BIOSYNTHESIS OF POLYENE ANTIBIOTICS AND PHYTOHORMONES BY *Streptomyces netropsis* IMV Ac-5025 UNDER THE ACTION OF EXOGENOUS INDOLE-3-CARBINOL

Mariia LOBODA[*], Lyudmyla BILIAVSKA[*], Galyna IUTYNSKA[*]

[*]*D.K. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Department of general and soil microbiology. Kyiv, Ukraine.*
Correnspondence author: Mariia Ivanivna Loboda, D.K. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Department of general and soil microbiology, Kyiv, Ukraine, zip code: 03143, phone: +380993718498, e-mail: marichka20loboda@gmail.com

Abstract. Soil streptomycetes are active producers of a wide range of metabolites. In a complex of biologically active substances synthesized by *Streptomyces netropsis* IMV Ac-5025 polyene antibiotics and phytohormones were detected. At present, the secondary metabolites' influence on the polyene antibiotics biosynthesis by soil streptomycetes is insufficiently explored. The aim of the work was to research the effect of exogenous auxin indole-3-carbinol on polyene antibiotics and phytohormones biosynthesis by *S. netropsis* IMB Ac-5025. The strain was cultivated in organic and synthetic liquid nutrient media; biomass accumulation, biosynthesis of polyene antibiotics, auxins and cytokinins were determined under the action of exogenous indole-3-carbinol.

It has been found the concentration of exogenous auxin by which quantity of polyene antibiotics was increased in 2.8 and 1.9 times, auxins – in 2.9 and 2.0 times, cytokinins – in 2.8 and 5.6 times respectively. Thus, exogenous indole-3-carbinol enhanced polyenes antibiotics and phytohormones biosynthesis by *S. netropsis* IMV Ac-5025. This indicates an important relationship between the biosynthesis of secondary metabolites by soil streptomycetes. The possibility to regulate the amount of biologically active substances opens opportunities for elaboration of bioproduct with protective and plant stimulating properties.

Keywords: *Streptomyces netropsis*; indole-3-carbinol; polyene antibiotic; phytohormone.

INTRODUCTION

Actinobacteria are saprotrophic gram-positive bacteria with a wide biochemical and morphological diversity. They have various beneficial properties such as plant growth promoting, plant pathogens suppression, decomposition of organic residues in a soil, phytoremediation etc. Most commonly, their presence observes in soil, marine water and compost. *Actinobacteria* of *Streptomyces* genus are active producers of a wide range of biologically active metabolites. The complex of synthesized biologically active substances determines the regulation of plant growth and induction of the resistance against stress factors, provide antagonism against phytopathogenes and parasites. Besides, these substances have antiviral and antitumor properties [2, 16].

In previous years the new *S. netropsis* IMV Ac-5025 strain was isolated by the researchers from the Institute of Microbiology and Virology, NAS of Ukraine. It produces a wide range of biologically metabolites, such as antibiotics (including polyene macrolides), phytohormones (auxins, cytokinins, gibberellins), lipids (phospholipids, sterols), amino acids, peptides, etc. [5].

In the biomass of this soil streptomycete it was found the accumulation of antibiotics of different classes, that provide antibacterial and antifungal properties. The major components in the antibiotic complex were heptaene candidine and new tetraene antibiotic, about which is absent information in NCBI antibiotics database. Due to the polyene antibiotics biosynthesis, the strain is an active antagonist against phytopathogenic fungi (*Alternaria, Fusarium, Cladosporium* genera) and antibiotics of other classes provided activity against phytopathogenic bacteria (*Pseudomonas, Xanthomonas, Clavibacter* genera) [3, 4].

It is known that polyene antibiotics interact with ergosterols in fungal cells membranes and cause the creation of channels through which leakage of elements is occur that leads to the death of the target. These antibiotics are synthesized by polyfunctional enzymes polyketide synthases (PKSs) type II whose molecules are often associated with aminoglycoside group – d-mycosamine [15, 21].

Other physiologically active substances such as phytohormones auxins, cytokinins, gibberellins, and a small quantity of abscisic acid also accumulated in the biomass of tested strain. They affect on secondary metabolites biosynthesis, spore formation and development of air mycelium, cell division, and differentiation [5, 8]. However, the role of phyto-hormones for streptomycetes needs further study. Earlier we revealed that among the full range of biologically active substances synthesized by this strain, the production of polyene antibiotics is the most correlated with the biosynthesis of cytokinins, auxins and sitosterols. Indole-3-carbinol had the greatest influence on the polyene antibiotics biosynthesis among auxins [18].

Nowadays, the effect of auxins on polyene antibiotics biosynthesis by soil streptomycetes is little remains known. Therefore, the effect of exogenous indole-3-carbinol on the polyene antibiotics and phytohormones biosynthesis by *S. netropsis* IMV Ac-5025 is important to research.

