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T.P. PIROG[1,2*], D.V. PIATETSKA[1], N.O. KLYMENKO[1], H.O. IUTYNSKA[2]

[1] The National University of Food Technologies,
68 Volodymyrska Str., Kyiv, 01601, Ukraine

[2] Zabolotny Institute of Microbiology and Virology, NAS of Ukraine,
154 Akademika Zabolotnoho Str., Kyiv, 03143, Ukraine

* Author for correspondence; e-mail: tapirog@nuft.edu.ua

WAYS OF AUXIN BIOSYNTHESIS IN MICROORGANISMS

Among plant hormones, auxins, in particular indole-3-acetic acid (IAA), are the most studied and researched. Almost all groups of soil microorganisms, both plant-associated and non-plant-associated bacteria, fungi, and phytopathogenic microorganisms are capable of producing auxins. The development of preparations for crop production is directly related to the production of bacterial strains with high auxin-synthesizing potential, which is possible only with a full understanding of the ways of regulation and synthesis of auxins in bacteria. The synthesis of auxins in microorganisms can take place in two ways: by the gradual conversion of tryptophan to IAA (tryptophan-dependent pathway) or by the use of other intermediates (tryptophan-independent pathway). The latter is poorly clarified, and in the literature available today, there is only a small amount of information on the functioning of this pathway in microorganisms. The review presents literature data on the ways of auxin biosynthesis in different groups of microorganisms, as well as approaches to the intensification of indole-3-acetic acid synthesis. The formation of IAA from tryptophan can be carried out in the following ways: through indole-3-pyruvate, through indole-3-acetamide, and through indole-3-acetonitrile. The vast majority of available publications are related to the assimilation of tryptophan through the formation of indole-3-pyruvate as this pathway is the most common among microorganisms. Thus, it functions in rhizospheric, symbiotic, endophytic, and free-living bacteria. The concentration of synthesized IAA among natural strains is in the range from 260 to 1130 μg/*mL. Microorganisms in which the indole-3-acetamide pathway functions are characterized by lower auxin-synthesizing ability compared to those that assimilate tryptophan through indole-3-pyruvate. These include bacteria of the genera Streptomyces, Pseudomonas, and Bradyrhizobium and fungi of the genus Fusarium. The level of synthesis of IAA in such microorganisms is from* $1.17 \cdot 10^{-4}$ *to 255.6* μg/*mL. To date, only two strains that assimilate tryptophan via the indole-3-acetonitrile pathway and form up to 31.5* μg/*mL IAA have been described in the available literature. To intensify the synthesis of indole-3-acetic acid, researchers use two main approaches: the first consists in introducing into the culture medium of exogenous precursors of biosynthesis (usually tryptophan, less often indole-3-pyruvate, indole-3-acetamide, and indole-3-acetonitrile); the second — in increasing the expression of the corresponding genes and creating recom-*

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binant strains-supersynthetics of IAA. The largest number of publications is devoted to increasing the synthesis of IAA in the presence of biosynthesis precursors. Depending on the type of bacteria, the composition of the nutrient medium, and the amount of exogenously introduced precursor, the synthesis of the final product was increased by 1.2—27 times compared to that before the intensification. Thus, in the presence of 11 g/L tryptophan, Enterobacter sp. DMKU-RP206 synthesized 5.56 g/L, while in a medium without the precursor, it yielded only 0.45 g/L IAA. Recombinant strains Corynebacterium glutamicum ATCC 13032 and Escherichia coli MG165 formed 7.1 and 7.3 g/L IAA, respectively, when tryptophan (10 g/L) was added to the culture medium. The level of auxin synthesis in microorganisms may be increased under stress conditions (temperature, pH, biotic and abiotic stress factors), but in this case, the IAA concentration does not exceed 100 mg/L, and therefore this method of intensification cannot compete with the others above.

Keywords: *tryptophan, indole-3-pyruvate, indole-3-acetamide, indole-3-acetonitrile, indole-3-acetic acid, intensification of phytohormone synthesis.*

In the current conditions, the use of phytohormonal drugs in agriculture, crop production, and forestry is becoming increasingly important. This is due to the prospects of fuller realization of the genetic potential of crops by increasing the resistance of plants to stressors of biotic and abiotic nature, as well as by increasing and improving crop quality [1].

