 280 nm and fixation of absorption spectra in the 191–700 nm range. Agilent G1956B mass spectrometric detector (*Agilent Technologies*, USA) was used to determine the molecular weights of the tested compounds. Ionization was performed in ESI and APCI mode with positive ion fixation in SCAN mode in the range of 100–1200 m/z. The calibration was performed using standard auxin solutions.

Study of tryptophan transaminase activity

Obtaining of cell-free extracts. To obtain cell-free extracts the culture broth after cultivation of *R. erythropolis* IMV Ac-5017 in the liquid mineral medium with crude glycerol was centrifuged (4000 g, 15 min, 4° C). The cell pellet was washed twice from the residual medium with 0.05 M K+ phosphate buffer (pH 7.0) by centrifuging (4000 g, 15 min, 4° C). The washed cells were resuspended in 0.05 M K+ phosphate buffer (pH 7.0) and destroyed by ultrasound (22 kHz) 3 times for 60 s at 4° C on an UZDN-1 apparatus. The disintegrated cells were centrifuged (12000 g, 30 min, 4° C), the precipitate was discarded and the supernatant was used as cell-free extract.

Analysis of 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-3-pyruvate from L-tryptophan and 2-oxoglutarate, which was analyzed spectrophotometrically at 330 nm [15].

Statistics. All the experiments were repeated three times, and the number of analytical measurements in each experiment was 3–5. The statistical processing of the experimental data was carried out in accordance with the algorithm described in [5]. Differences of mean indicators were deemed as reliable at the significance level $p<0.05$.

Results. Previous studies have shown that the synthesis of auxin metabolites was dependent on the nature of the carbon source in the culture medium of *R. erythropolis* IMV Ac-5017 [5].

In this work, the choice of substrates (ethanol and waste oil after frying meat) for the cultivation of *R. erythropolis* IMV Ac-5017 with the aim of intensifying auxin synthesis was due to the following reasons. Firstly, the strain *R. erythropolis* IMV Ac-5017 synthesized the highest amount of auxins under conditions of growth on waste oil (91.3 μg/l) and ethanol (84.3 μg/l) compared to that on other substrates [5]. Secondly, the complex microbial preparation should be characterized by high antimicrobial activity against phytopathogenic bacteria, and previously [16] it was shown that such properties are inherent to the surfactants synthesized during the cultivation of IMV Ac-5017 strain on waste oil. Thirdly, waste oil is toxic, its remnants are not disposed in Ukraine, and its use as a substrate will simultaneously allow to transform hazardous waste and use exometabolites of *R. erythropolis* IMV Ac-5017 strain for a new purpose.

The data presented in Table 1 show that regardless of the tryptophan introduction moment into IMV Ac-5017 strain culture medium with waste oil, a significant increase in auxin synthesis was observed compared to the indicators on the medium without this precursor. Indole-3-acetic acid (IAA), indole-3-carboxylic acid and indole-3-butyric acid were identified as compounds of auxin nature, but the highest quantitative content among all auxins was in IAA, whose precursor is tryptophan.

The highest level of synthesis was observed when 300 mg/l of tryptophan was added at the end of the exponential growth phase (2398.14 μg/l compared to 9.85 μg/l on medium without precursor). Such data are consistent with our assumption that induction of phytohormone synthesis will occur under such conditions, because phytohormones are secondary metabolites.

At the same time, in the case of introducing 300 mg of tryptophan to the medium with ethanol, the amount of synthesized auxins by *R. erythropolis* IMV Ac-5017 was 5634.22 μg/l (Table 2) and was twice higher than under similar conditions of cultivation on waste oil (2398.14 μg/l, see Table 1). As in the cultivation of *R. erythropolis* IMV Ac-5017 on waste oil, during the cultivation of the

Table 1

The effect of tryptophan on the auxins synthesis under cultivation of *R. erythropolis* IMV Ac-5017 on waste oil

Auxins	Amount of auxins (μg/l) in the presence of tryptophan (mg/l)				
	Without tryptophan (control)	200		300	
		a	b	a	b
IAA	2.49	565.248	374.798	424.888	1876.0
ICA	5.4	606.042	273.08	320.221	522.144
IButA	1.96	–	–	–	–
Total	9.85	1171.29	647.88	745.11	2398.14

Legend: The tryptophan was added at the lag phase (a) or at the end of exponential phase (b); "–" – not found. The error did not exceed 5%.