MATERIALS AND METHODS

Some aspects of the biosynthetic ability of *S. netropsis* IMV Ac-5025 were researched, gained from

the collectin of D.K. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Department of general and soil microbiology. To obtain the biosynthesis of antibiotics and other biologically active substances under the action of exogenous indole-3-carbinol, the strain was cultivated in-depth method in synthetic (starch-ammonia) and organic (soy) nutrient media [23].

The strain was stored in oaten (ISP-3) agar at +4 °C temperature [11]. To receive the inoculum, *S. netropsis* IMV Ac-5025 was introduced into flasks with nutrient medium, containing soy broth 15 g/L, yeast extract 5 g/L, glucose 20 g/L and incubated on a rotary shaker for 2 days at the temperature of +28±1 °C, n = 240 rpm (revolutions per minute). The inoculum was added in an quantity of 5% of the appropriate nutrient media volume and incubated under the same conditions for the next 7 days. Concentrations of exogenous indole-3-carbinol were 0.1 mg/L, 0.5 mg/L, 2.5 mg/L, 5 mg/L, 25 mg/L, and control variant without auxin addition. On the seventh day in the stationary phase of growth we measured the biomass accumulation and detected the polyene antibiotics, auxins, cytokinins biosynthesis in it. Biomass was taken into account by the gravimetric method and expressed in grams of absolutely dry biomass (ADB) per 1 liter of nutrient medium [5].

The polyene antibiotics were extracted from the producer's wet biomass by 96° ethanol after 24 hours. Ethanol extracts were applied on chromatographic plates TCL Silica gel 60 F_{254} (“Merck”, Germany). Chromatography of the extracts was carried out in a system of solvents – butanol: acetic acid: water (3:1:1). The spots formed on the chromatographic plates were compared with the control. As a control were used pre-purified heptaene fraction (candidine) and tetraene compound synthesized by this producer. Purification of the compounds was performed by chromatographic separation on chromatographic plates TCL Silicagel 60 F254 (“Merck”, Germany) in a solution system for polyene antibiotics. After the separation of substances in the chromatographic chamber it was carried out the extraction of antibiotics by 60° ethanol. After that, the antibiotics were re-washed from the walls of the epindorphs, centrifuged and the obtained extracts were used as controls. The spots formed at the appropriate height of the plate were analyzed on the device spectrodensitometer “Sorbfill” in the UV filter with a wavelength of 365 nm using software and identified as corresponding to each of the fractions. The device and software responded to the height of the peak, which corresponded to the fraction of candidine or tetraene compounds. The peak area indicated the amount of antibiotic and was supplemented with control. We determined the amount of polyene antibiotics by summing the quantity of two indicated fractions [3]. Antibiotics were extracted from the wet biomass and then were transferred to 1 g of completely dry biomass (ADB).

The content of phytohormones was determined in ethanolic extracts of *S. netropsis* IMV Ac-5025 biomass. For this purpose, purification of phytohormones was carried out in three stages in a mixture of solvents used sequentially: chloroform (the first step), 12.5% ammonia (the 2nd step) and ethyl acetate: acetic acid (20:1) (the 3rd step) on plates with silica gel “Silufol UV_{254}” (“Chemapol”, Czech Republic). After the 2nd stage of chromatography, we quantitatively applied samples and controls of cytokinins (was used zeatin) and auxins (was used indole-3-acetic acide) on chromatographic plates for the following separation of phytohormones. Then, for each sample, a silica gel layer corresponding to cytokinins and indole compounds was removed from the plate and transferred to epindorphs. Extraction of cytokinins was carry out by 96° ethanol and auxins – by ethyl acetate. Samples were and placed in a refrigerator for 1 day at a temperature of +4°C. On the second day, the samples were stirred and centrifuged. The obtained supernatant was transferred into epindorphs of smaller volume and left for 1 day in a thermostat (temperature +28°C) for the evaporating and concentrating of phytohormones. On the third day, the phytohormones were quantitatively washed from the walls of the epndorphs with appropriate solvents (as described above) and applied on the chromatographic plates for quantitative analysis. The purified extracts were separated on silica plates (“Merck”, Germany) for indole compounds and on alumina plates for cytokinins (“Merck”, Germany). Separation of phytohormones was carried out in a solvent mixture of chloroform: ethyl acetate: acetic acid (100:100:1) for the determination of auxins and chloroform: acetic acid (19:1) for cytokinins determination. Quantitative detection of phyto-hormones was performed by using a spectro-densitometer “Sorbfil”. As standards were used chemically pure cytokinins: zeatin, zeatinribozid, isopentenyladenine, isopentenyladenosine (“Sigma-Aldrich”, Germany) and auxins: indole-3-acetic acid (IAA), indole-3-acetic acid hydrazide, indole-3-carbinol, indole-3-butyric acid, indole-3-carbox-aldehyde, indole-3-carboxylic acid (“Sigma-Aldrich”, Germany) [22].