One of the most common and well-studied natural hormones is auxin. Biologically active substances with growth-stimulating properties in plants appeared more than a hundred years ago [2]. In particular, in 1924—1928 the Ukrainian scientist M.H. Kholodnyi for the first time isolated indole-3-acetic acid from the roots, stems, and leaves of plants. It was the first auxin later called plant growth hormone.

In addition to plants, both plant-associated microorganisms [3—11] and phytopathogenic bacteria [12—15], as well as fungi [16—18], are able to form IAA in high enough concentrations. Bacterial IAA stimulates the stretching of cells in the coleoptiles and shoots of the plant, activates the division of cambium cells, accelerates fruit set, and promotes root formation, increasing the number and length of additional and main roots.

However, in some cases, the ability to synthesize IAA is a factor in the pathogenicity of bacteria and fungi against plants because at high concentrations of IAA, uncontrolled plant growth begins with subsequent damage to integumentary tissues, resulting in phytopathogens penetrating into the host plant through the formed gaps [19].

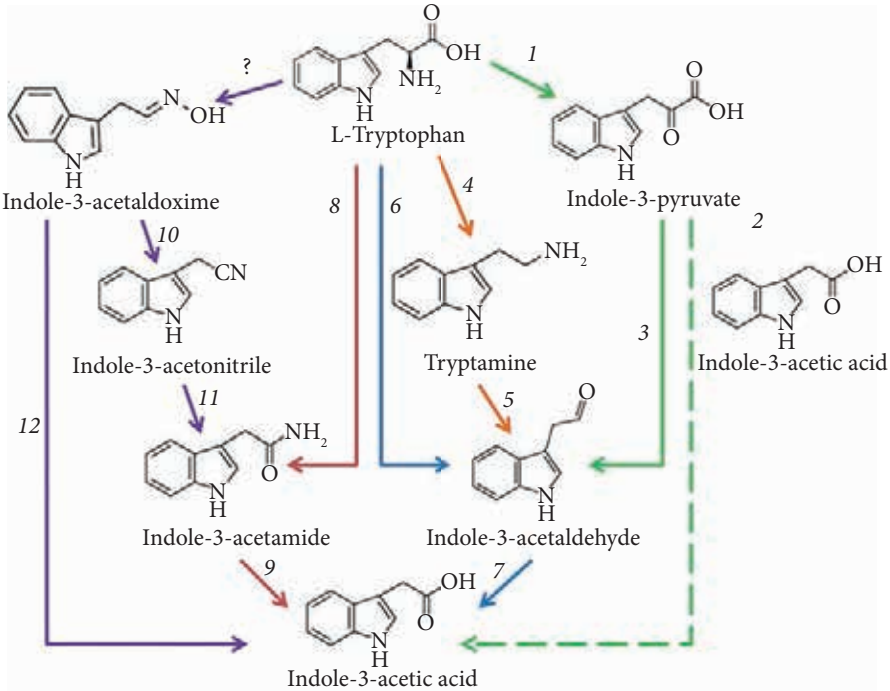
It is known from the literature [20] that the main precursor of IAA synthesis is heterocyclic amino acid L-tryptophan, so the synthesis of auxins can take place in two ways: by stepwise conversion of tryptophan to IAA (tryptophan-dependent pathway) or by using other intermediates such as acetonitrile (tryptophan-independent pathway). Note that the latter is poorly studied, and in the literature available today there is no information on the functioning of this pathway in microorganisms. The development of plant-based products is directly related to the production of bacterial strains with high IAA-synthesizing potential, which is possible only with a full understanding of the ways of regulation and synthesis of auxins in bacteria.

In connection with the above, the aim of the review is to summarize the current literature on the ways of auxin biosynthesis by different groups of microorganisms and ways to intensify the synthesis of these phytohormones.

Tryptophan-dependent pathway of auxin biosynthesis. Depending on the formed intermediates, there are three options for the assimilation of tryptophan in microorganisms, namely indole-3-pyruvate (IP), indole-3-acetamide (IAM), and indole-3-acetonitrile (IAN) pathways [19].