Table 2

Synthesis of auxins by *R. erythropolis* IMV Ac-5017 in the presence of tryptophan in the medium with ethanol

Auxins	Amount of auxins (μg/l) in the presence of tryptophan (mg/l)				
	Without tryptophan (control)	200		300	
		a	b	a	b
IAA	110.31	3302.0	2189.0	4404.0	4597.0
ICA	25.63	452.016	370.752	979.48	977.54
IButA	–	41.564	38.014	54.755	59.678
Total	135.94	3795.58	2597.766	5438.235	5634.22

Legend: The tryptophan was added at the lag phase (a) or at the end of exponential phase (b); "–" – not found. The error did not exceed 5%.

strain on ethanol, an increase in the concentration of tryptophan in the medium was accompanied by an increase in the amount of synthesized auxins.

However, it should be noted that the level of auxin synthesis on ethanol did not depend on the time of tryptophan introduction. Auxins amount was almost the same after tryptophan addition both at the beginning of the cultivation process and at the end of the exponential growth phase (Table 2). Our further research will be devoted to clarification of these questions.

In general, the introduction of tryptophan into the medium with both substrates allowed to increase the total amount of auxins by 20–240 times compared to that without a precursor of biosynthesis. It is possible that a further increase in the amount of tryptophan will be accompanied by an intensification of auxin synthesis. However, at this stage, for the creation of an effective microbial preparation with growth-stimulating properties it is unnecessary, because at the achieved concentration of auxins (2–5 mg/l, see Tables 1 and 2) the culture broth of *R. erythropolis* IMV Ac-5017 with the purpose of seed or roots treatment of plants seedlings must be diluted at least in 400–500 times.

Literature data [17] show that the induction by tryptophan introduction is due to the fact that in microorganisms this amino acid is the precursor of IAA biosynthesis (Fig. 1).

Conversion of tryptophan to IAA can be accomplished in three ways:

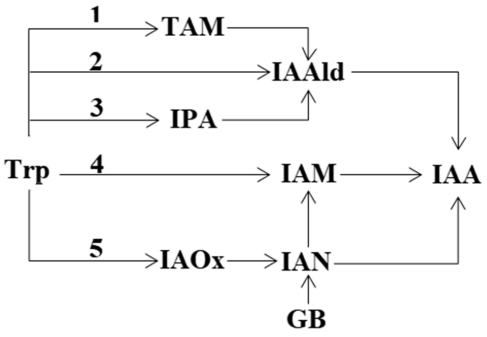
− Synthesis through indole-3-pyruvic acid and indole-3-acetic aldehyde. This is the main pathway characteristic of fungi and bacteria;

− Conversion of tryptophan to indole-3-acetic aldehyde may involve an alternative pathway of synthesis in which tryptamine is formed. This pathway is found in mycorrhizal fungi and cyanobacteria.

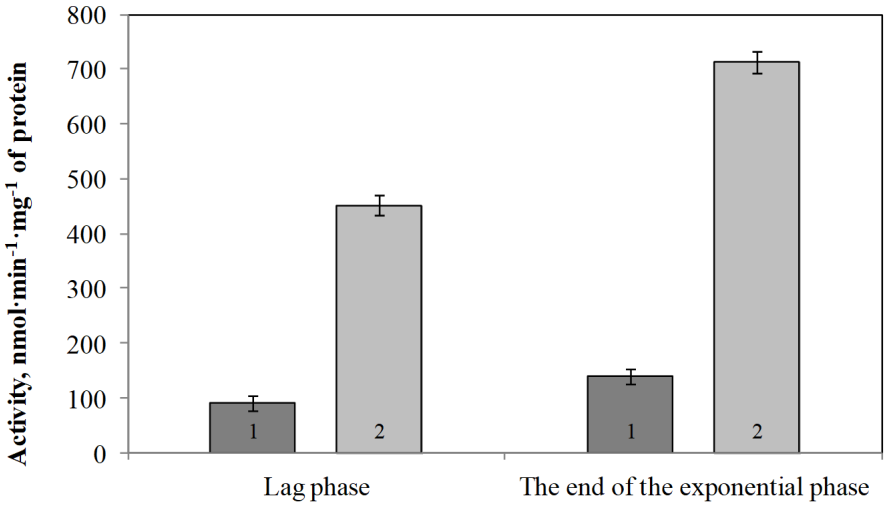
− IAA formation via indole-3-acetamide. This is characteristic of phytopathogenic bacteria and fungi.