RESULTS

In our study we compared the biosynthetic ability of *S. netropsis* IMV Ac-5025 cultivated in different nutrient media – synthetic (ammonia starch) and organic (soy). The synthetic nutrient medium is important to use for determine the quantity of biologically active substances synthesized *de novo*. However, the organic medium contains organic components (corn extract, yeast extract, etc.), so it is rich of growth factors and metabolites precursors and it make be possible to receive much more biomass and accordingly some metabolites. Organic media are used

in biotechnology, and main components are cheaper than chemically synthesized compounds.

Among the metabolites of tested strain, two fractions of polyene macrolides were identified: heptaene candidine and a new tetraene compound. The biosynthesis of both compounds increased under the action of exogenous indole-3-carbinol. The total quantity of polyene antibiotics ranged from 481 to 1373 µg/g ADB (absolutely dry biomass) in synthetic and from 4083 to 7888 µg/g ADB in organic medium. The biggest quantity of polyenes was accumulated at auxin concentrations 5 mg/L in synthetic and 25 mg/L in organic nutrient media, there is in 2.8 and 1.9 times respectively more than in the control variant (Fig. 1).

The following dynamics of the biomass accumulation was observed in the synthetic nutrient medium (figures are indicated in ascending order of indole-3-carbinol concentration): 2.34 g/L; 2.6 g/L; 2.78 g/L; 3.22 g/L; 1.32 g/L; 1.76 g/L; and in an organic nutrient medium - 6.98 g/L; 9.52 g/L; 10.4 g/L; 8.02 g/L; 6.62 g/L; 6.6 g/L. Thus, in the starch-ammonia nutrient medium, the quantity of accumulated biomass was the lowest under the action of 5 mg/L indole-3-carbinol (which is on 56 % less that in control variant), in the soy medium- under the action of 25 mg/L of exogenous substance.

Previous studies have shown that the biosynthesis of polyene antibiotics is highly correlated with the formation of auxins [16]. Therefore, it was important to analyze the effect of exogenous indole-3-carbinol on auxins accumulation. In conditions of deep cultivation under the action of indole-3-carbinol in both synthetic and organic media the strain produced IAA, indole-3-butyric acid, indole-3-acetic acid hydrazide, indole-3-carboxaldehyde, indole-3-carboxylic acid, which are the products of IAA transformation. It was found the increase of auxins production in both nutrient media with the increase of exogenous auxin concentration.

In the starch-ammonia medium, the amount of auxins ranged from 20.4 to 40.7 µg/g ADB and was the highest at the concentration of exogenous indole-3-carbinol 5 mg/L. The biosynthesis of active forms of auxins was increased to a greater extent: indole-3-acetic acid hydrazide and indole-3-butyric acid (the latter is a more stable compound than IAA and may be a spare form). In soybean nutrient medium, the auxins amount ranged from 92.1 to 358.8 µg/g ADB, which is almost in 9 times more than in synthetic. At 25 mg/L of exogenous indole-3-carbinol, as in starch-ammonia nutrient medium, was identified the highest amount of indole-3-acetic acid hydrazide, indole-3-butyric acid and indole-3-carboxylic acid – the decomposition product of indole-3 carbinol (Fig. 2). Indole-3-carbinol, indole-3-carboxaldehyde, and indole-3-carboxylic acid are inactive auxins that are formed as result of IAA degradation.

In ethanolic extracts of producer's biomass were found active forms of cytokinins: zeatin and isopentyladenine, as well as zeatin-riboside and isopentenyladenosine, which are formed by attaching of the ribose residue to the purine ring in the N^9-position. The last two forms of cytokinins have lower activity than free forms, but they are stable, spare and can be quickly transformed into more active forms. The addition of exogenous indole-3-carbinol into the culture medium caused an increase of the accumulation