The scheme of the tryptophan-dependent pathway of IAA synthesis in microorganisms is shown in Figure.

Indole-3-pyruvate pathway. The IP pathway is the main pathway for the synthesis of indole-



Ways of IAA synthesis in bacteria. Green, orange and blue lines — IP pathway of synthesis of IAA, red — IAM pathway, purple — IAN pathway. The dashed lines indicate non-enzymatic reactions. Enzymes: *1* — tryptophan aminotransferase; *2* — indolyl lactate dehydrogenase; *3* — indolyl-3-pyruvate decarboxylase; *4* — tryptophan decarboxylase; *5* — aminoxidase; *6* — tryptophan side chain oxidase; *7* — indolyl-3-acetaldehyde oxidase; *8* — tryptophan-2-monooxygenase; *9* — indlylacetamine hydrolase; *10* — acetaldoxime dehydratase; *11* — nitrile hydratase; *12* — nitrilase; the question mark indicates an unknown enzyme

acetic acid, which functions in both plants and bacteria.

In the first stage, L-tryptophan is deaminated to IP by the enzyme tryptophan aminotransferase (EC 2.6.1.99), then indolyl-3-pyruvate decarboxylase (EC 4.1.1.74), a key enzyme of the IP pathway, converts IP into indole-3-acetaldehyde, which in turn is oxidized to IAA by indolyl-3-acetaldehyde oxidase (EC 1.2.3.7) [21, 22].

The largest group of bacteria with high auxin-synthesizing ability is plant- growth-promoting rhizobacteria (PGPR). This is primarily due to the fact that PGPR inhabit the rhizosphere of plants and are in close contact with the host plant. That is why the synthesis of IAA by this group of bacteria plays an important role in the bacteria-plant relationship [3]. The PGPR group includes symbiotic as well as rhizosphere and free-living microorganisms.

Symbiotic bacteria. Such microorganisms are characterized by the ability to form a mutually beneficial symbiosis with the host plant [3].

Imada et al. [3] found that the symbiotic nitrogen-fixing strain *Rhizobium tropici* CIAT 899 forms 33.03 μg/mL IAA in the presence of tryptophan in the culture medium. The *y4wE*, *lao*, and *iorA* genes were identified by analysis of the *R. tropici* CIAT 899 genome, indicating the possible involvement of these genes in the IAA biosynthesis via IP. In addition, when the strain was grown in the presence of tryptophan, the transcriptional activity of the *y4wE, lao*, and *iorA* genes increased by 1.33, 1.45, and 2.21-fold, respectively, compared to the culture grown in the absence of precursor.

Rhizospheric, phyllospheric, and endophytic bacteria. In bacteria of the genus *Enterobacter*,

the functioning of the IAA biosynthesis pathway has been established in [7, 25].

Scientists from India [7] have isolated three strains of *Enterobacter* sp. bacteria from the rhizosphere of *Abrus precatorius*: A27CK, A3CK, and A7CK. In the study of the effect of carbon and nitrogen sources on the auxin-synthesizing ability of strains, the maximum concentration of IAA was achieved by culturing A3CK on a medium with 0.5% mannitol and 0.2% L-asparagine, which is 1.7 times more than when growing this strain on a basic medium without additional introduction of mannitol and the mentioned above essential amino acid.

Another study [25] described a new strain of *Enterobacter xiangfangensis* BHW6 isolated from the rhizosphere of maize, in which the *ipdC* sequence encoding a key enzyme of the IP pathway was detected. Upon introducing 15 g/L tryptophan into the culture medium of strain BHW6, the level of IAA synthesis was 1.13 mg/mL.

In 2018, a paper was published [10], in which scientists described a method of regulating IAA synthesis by introducing into the culture medium of *Azospirillum brasilense* Sp245, *A. brasilense* Az39, and A. *brasilense* Cd aromatic and linear amino acids. It was also found that the studied strains are able to synthesize IAA.