To confirm that exogenous tryptophan is involved in the biosynthesis of auxins, the activity of one of the key enzymes in the synthesis of IAA (tryptophan transaminase) was analyzed. Tryptophan transaminase catalyzes the reaction of the formation of indole-3-pyruvic acid from L-tryptophan and 2-oxoglutarate.

According to the data shown in Fig. 2, in the case of *R. erythropolis* IMV Ac-5017 cultivation on medium with ethanol and 300 mg/l of tryptophan, the activity of this enzyme was higher than on the medium without this precursor. In addition, it



F i g. 1. Ways of synthesis of indole-3-acetic acid from tryptophan in bacteria: TAM – tryptamine; IAAld – indole-3-acetaldehyde; IPA – indole-3-pyruvate; IAM – indole-3-acetamide; IAOx – indole-3-acetaldoxime; IAN – indole-3-acetonitrile; GB – glucobrassicin; IAA – indole-3-acetic acid. 1 – through TAM; 2 – bypass tryptophan pathway; 3 – through IPA; 4 – through IAM; 5 – through IAN



F i g. 2. The effect of tryptophan on the activity of tryptophan transaminase of *R. erythropolis* IMV Ac-5017: 1 – without tryptophan; 2 – tryptophan, 300 mg/l

should be noted that when introducing tryptophan at the end of the exponential growth phase, the activity of tryptophan transaminase was 1.6 times higher than when introducing in the lag phase, which is consistent with the data in Table. 1, regarding the concentration of formed auxins.

The obtained results allow us to assume that the IAA biosynthesis in *R. erythropolis* IMV Ac-5017 is due to the formation of indole-3-pyruvate.

Discussion. Earlier we showed the ability of surfactant producers *R. erythropolis* IMV Ac-5017, *Acinetobacter calcoaceticus* IMV B-7241 and *Nocardia vaccinii* IMV B-7405 to synthesize phytohormones of auxin nature [5]. In 2016 Polish researchers reported that IAA was formed by bacteria (mainly *Rhodococcus* species) isolated from contaminated hydrocarbons and heavy metals [18]. In 2018–2019, papers [19–21] were published in which the ability of producers of surface-active lipopeptides and rhamnolipids to synthesize phytohormones of auxin nature was established. Thus, endophytic strain *Bacillus* sp. Fcl1 [19] synthesize iturin A and surfactin, which had antimicrobial effects on the phytopathogenic fungi of the genera *Fusarium, Phytophthora, Sclerotium, Corynespora*, as well as IAA, presence of which in the culture broth was determined by a qualitative reaction with a Salkowski reagent. The authors did not analyze the concentration of synthesized lipopeptides and IAA.

Bacillus sp. B19, *Bacillus* sp. P12 and *B. amyloliquefaciens* B14, isolated from the soil, synthesize a complex of antimicrobial compounds (surface-active lipopeptides kurstakin, surfactin, iturin, fengycin and antibiotic polymyxin), as well as auxins [20]. The concentration of auxins synthesized by strains B19 and P12 was 5.71 and 4.90 mg/l, re-

spectively. Tryptophan was not added to the culture medium. However, tryptone which contains tryptophan was used as a carbon source for cultivation of producers.

Pseudomonas aeruginosa L10 endophytic strain [21] under the cultivation on diesel fuel (5 g/l) synthesized rhamnolipids, which reduced the surface tension to 29.5 mN/m, and IAA at a concentration of 27 μg/l. It should be noted that in this work the authors did not try to increase the synthesis of IAA.

Probably, our work is one of the first to report the possibility of induction of auxin synthesis by surfactant producers by introducing a precursor of biosynthesis into the culture medium – tryptophan. On the other hand, there are reports of increased IAA synthesis by other soil microorganisms [22–32].

Liu et al. [22] showed that cultivation of *Burkholderia pyrrocinia* JK-SH007, a poplar endophyte, on Tryptone-soy medium in the presence of 1 g/l of tryptophan allowed to increase the synthesis of IAA from 0.795 mg/l to 6.621 mg/l. In our opinion, the researchers failed to achieve the required degree of transformation of tryptophan into IAA.