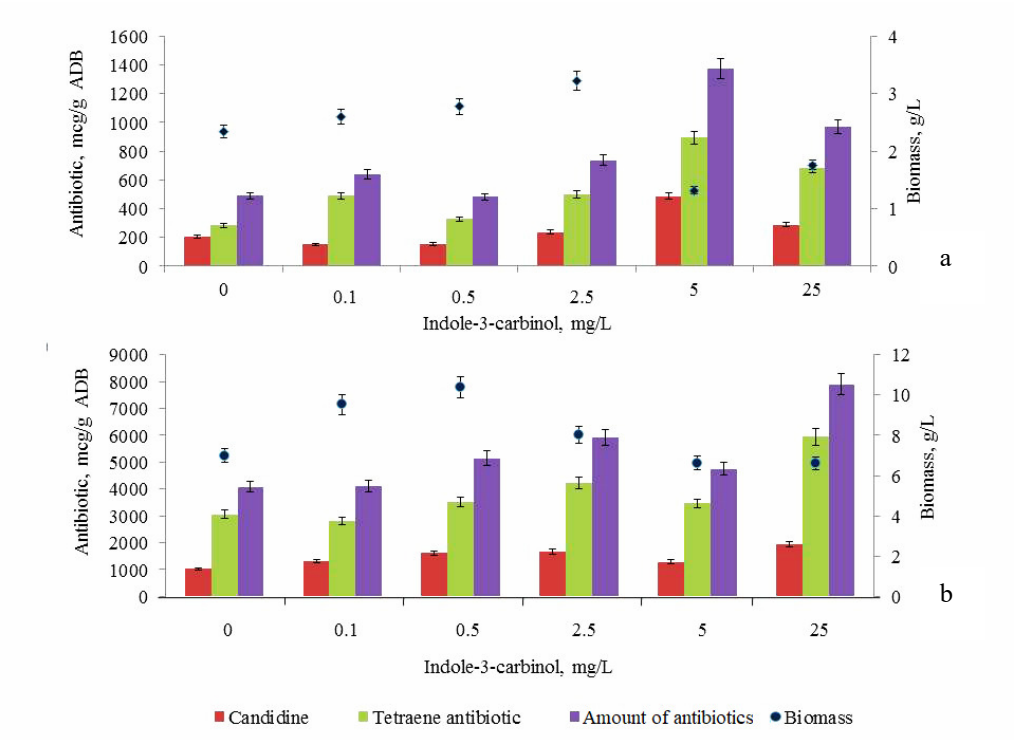

Figure 1. Biosynthesis of polyene antibiotics and biomass accumulation by *S. netropsis* IMB Ac-5025 under the action of exogenous indole-3-carbinol in synthetic (a) and organic (b) nutrient media

of total cytokinins in producer's biomass. It was detected the largest increase of spare cytokinins – zeatin-riboside and isopentenyladenosine in both media. At a concentration of 25 mg/L of indole-3-carbinol, the total amount of cytokinins was raised in 2.8 times in the synthetic and in 3.2 times in the organic medium compared with the control (Fig. 3).

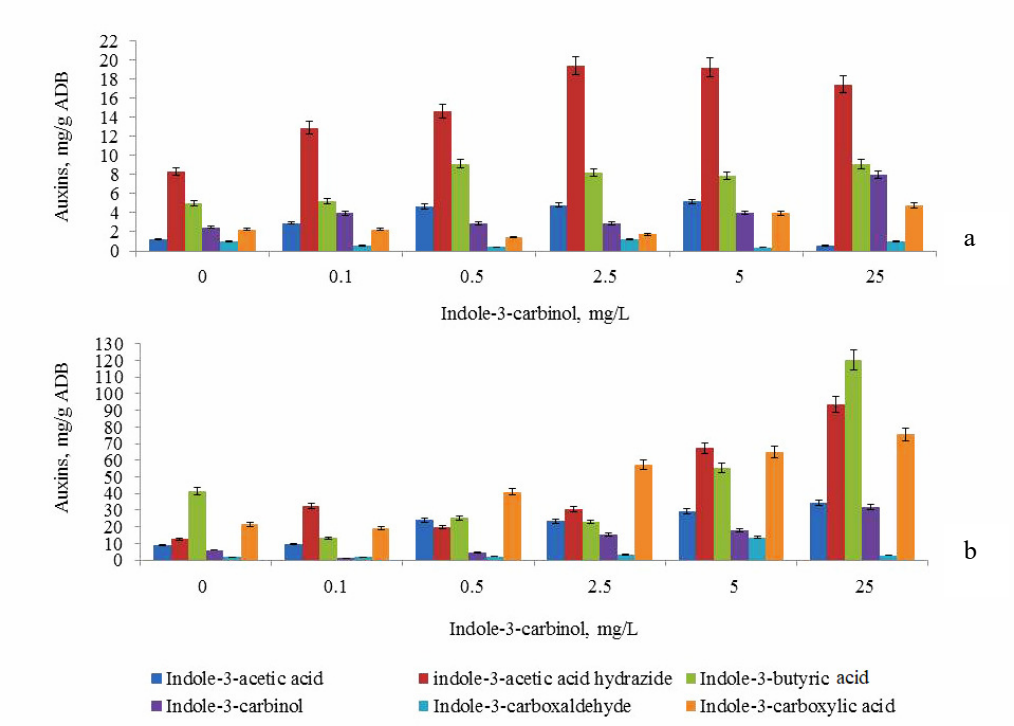

Figure 2. Auxins biosynthesis by *S. netropsis* IMV Ac-5025 under the action of indole-3-carbinol in synthetic (a) and organic (b) media