In [8], the authors noted that the endophytic strain *Gluconacetobacter diazotrophicus* PAL5, which colonizes the roots and stems of sugar cane, forms IAA through the IP pathway. This was determined by the creation of two mutant strains Gdiaa34 and Gdiaa01, which due to the mutation did not form IP, indole-3-lactate (intermediates of the IP pathway), and IAA. In contrast, when growing wild strain PAL5 on a medium with 0.1 g/L tryptophan, the total concentration of indole compounds (IAA, IP, and indole-3-lactate) was 117.7 μg/mL.

In 2018, Estenson et al. devoted their work [9] to the study of the synthesis of IAA using the rhizosphere strain of *Pantoea* sp. YR343, which was isolated from the root zone of the *Populus deltoides*. Genome analysis of strain YR343 showed that in the presence of 1 mM of tryptophan, 7 transferases of aromatic amino acids and 17 aldehyde dehydrogenases, which catalyze the conversion of tryptophan to IAA through IP, were active. Unfortunately, the authors did not determine the concentration of synthesized phytohormones.

Scientists from Taiwan [4] found that the conditionally pathogenic strain *Acinetobacter baumanii* ATCC19606 produces up to 6.5 μM of IAA in the presence of 1 g/L tryptophan. In addition, other compounds of the indole structure have been identified in the culture broth: indole-3-lactate and indole-3-ethanol, which are intermediates of the IP pathway of IAA synthesis. To confirm the functioning of the IP pathway, the researchers determined the expression of the *ipdC* and *iacH* genes, responsible for the formation of enzymes in this pathway. Their activity was increased by 208 and 61 times, respectively, in the presence of tryptophan in the medium compared to the medium containing pyruvate.

The *Pantoea agglomerans* C1 strain was isolated from the lettuce phyllosphere and its genome was analyzed for the presence of enzymes involved in IAA biosynthesis (11 sequences) [26]. The analysis revealed that of all the analyzed sequences, there are only 3 that are responsible for the synthesis of enzymes of the IP pathway. The researchers optimized the cultivation conditions of the strain, in particular, by selecting the composition of the nutrient medium, the carbon source, and the physiological state of the inoculum.

Pan et al. [27] performed complete sequencing of the *Mycobacterium* Mya zh01 genome and identified 7 gene sequences encoding genes associated with IAA biosynthesis. Among them, 3 indicate the probable functioning of the IP pathway, namely IP monooxygenase, IP decarboxylase, and aldehyde dehydrogenase. Due to the formation of 16.18 μg/mL IAA, strain Mya zh01 had a positive effect on plant growth and seed germination.

Note that such studies are necessary to understand the unique regulatory mechanisms of phytohormone synthesis and can be the basis for the development of technology for their industrial production.

Indole-3-acetamide pathway. Another tryptophan-dependent route of synthesis of IAA is IAM. This pathway is less common because it is characteristic of some symbiotic microorganisms [28], associative ones [29—32], and the vast majority of phytopathogens [13, 15, 16, 33, 34].

Symbiotic nitrogen-fixing bacteria. In [28], scientists studied the ability of nitrogen-fixing bacteria *Bradyrhizobium japonicum* E109 and *Bradyrhizobium elkanii* 5019 to form IAA, but the concentrations of synthesized IAA were extremely low. The absence in the genome of bacteria of the genetic sequence *ipdC*, which encodes a key enzyme of the IP pathway, and the presence of IAM (0.3—0.55 nmol/L) in the culture broth indicates the functioning of the IAM pathway of indole-acetic acid biosynthesis.

Rhizospheric and endophytic microorganisms. In 2008, the strain *Pseudomonas fluorescence* Psd was isolated from the rhizosphere of black pea (*Vigna mungo*) [29]. Later the same scientists [30] established the ability of the *P. fluorescence* Psd, in addition to antibiotics, to synthesize phytohormones, in particular IAA. In their further research [34], Kochar et al. found that the strain of *P. fluorescence* Psd synthesizes IAA through the IAM pathway. Due to the excessive expression of tryptophan monooxygenase (*iaaM*) and IAM hydrolase (*iaaH*) genes, involved in the IAM pathway, the Psd strain produced 13 times more IAA, which also had a detrimental effect on the sorghum root development.