In other works [23–27, 30–32] much more efficient conversion of the precursor to auxin was observed. Thus, the pathogen of tomatoes *Pseudomonas syringae* DC3000 synthesizes IAA through the formation of indole-3-acetaldehyde from indole-3-pyruvate (see Fig. 1) [23]. When 0.1 g/l of tryptophan was added to the culture medium, the concentration of IAA was 2.7 mg/l, which are three orders of magnitude higher than without the introduction of biosynthesis precursor (0.03 mg/l).

At the same time, the researchers examined the effect of other synthesis precursors on the formation of IAA, in particular, indole-3-acetaldehyde and indole-3-acetonitrile, when making which in the culture medium the concentration of auxin at 48 h of cultivation was 11.7 and 14.1 mg/l, respectively.

Shim et al. [24] from the heavy metal-contaminated rhizosphere of plants isolated *Bacillus* sp. JH 2-2 strain, which in the presence of 0.5 g/l tryptophan on sucrose medium formed 6.44 mg/l IAA, while in the absence of this amino acid in the culture medium auxins were not detected at all.

The above microorganisms are capable of synthesizing IAA in the range of 1.0–6.4 mg/l. Such indicators are at the level obtained by us in the cultivation of *R. erythropolis* IMV Ac-5017 (1.88–4.59 mg/l IAA, see Tables 1 and 2). It should be noted that the previously described [22, 24] and described below producers [25, 26, 31] are in a certain interaction with plants – as endophytes [22, 31] or as associated rhizosphere microorganisms [24–26]. This causes the formation by these strains of higher concentrations of phytohormones to ensure beneficial interaction with plants. At the same time, our studied *R. erythropolis* IMV Ac-5017 strain belongs to the free-living soil bacteria, for which the synthesis of compounds that stimulate plant growth is not typical.

Scientists from Thailand [25] isolated an unidentified strain number DPY-05 from the rhizosphere of orchids (*Dendrobium pulchellum*), which on mannitol-yeast medium formed IAA at a concentration of 67.18 mg/l (in the presence of 0.5 g/l of tryptophan), which is almost 9 times higher than without the introduction of the biosynthesis precursor (11.48 mg/l). In Kumari et al. paper [26] it was noted that the concentration of IAA synthesized by *Bacillus subtilis* DR2 strain, which was isolated from the rhizosphere of *Eragrostis cynosuroides*, increased almost 1.7 times (168.1 mg/l compared to 100.26) in a medium with mannitol and 1.2 g/l tryptophan.

However, the authors of these works determined the concentration of IAA by spectrophotometric methods using Salkowski's reagent [25] and Kovac's reagent [26], which show the total content of indole compounds in the sample, not only IAA [27, 28]. Chromatographic methods are more specific and allow to determine the quantitative and qualitative composition of auxins, so the final concentration of IAA may actually be lower, as shown in De-Bashan et al. [29]. Thus, in the determination of exogenous auxins in the culture medium of *Azospirillum brasilense* Cd with 0.2 g/l of tryptophan using Salkowski's reagent 91.33 mg/l of indole compounds were identified, and by HPLC – 44.01 mg/l IAA.

The formation of an order of magnitude greater amount of IAA, determined by HPLC, was reported by Gang et al. [30]. *Klebsiella* SGM 81 strain was isolated from the rhizosphere of garden carnation (*Dianthus caryophyllus*) and is capable of endophytic root colonization. When determining the content of indole compounds in the culture broth obtained after culturing *Klebsiella* SGM 81 on medium with peptone and 5 g/l of tryptophan, 960.0 mg/l IAA was detected. Since IAA was not identified in the absence of tryptophan, the authors hypothesized that the *ipdC* gene responsible for indole-3-pyruvate decarboxylase formation has high substrate specificity.

There are studies [27, 31, 32] that aim to create potential industrial strains producing IAA in order to obtain high concentrations of synthesized auxin. To date, methods of genetic engineering are the most widely used for the construction of organisms with the necessary properties. Obtaining of high efficient producers of IAA is not an exception.