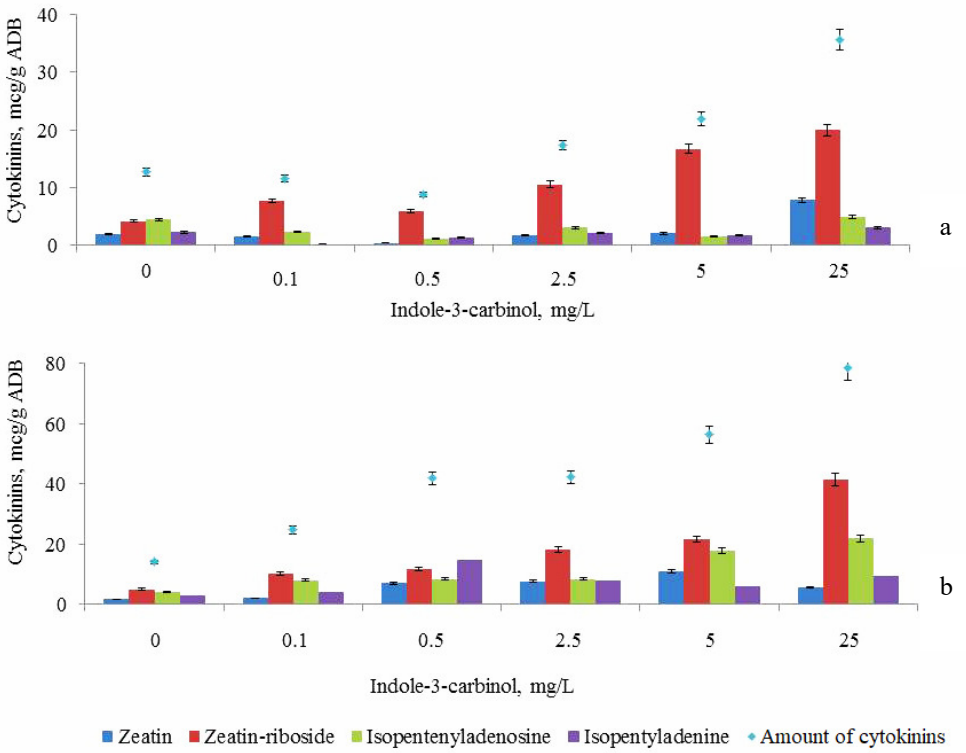

Figure 3. Biosynthesis of cytokinins by *S. netropsis* IMV Ac-5025 under the action of exogenous indole-3-carbinol in synthetic (a) and organic (b) nutrient media

DISCUSSION

The search of conditions for increasing of the microbial biosynthesis of biologically active compounds is an important issue of fundamental and biotechnological importance. Organic nutrient medium has been found to has the advantages of using for *S.netropsis* IMV Ac-5025 cultivating under the action of exogenous indole-3-carbinol, which contributed to the accumulation of more polyene antibiotics, auxins, cytokinins. As follows, in the organic nutrient medium producer accumulated more than in a synthetic medium: polyene antibiotics – in 6 times, auxins – in 9 times, and cytokinins – in 2.2 times This occurs in result of presence of a source of tryptophan in the organic medium. It is known that microorganisms use tryptophan dependent path of auxins biosynthesis. Tryptophan is a component of the soybean medium, in contrast, during the growth of streptomycetes in a synthetic nutrient medium, the auxins biosynthesis occurs *de novo*, which requires additional energy costs [14].

Up to date, polyene antibiotics proved to be the most effective antifungal agents due to their broad spectrum of potent fungicidal activity, and relatively low frequency of resistance among the fungal phytopathogens [21]. Among phytohormones, an important regulatory role in various growth processes of both plants and microorganisms belongs to auxins, which are chemically derived from indole. Auxins are necessary for soil microorganisms to grow, develop, cooperate with plants and interact with other microorganisms [19, 20].

Three major pathways for bacterial IAA biosynthesis have been known that remove the amino and carboxyl groups from the α-carbon of tryptophan via the intermediates indolepyruvate, indoleacetamide, or indoleacetonitrile; the oxidized end product IAA is typically secreted. Involvement of these oxidized products in the formation of indole-3-acetic acid is a mechanism of detoxification during which amino groups are released and involved to nitrogen metabolism. This indicates an important physiological role of auxins in the cell of bacteria (particularly streptomycetes) [24].

Cytokinins accumulated in the biomass of *S. netropsis* IMV Ac-5025 and derived from adenine, they are similar in structure, but have different biological activity. According to the functional value, cytokinins can be divided into reactive forms that bind to the receptor and cause a physiological response and reserve cytokinins that convert into active forms as needed. The increase of the cytokinins biosynthesis under the action of exogenous indole-3-carbinol had a positive effect on the cells division of tested strain, which was reflected in an increase of the amount of biomass and biosynthetic capacity [12].

Due to analyzing the biosynthetic pathways of secondary metabolites in streptomycetes, we can propose a probable scheme of the relationships between the biosynthesis of polyene antibiotics, auxins and cytokinins by soil streptomycetes (Fig. 4) [13]. It is known that polyene antibiotics are formed as a result of condensation of carbon chain with the addition of polyketide sugars, mevalonate and alkyl groups [1, 9]. The acetyl-CoA is a starting metabolite for the biosynthesis of polyene antibiotics, and cytokinins [7, 9].