In [32, 33], researchers found the presence in the genome of microorganisms of the active genetic sequences *iaaM* and *iaaH* responsible for the synthesis of key enzymes of the IAM pathway, namely tryptophan 2-monooxygenase and amidase, respectively. Lin et al. [32] isolated the strain *Streptomyces* En1 from *Taxus chinensis*, which formed IAA by culturing in trypton-soy broth in the presence of 5 mM tryptophan.

In 2019, a paper was published [33] in which the authors investigated the ability of *Streptomyces* sp. NEAU-S7GS2, isolated from the rhizosphere and roots of soybean (*Glycine max*), to IAA synthesis, as well as its positive effect on plant growth. Under conditions of growth of the strain on a medium with 1 g/L tryptophan, it formed up to 17.5 μg/mL IAA. Thus, during the pre-sowing treatment of corn seeds with culture broth of strain NEAU-S7GS2, the root length , its fresh and dry weight increased by 55.9, 107.9, and 151.7%, respectively. The authors note that the results indicate the prospects for the use of *Streptomyces* sp. NEAU-S7GS2 in the composition of preparations for crop production.

Phytopathogenic microorganisms. A large group of microorganisms that synthesize IAA through the IAM pathway is actinobacteria of the genus *Streptomyces* [32, 33, 35, 37] including strains *Streptomyces violaceus* 44 and *Streptomyces griseus* 20, isolated from potato scab, as well as phytopathogens *Streptomyces scabies* RL 840170 and *Streptomyces exfoliatus* RL 830103, which cause cortical lesions on potato tubers (*Solanum tuberosum*) [35]. In addition, the synthesis of IAA through IAM occurs among the genera *Agrobacterium* [15], *Pseudomonas* [13], and also in microscopic fungi of the genus *Fusarium* [16].

In the review [37], scientists summarized the available information from the beginning of the twentieth century until 2016 on the properties of metabolites synthesized by streptomycetes and their application in agriculture. Analysis of the genome of potato scab pathogen *Streptomyces scabiei* showed the presence of coding sequences *iaaM*/*iaaH*, the participation of which in the biosynthesis of IAA was confirmed by the gene destruction [17].

In [13], it was found that the causative agent of nodular disease of European olive (*Olea europaea*) *Pseudomonas savastanoi* pv. *savastanoi*

NCPPB 3335 formed up to 5.56 mg IAA/g DB under culturing on tryptone. With the removal of the *iaaM*/*iaaH* operon, the level of IAA synthesis decreased by 40 times, indicating the role of the IAM pathway in the synthesis of auxins in strain NCPPB 3335. Note that this strain is one of the most promising producers of IAA among all the above bacteria because it forms IAA of high concentrations without the additional introduction of such synthesis precursors as tryptophan or IAM into the culture medium.

Tsavkelova et al. [16] studied the synthesis of IAA among members of the genus *Fusarium*. It was found that the highest concentration of IAA was achieved by culturing the strain *Fusarium proliferatum* ET1 on sucrose in the presence of tryptophan. Also, the scientists constructed a recombinant strain of *Fusarium fujikuroi* #T2, which synthesized 255.6 mg/L IAA, by transferring *iaaM* and *iaaH* genes from *F. proliferatum* ET1.

Analysis of the literature showed that the IAM pathway for the synthesis of IAA is found in plant-associated bacteria (symbiotic nitrogen-fixing, rhizospheric, endophytic), as well as in phytopathogenic microorganisms. To date, this pathway of auxin biosynthesis is well studied, as evidenced by a sufficient amount of work on the analysis of the genome of IAA-synthesizing microorganisms, as well as attempts to regulate IAA synthesis and the creation of recombinant strains of supersynthetics.

Synthesis of IAA through indole-3-acetonitrile. The synthesis of IAA through IAN is characteristic mainly for higher plants, but a small number of reports [11, 18] are devoted to the biosynthesis of IAA through IAN by microorganisms.

The first report [18] on the synthesis of IAA via the IAN pathway by *Fusarium* spp. appeared in 2016. The maximum concentration of IAA was achieved by growing the strain *Fusarium graminearum* DAOM 233423 on a medium with carboxymethylcellulose in the presence of 0.2 mM IAN as a precursor of the synthesis.