Guo et al. [31] de novo developed an indole-3-pyruvate route of IAA synthesis in the genome of *Escherichia coli* RARE strain, which is incapable of synthesizing phytohormones of auxin nature. This pathway comprised three gene products: *Saccharomyces cerevisiae* aminotransferase ARO8 for the conversion of L-tryptophan to indole-3-pyruvic acid, *S. cerevisiae* decarboxylase KDC for the decarboxylation of indole-3-pyruvic acid to indole-3-acetaldehyde, and *E. coli* AldH for the oxidation of indole-3-acetaldehyde to the corresponding IAA. The newly formed *E. coli* DG121 strain produce up to 387 mg/l IAA in the presence of 0.5 g/l tryptophan on glucose medium (the concentration of IAA without tryptophan was not determined). Tsavkelova et al. [27] studied the synthesis of IAA among species of the genus *Fusarium*. The highest level of synthesis was achieved under cultivation of *F. proliferatum* ET1 endophytic strain isolated from the roots of a tropical orchid. Thus, subject to the introduction of 0.8 g/l of tryptophan in a medium with sucrose, the strain formed 13.8 mg/l IAA. In addition to IAA, indole-3-acetamide was detected in the culture broth, indicating the functioning of the auxin biosynthesis pathway via IAM in *F. proliferatum* ET1 (see Fig. 1). The researchers then transferred the *IAM1* and *IAH1* genes, which encode IAM pathway enzymes, from *F. proliferatum* ET1 to recipient *F. fujikuroi* and overexpressed them. Thus, a recombinant strain *F. fujikuroi* #T2 was constructed, which produced up to 255.6 mg/l IAA in the presence of 0.8 g/l tryptophan and 7.66 g/l in its absence.

Such synthesis parameters (255–387 mg/l IAA) reported in [27] and [31] do not exceed the concentrations obtained under cultivation of natural plant growth promoting bacteria (PGPB) strain *Klebsiella* SGM 81, which formed up to 960 mg/l IAA [30]. This may be due to the amount of tryptophan in the culture medium, which was 0.5–0.8 and 5 g/l, respectively.

However, there is report [32] that the endophyte of rice *Enterobacter* sp. DMKU-RP206 is capable of synthesizing indole-3-acetic acid at the level of 415 mg/l without adding tryptophan to the culture medium. Further, when culturing the strain in flasks, the highest concentration of phytohormones was obtained on medium with lactose (8.5 g/l) and with 11 g/l tryptophan (3.804 mg/l IAA). Further scaling of the process in the fermenter and selection of the optimal aeration of the medium (2 l/l ·min) allowed to increase the yield of auxin to 5.56 g/l, which is 13.4 times more than in the conditions of cultivation without a precursor. This concentration allows considering the strain DMKU-RP206 as a promising producer of auxin for the development of relative technology. We note that in the literature there are only a few publications about strains capable of synthesizing such a concentration of IAA (several g/l). Thus, in the review [12] we provided information about the bacterial strain *Pantoea agglomerans* PVM, which on a medium with sucrose and tryptophan (1 g/l) synthesized 2.19 g/l IAA, as well as the yeast strain *Rhodosporidium paludigenum* DMKURP301, which on sucrose in the presence of 4 g/l of tryptophan formed 1.63 g/l of indole-3-acetic acid.

Analysis of literature data [22–27, 30–32] showed that the introduction of biosynthesis precursors is effective for increasing the synthesis of extracellular phytohormones. However, the authors of [30–32], in which it was reported that high concentrations of IAA were obtained, introduced very high amounts of tryptophan (2–11 g/l) into the medium for culturing microorganisms. And the degree of intensification was not more than 20 times. The use of large amounts of tryptophan as a component of the nutrient medium is economically impractical. Our studies have shown the possibility of intensifying the IAA synthesis more than 40 times on medium with ethanol and more than 700 times on medium with waste oil (provided that only 0.3 g/l of tryptophan was introduced at the end of the exponential growth phase). In addition, most researchers analyze the ability to synthesize phytohormones on rich nutrient media, which contain as a source of carbon trypton [22], mannitol [26], sucrose [27], peptone [30], glucose [31] and lactose [32]. Such media for growing phytohormone producers are expensive, so there is a need to reduce their cost, in particular by finding cheaper carbon substrates. Our research has for the first time shown the possibility of the formation of auxins on a cheap medium using fried oil as a substrate.

Therefore, as the result of this work it was established the possibility of increasing by two or three orders the amount of synthesized auxins in the case of low concentrations of their precursor biosynthesis in the culture medium

of *R. erythropolis* IMV Ac-5017 not only with ethanol but also with industrial waste (waste oil). The obtained results are the basis for increasing the efficiency of the exometabolites of *R. erythropolis* IMV Ac-5017 strain with growth-stimulating properties in crop production.