Isoprenoid biosynthesis is essential for cell survival and these molecules are involved in a wide variety of vital biological functions. Isoprenoids may be synthesized via the classical mevalonate pathway in bacteria. The mevalonate pathway starts with the condensation of three acetyl-CoA molecules and a subsequent reduction to mevalonate. The mevalonate is then phosphorylated twice to form mevalonate-5-diphosphate, and another phosphorylation with subsequent decarboxylation-driven dephosphorylation yields isopentenyl diphosphate, which can be isomerized to dimethylallyl pyrophosphate (DMAPP), serving as a cytokinins precursor [12].

Alkyl groups are involved in the biosynthesis of cytokinins, such as α-methyl, 2-ethyl-, 3-methoxy, 4-hydroxy-, amino groups, etc. They are also used in the biosynthesis of polyketides to crosslink α-carbon chains [6, 21]. Probably, the biosynthetic pathways of polyene antibiotics and cytokinins intersect at this point in *S. netropsis* IMV Ac-5025.

DMAPP is an isomer of isopentenyl pyrophosphate (IPP). The enzyme isopentenylpyrophosphate isomerase catalyzes isomerization between DMAPP and IPP [25]. Subsequent metabolic transformations of DMAPP lead to the formation of isopentinyladenosine, from which zeatin riboside is formed, and isopentinyladenin, from which zeatin is formed. Zeatin and zaetin riboside are mutually transient forms of cytokinins [17]. Besides, auxins are known to regulate the biosynthesis of cytokinins [18]. Therefore, the addition of indole-3-carbinol in the nutrient medium caused an increase of cytokinins biosynthesis at our experimental conditions.

It is known that the shikimate pathway contributes to assemble the basic building blocks for the range of aromatic amino acids in bacteria. This pathway also gives rise to many secondary metabolites and known to give precursors for the polyene antibiotic biosynthesis, which was confirmed by the results of our research. It is a seven step metabolic route used by bacteria for the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan). The shikimate pathway is noted as a specialized biosynthesis pathway for benzoid aromatic compounds (very often, along with the shikimate pathway, the polyketide (acetate-malonate) mechanism for constructing benzene nuclei is also noted – the closure (zip-assembly) of aromatic systems via intramolecular condensation [10, 12]. In our experiment, exogenous indole-3-carbinol, probably, activated this metabolic pathway and led to an increase of polyene antibiotics biosynthesis with polyketide structure. At the same time, an increase of

the tryptophan accumulation could be directed to the phosphoenolpyruvate pathway for auxins biosynthesis. Therefore, due to the introduction of exogenous phytohormone, the biosynthesis of indole-3-acetic acid, indole-3-acetic acid hydrazide and indole-3-butyric acid were increased. Subsequent metabolic transformations of these auxins led to an increase of other auxins accumulation such as indole-3-carboxyl aldehyde, indole-3-carbinole, indole-3-carboxylic acid and the pool of indole rings, which used in the construct of the macrolide ring of polyene antibiotics. Thus, we first showed the relationship between the biosynthesis of auxins and polyene antibiotics it soil streptomycetes.

The scheme is hypothetical and promising for further research. These data demonstrate the importance of interconnection between antibiotics and phytohormones biosynthesis in streptomyces' cells. *S. netropsis* IMV Ac-5025 is the promising producer of biologically active substances and exogenous indol-3-carbinol is proved to enhance it.

In this work we have established the increase of the biosynthesis of polyene antibiotics (candidine and new tetraene compound), auxins (IAA, indole-3-acetic acid hydrazide, indole-3-carbinol, indole-3-butyric acid, indole-3-carboxaldehyde, indole-3-carboxylic acid) and cytokinins (zeatin, zeatinribozid, isopentenyl-adenine, isopentenyladenosine) by *S. netropsis* IMV-Ac-5025 under the action of exogenous indole-3-carbinol. This indicates an important relationship between the biosynthesis of secondary metabolites by soil streptomycetes. The possibility to regulate the amount of biologically active substances opens opportunities for elaboration of bioproduct with protective and plant stimulating properties.

At the Department of general and soil microbiology of the Institute of Microbiology and Virology of NASU was developed a metabolic bioformulation with the commercial name "Phytovit". Based on biologically active substances synthesized by *S. netropsis* IMV Ac-5025, bioproduct exhibits antagonistic properties against phytopathogenic microorganisms and has a stimulating and adaptogenic effect on plants. We were interested in the effect of indole-3-carbinol on the formation of polyene antibiotics by soil streptomycete for two reasons. Firstly, nowadays the effect of auxins on polyene antibiotics biosynthesis by soil streptomycetes is little remains known. Secondly, the results of the study are fundamental for understanding the possible interrelationships in the biosynthesis of biologically active substances by soil streptomycetes. The search of substances to increase the formation of a particular metabolite, in this case, polyene antibiotics and phytohormones is an effective biotechnological tool to improve the bioprodut.