In [11] Sun et al. found that the rhizospheric strain *Variovorax boronicumulans* CGMCC 4969 synthesized 0.26 g/L IAA in the presence of 0.1 g/L IAN. The scientists also established that the precursor of IAA synthesis in *V. boronicumulans* CGMCC 4969 is IAN, and the synthesis occurs through IAM.

To date, there are only a few reports on the functioning of the IAN pathway in microorganisms, which indicates a lack of study. Therefore, further studies of the IAN pathway will provide a better understanding of the synthesis stages and mechanisms of IAA regulation.

Genetically modified IAA producers. To date, the most widely used methods for the construction of organisms with the necessary properties are genetic modification. Obtaining IAA overproducers is no exception.

IP pathway. Three enzymes are required for the biosynthesis of IAA from L-tryptophan via the IP pathway: aminotransferase, which converts L-tryptophan to indole-3-pyruvic acid, IP decarboxylase, which performs decarboxylation of IP to indole-3-acetaldehyde, and indole-3-acetate dehydrogenase, which is responsible for the oxidation of indole-3-acetaldehyde to IAA. In [23, 38], the original strains were not capable of synthesizing phytohormones of auxin nature, so the IP route of IAA synthesis in the genome of recipients was created *de novo* by inserting genes encoding the corresponding enzymes.

Guo et al. [23] created a genetically modified strain of *Escherichia coli* DG121, which formed up to 387 mg/L IAA in the presence of 0.5 g/L tryptophan. The process of genetic modification consisted of three gene products: ARO8 aminotransferase from *Saccharomyces cerevisiae*, KDC decarboxylase, and *E. coli aldH* gene sequence.

The *aspC, ipdC,* and *iad1* genes from *Escherichia coli*, *Enterobacter cloacae,* and *Ustilago maydis*, which were expressed under the control of the *tac, ilvC,* and *sod* promoters, were used to establish the biological pathway of IAA biosynthesis in *C. glutamicum* ATCC 13032 [38]. The

final genetically engineered strain when cultured on tryptone in the presence of 10 g/L tryptophan formed 2.3 g/L IAA in a flask and 7.3 g/L IAA in a five-liter fermenter.

IAM pathway. In 2020, Wu et al. [39] developed and incorporated two pathways of IAA biosynthesis in *E. coli* MG165 using the whole-cell catalysis via tryptamine and via IAM. Thus, when culturing the MIA-6 strain by the IAM pathway on a medium with glucose and 10 g/L tryptophan, IAA synthesis was achieved at the level of 7.1 g/L. At the same time, as for the MTAI-5 strain, in which the TAM pathway functioned, the concentration of IAA was 98.4 times lower (<0.1 g/L).

Therefore, the following conclusions can be drawn from the works described above. Firstly, the vast majority of soil bacteria are characterized by the IP route of IAA synthesis. Secondly, it is possible to regulate the synthesis of auxins at the level of expression of genes responsible for the formation of necessary enzymes.

Generalized data on the IAA producers are given in Table 1. They indicate that high concentrations of IAA (from 260 to 1130 μg/mL among natural strains) are formed by microorganisms in which tryptophan is involved in metabolism through IP. At the same time, the genetic modification of bacteria makes it possible to obtain supersynthetic strains that produce up to 7.3 g/L IAA [38]. The IAM and IAN pathways are less common and mostly function in endophytic and phytopathogenic microorganisms.

Introduction of biosynthesis precursor into the medium. One of the first works to intensify the synthesis of auxins by introducing a precursor of biosynthesis into the culture medium was published by Belgian scientists Costacurta and Vanderleyden in 1995 [40]. However, the issue of intensification of phytohormones still remains relevant, so we focus on publications in recent years.

Over 2013—2019, information appeared on the synthesis of auxins by both associated [42—45] and non-associated [12, 46] with plant bacteria.

Intensification of IAA synthesis in plant-associated bacteria. Phytohormones synthesized by associative microorganisms are functionally equivalent to hormones synthesized by the plant itself, which was the reason for the interest of scientists from around the world in IAA producers.

Rhizosphere microorganisms. The largest number of publications [42—45, 49, 51, 52] is devoted to rhizosphere bacteria that synthesize phytohormones, in particular IAA.