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ІНДУКЦІЯ СИНТЕЗУ АУКСИНІВ *RHODOCOCCUS ERYTHROPOLIS* ІМВ АС-5017 ПРИ ДОДАВАННІ ТРИПТОФАНУ В СЕРЕДОВИЩЕ КУЛЬТИВУВАННЯ

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Резюме

Здатність продуцентів поверхнево-активних речовин (ПАР) до синтезу фітогормонів розширює сфери їх практичного застосування і окреслює перспективи для створення мікробних препаратів з ріст-стимулювальними властивостями. Можливість інтенсифікації синтезу штамами фітогормонів-стимуляторів підвищує ефективність використання таких препаратів. **Мета**. Дослідити можливість індукції синтезу позаклітинних ауксинів за присутності триптофану в середовищі культивування продуцента ПАР *Rhodococcus erythropolis* IMB Ac-5017 та встановити оптимальну концентрацію і час внесення у середовище для забезпечення максимального синтезу ауксинів. **Методи**. Біохімічні, мікробіологічні, біотехнологічні. Культивування проводили у рідкому мінеральному середовищі з внесенням субстратів етанолу та відпрацьованої соняшникової олії. Триптофан додавали в середовище у вигляді 1% розчину в кількості 200 або 300 мг/л на початку процесу або в кінці експоненційної фази росту. Фітогормони виділяли шляхом послідовної екстракції органічними розчинниками із супернатанту культуральної рідини після екстракції ПАР. Попереднє очищення і концентрування фітогормонів здійснювали методом тонкошарової хроматографії. Якісне та кількісне визначення ауксинів проводили за допомогою високоефективної рідинної хроматографії. **Результати**. Встановлено, що незалежно від концентрації і часу внесення триптофану в середовище культивування *R. erythropolis* IMB Ac-5017 з обома субстратами, спостерігали значне підвищення (на 2−3 порядки) кількості синтезованих ауксинів у порівнянні з показниками на середовищі без триптофану. Найвища концентрація ауксинів (5552−5634 мкг/л) спостерігалася при додаванні 300 мг/л триптофану у середовище культивування з етанолом, у той час як без попередника їх кількість становила всього 143 мкг/л. На відміну від культивування штаму на етанолі, де синтез ауксинів не залежав від часу внесення триптофану, на відпрацьованій олії *R. erythropolis* IMB Ac-5017 утворював максимальну кількість ауксинів при внесенні 300 мг/л триптофану наприкінці експоненційної фази росту (2398 мкг/л порівняно з 9.8 мкг/л на середовищі без попередника). Серед ауксинів ідентифіковано індол-3-оцтову кислоту, індол-3-карбонову кислоту та індол-3-масляну кислоту. Проте було синтезовано найбільшу кількість саме індол-3-оцтової кислоти, попередником якої і є триптофан. Синтез цього ауксину (найпоширеніший рослинний ауксин) за наявності 300 мг/л триптофану збільшувався більш, ніж у 40 разів на середовищі з етанолом і більш, ніж у 700 разів на середовищі з відпрацьованою олією. Індукція синтезу ауксинів штамом *R. erythropolis* IMB Ac-5017 корелювала зі зміною активності триптофантрансамінази: при культивуванні на етанолі без триптофану вона становила 138 нмоль·хв$^{-1}$·мг$^{-1}$ білка, у той час як за наявності попередника підвищувалася у 5,2 рази (до 714 нмоль·хв$^{-1}$·мг$^{-1}$ білка). Отримані результати дають підставу припустити, що біосинтез індол-3-оцтової кислоти у штаму IMB Ac-5017 відбувається через утворення індол-3-пірувату. **Висновки**. Встановлена можливість підвищення на два-три порядки кількості позаклітинних ауксинів у разі внесення невисоких концентрацій попередника їх біосинтезу в середовище культивування *R. erythropolis* IMB Ac-5017 не тільки з етанолом, а й з відпрацьованою олією. Отримані результа-

ти можна розглядати як перспективні для використання у рослинництві екзометаболітів штаму *R. erythropolis* IMB Ac-5017 з ріст-стимулювальними властивостями.

Ключові слова: продуцент ПАР *Rhodococcus erythropolis* IMB Ac-5017, позаклітинні ауксини, триптофан, триптофантрансаміназа, індукція синтезу ауксинів.

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