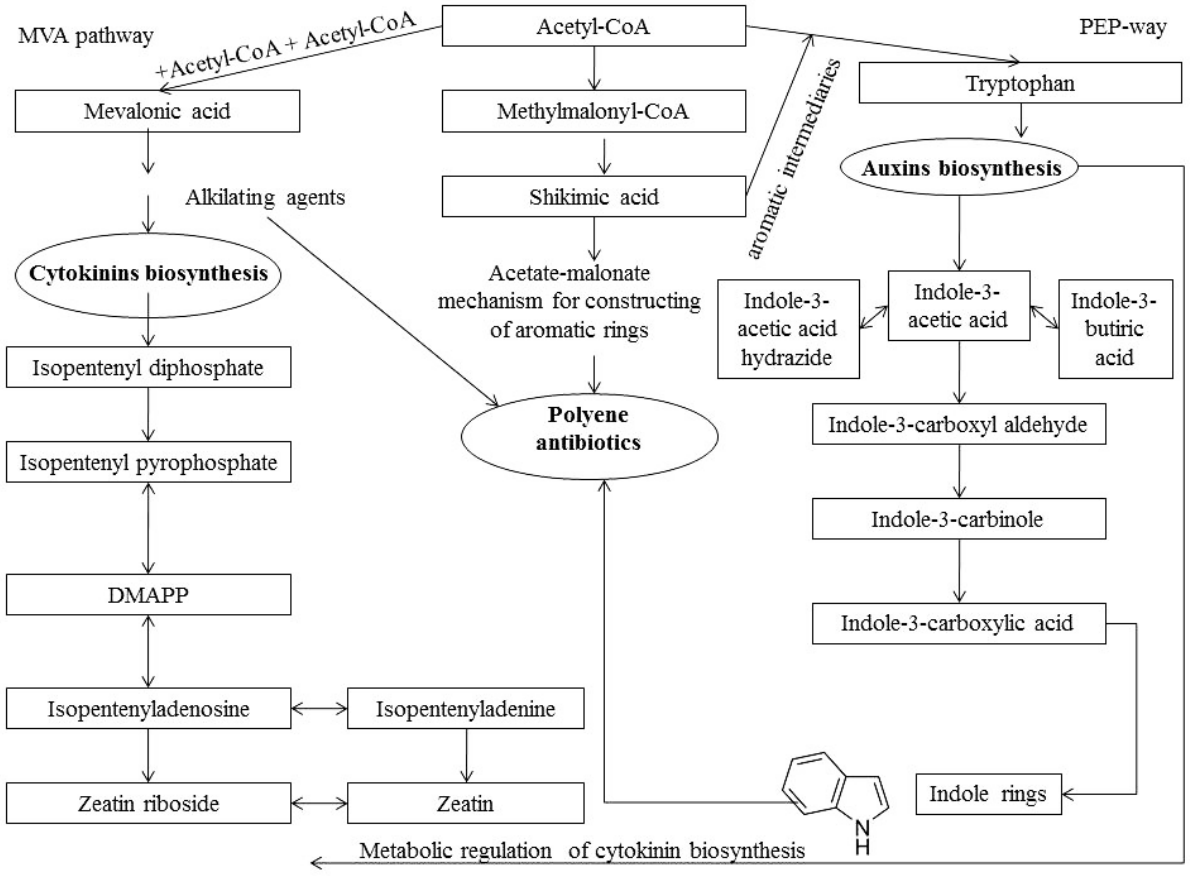

Figure 4. Scheme of the probable relationships between the biosynthesis pathways of polyene antibiotics, auxins and cytokinins in soil streptomycetes

REFERENCES

[1] Bacher, A., Eisenreich, W., (2010): Metabolic studies using the retrobiosynthesis concept – theory, technology, and examples. Comprehensive Natural Products II. Chemistry and Biology, 9: 675-694.

[2] Biliavska, L.O., (2015): Auxins biosynthesis by soil streptomyces – the antagonists of phytopathogenic microorganisms and nematodes. Microbiology and Biotechnology, 1: 36-43.

[3] Biliavska, L.O., Efremenkova, E.V., Zenkova, V.A., Kozyrytska, V.E., Iutynska, G.O., (2015): Soil streptomycete *Streptomyces netropsis* – producer of fungicidal substances. Modern mycology in Russia: The 3rd International Mycological Forum, Russia.

[4] Biliavska, L.O., Efymenko, T.O., Efremenkova, O.V., Kozyrytska V.E., Iutynska G.O., (2016): Identification and antagonistic properties of the soil streptomycete *Streptomyces sp.* 100. Microbiologichny zhurnal, 78: 27-38.