Wagi and Ahmed [49] found that the isolated from the rhizosphere of *Solanum nigrum* strain *Bacillus cereus* So3II b yielded on a tryptophan-free medium 23.3 mg/L IAA, while added with a precursor, it formed three times more concentrated IAA — 70.0 mg/L. The concentration of tryptophan, which provided the synthesis of such an amount of auxins, was not reported by the authors, but they used a precursor in a sufficiently high amount (5—40 mg/mL). Note that, in contrast to the strain *S. fradiae* NKZ-259 [46], *B. cereus* So3II was characterized by a significantly higher level of IAA synthesis, even under conditions of growth on a medium without a precursor.

In [50], it was noted that the concentration of IAA synthesized by *Bacillus subtilis* DR2 (associated with *Eragrostis cynosuroides*) was increased by almost 1.2 times in the presence of 1.2 g/L tryptophan and using trypton as a source of carbon.

A strain of *Bacillus* sp. JH 2-2 was isolated from the heavy-metal-contaminated rhizosphere of plants growing near an abandoned mine [52]. In the presence of 0.5 g/L tryptophan, the strain formed 5.9 μg/mL IAA, while in the absence of this amino acid in the culture medium, auxins were not detected.

Among all analyzed species of the genus *Bacillus*, the highest level of tryptophan transformation in IAA was achieved under the cultivation of strain JH 2-2, because with the addition of a small amount of precursor, the authors practically started the synthesis of auxins.

Naveed et al. [51] found that the concentration of IAA synthesized by *Burkholderia phy-*

Table 1. **Synthesis of IAA by different groups of microorganisms**

Producer	Group of microorganisms	Carbon source	Concentration of tryptophan, g/L	Concentration of IAA, μg/mL	Literature
IP pathway					
Rhizobium tropici CIAT 899	Symbiotic	Dextrose	0.1	33.03	[3]
Enterobacter sp. A3CK	Rhizospheric	Mannitol L-asparagine	1.0	430.0	[7]
Enterobacter xiangfangensis BHW6	Rhizospheric	Trypton	15	1130.0	[25]
Gluconacetobacter diazotrophicus PAL5	Rhizospheric	Glucose Glycerol	0.1	13.1	[8]
Pantoea sp. YR343	Rhizospheric	Glucose	0.2	n/d	[9]
Azospirillum brasilense Sp245	Rhizospheric	Glucose	0.1	5.73	[10]
Azospirillum brasilense Az39		Malate		6.09	
Azospirillum brasilense Cd				6.27	
Acinetobacter baumanii	Rhizospheric	Glycerol	1.0	1.14	[4]
Pantoea agglomerans C1	Phyllospheric	Saccharose	0.8	263.33	[26]
Mycobacterium Myazh01	Endophytes	Potato flour	0.2	16.18	[27]
Escherichia coli DG121	Free-living	Glucose	0.5	387.0	[23]
Corynebacterium glutamicum ATCC 13032	Free-living	Trypton	10.0	7300	[38]
IAM pathway					
Bradyrhizobium japonicum E109	Symbiotic	Mannitol	0.01	$1.17 \cdot 10^{-4}$	[28]
Bradyrhizobium elkanii 5019		Mannitol	0.01	$2.65 \cdot 10^{-4}$	
Pseudomonas fluorescence Psd	Rhizospheric	Succinate	1.0	80 μg IAA/OD[a]	[30]
Streptomyces sp. En1	Endophytes	Trypton	1.0	n/d	[32]
Streptomyces sp. NEAU-S7GS2	Endophytes	Trypton	1.0	17.5	[33]
Escherichia coli MG165	Free-living	Glucose	10.0	7.1	[39]
Streptomyces scabiei	Phytopathogens	Cellobiose	2.0	1.1 mg/g DB[b]	[17]
Pseudomonas savastanoi pv. *savastanoi* NCPPB 3335	Phytopathogens	Trypton	—	5.56 mg/g DB[b]	[13]
Fusarium proliferatum ET1	Phytopathogens	Saccharose	0.8	13.8	[16]
Fusarium fujikuroi #T2	Phytopathogens	Saccharose	0.8	255.6	[16]
IAN pathway					
Fusarium graminearum DAOM 233423	Phytopathogens	Carboxymethyl cellulose	—	31.5	[18]
Variovorax boronicumulans CGMCC 4969	Rhizospheric	Trypton	—	$2.61 \cdot 10^{5}$	[11]

[a] — optical density (OD); [b] — dry biomass (DB); n/d — the IAA concentration was not determined; — — tryptophan was not introduced.

tofirmans PsJN increased by 14 times with the introduction of tryptophan into the culture medium (concentration not specified).