[5] Biliavska, L.O., Pidlypska, V.A., Kozyrytska, V.E., Iutynska, G.O., (2015): Biosynthetical activity of soil streptomycetes – antagonists of plan-parasitic nematodes and phytopathogens. The 4th European Conference on Biology and Medical Sciences, Austria.

[6] Calderone, C.T., (2008): Isoprenoid-like alkylations in polyketide biosynthesis. Natural Product Reports, 25: 845-853.

[7] Frebort, I., Kowalska, M., Hluska, T., Frebortova, J., Galuszka, P., (2011): Evolution of cytokinin biosynthesis and degradation. Journal of Experimental Botany, 62: 2431-2452.

[8] Frugier, F., Kosuta, S., Murray, J.D., Crespi, M., Szczyglowski, K., (2008): Cytokinin: secret agent of symbiosis. Trends in Plant Science, 13: 115-120.

[9] Hamilton-Miller, J.M., (1973): Chemistry and biology of the polyene macrolide antibiotics. Bacteriological Reviews, 37: 166–196.

[10] Herrmann, K.M., (1995): The Shikimate pathway: early steps in the biosynthesis of aromatic compounds. The Plant Cell, 7: 907-919.

[11] Iutynska, G.O., Kozyrytska, V.E., Biliavska, L.O., (2014): Strain *S. marinolimosus* – antagonist of phytopathogenic microorganisms. Zabolotny Institute of Microbiology and Virology, NASU. Patent of Ukraine 107122.

[12] Kakimoto, T., (2003): Biosynthesis of cytokinins. Journal of Plant Research, 116: 233-239.

[13] Kanehisa, M., Goto, S., (2000): KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Research, 28: 27-30.

[14] Khamna, S., Yokota, A., Peberdy, J.F., Lumyong, S., (2010): Indole-3-acetic acid production by *Streptomyces* sp. isolated from some Thai medicinal plant rhizosphere soils. Eurasian Journal of BioSciences, 4: 23-32.

[15] Khan, N., Rawlings, B., Caffrey, P., (2011): Labile point in mutant amphotericin polyketidesynthases. Biotechnology Letters, 33: 1121-1126.

[16] Loboda, M.I., Voichuk, S.I., Biliavska, L.O., (2019): Correlation dependence of the antibiotic compounds biosynthesis and other biologically active substances in soil streptomycetes. Mikrobiolohichnyi Zhurnal, 81: 36-47.

[17] Morris, R.O., Blevins, D.G., Dietrich, J.T., Durley, R.C., Gelvin, S.B., Gray, J., Hommes, N.G., Kaminek, M., Mathews, L.J., Meilan, R., Reinbott, T.M., Sayavedra-Soto, L., (1993): Cytokinins in plant pathogenic bacteria and developing cereal grains. Functional Plant Biology, 20: 621-637.

[18] Nordstrom, A., Tarkowski, P., Tarkowska, D., Norbaek, R., Astot, C., Dolezal, K., Sandberg, G., (2004): Auxin regulation of cytokinin biosynthesis in *Arabidopsis thaliana*: a factor of potential importance for auxin-cytokinin-regulated development. Proceedings of the National Academy of Sciences of the United States of America, 101(21): 8039-8044.

[19] Patten, C.L., Blakney, A.J., Coulson, T.J., (2013): Activity, distribution and function of indole-3-acetic acid biosynthetic pathways in bacteria. Critical Reviews in Microbiology, 39: 395-415.

[20] Patten, C.L., Glick, B.R., (1996): Bacterial biosynthesis of indole-3-acetic acid. Canadian Journal of Microbiology, 42: 207-220.

[21] Risdian, C., Mozef, T., Wink, J., (2019): Biosynthesis of polyketides in Streptomyces. Microorganisms, 7: 124-142.

[22] Savinsky, S.V., Dragovoz, I.V., Pedchenko, V.K., (1991): Determination of zeatin, indolyl-3-acetic and abscisic acids from one plant sample using high-performance liquid chromatography. Physiology and biochemistry of cultural plants, 23: 611-618.

[23] Semenow, S.M., (1990): Laboratory media for actinomycetes and fungi. Directory. Moscow, Agroprom Publishing House, pp.140-148.

[24] Solans, M., Vobis, G., Cassan, F., Luna, M., Wall, L., (2011): Production of phytohormones by root-associated saprophytic actinomycetes isolated from the actinorhizal plant *Ochetophila trinervis*. World Journal of Microbiology and Biotechnology, 27: 2195-2202.

[25] Wang, W., Oldfield, E., (2014): Bioorganometallic chemistry with IspG and IspH: structure, function, and inhibition of the Fe_4S_4 proteins involved in isoprenoid biosynthesis. Angewandte Chemie International Edition, 53: 4294-4310.

Received: June 21, 2020
Accepted: September 3, 2020
Published Online: September 7, 2020
Analele Universităţii din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House