In [42—44], the authors did not provide data on the synthesis of IAA by rhizosphere microorganisms in the absence of tryptophan. Therefore, it remains unknown to what extent the synthesis of this auxin was intensified.

Jeyanthi and Ganesh [42] reported that when 0.5 g/L tryptophan was introduced into the culture medium of *Pseudomonas fluorescence* sp., the concentration of auxins reached its maximum in the stationary phase of growth (58 μg/mL). It was also noted that the increase in the concentration of biosynthesis precursor to 0.6 g/L reduced the concentration of auxins by 8 μg/mL.

The authors [43] noted that the rhizosphere strain *Enterobacter cloacae* SN19 (isolated from the tropical legume *Teramnus labialis* (L.f.) Spreng) in the presence of 1 g/L tryptophan synthesized IAA with a concentration of 382.23 μg/mL.

Ozdal et al. [44] isolated the strain *Arthrobacter agilis* A17 from the rhizosphere of *Verbascum vulcanicum,* and it synthesized 75 mg/L IAA in the presence of 1 g/L tryptophan. These researchers also immobilized *A. agilis* A17 cells in the alginate layer, which increased the IAA concentration to 520 mg/L.

In 2020, Lebrazi et al. optimized the cultivation conditions for four rhizosphere bacteria (*Phyllobacterium* sp., *Bacillus* sp., *Agrobacterium* sp., and *Rhizobium* sp.) to obtain maximum IAA synthesis. It was determined that 2 g/L of tryptophan should be in the medium with mannitol to achieve maximum IAA synthesis [53].

Endophytes. Scientists from Thailand [54] isolated from the roots of *Dendrobium pulchellum* an unidentified strain number DPY-05, which formed IAA with a concentration of 67.18 μg/mL, which is almost 27 times higher than that without the introduction of the biosynthesis precursor.

Phyllospheric bacteria. The strain *Enterobacter* sp. DMKU-RP206 isolated from the surface of rice leaves [45] synthesizes, 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. Although the strain DMKU-RP206 is the most efficient amongst all described above [42—44, 49, 51, 52], we think that the presence of such a high amount of precursor in the cultivation medium is economically unprofitable. But it should be noted that even under cultivation without tryptophan, the IAA concentration is still pretty high (0.415 g/L) because *Enterobacter* sp. DMKU-RP206 is a more promising producer than other prokaryotes.

Intensification of IAA synthesis by microorganisms not associated with plants. The ability to synthesize phytohormones has also been established among free-living bacteria that are not directly involved in plant life [12, 46].

In [12], researchers noted that the causative agent of bacterial spot of plants *P. syringae* pv. *tomato* DC3000 synthesizes up to 2.76 μg/L IAA in the presence of 0.25 mM tryptophan in the culture medium. In particular, the introduction into the culture medium of 0.25 mM of IP instead of tryptophan helped to increase the concentration of IAA to 14.1 μg/L.

Also, Mon Myo et al. [46] found that the producer of neomycin *Streptomyces fradiae* NKZ-259 synthesized on a tryptophan-free medium 4.876 mg/L IAA, while in the case of tryptophan introduction, the level of synthesis increased by 20 times.

In our opinion, given the mode of existence and relatively low concentration of IAA, the synthesis of this class of phytohormones in the microorganisms described above [12, 46] may be one of the means of bacterial protection under adverse environmental conditions. In particular, in another study [9], researchers noted that the presence of exogenous IAA contributed to cell growth, and the presence of endogenous IAA — to their survival under stress.

The effect of tryptophan on IAA synthesis in eukaryotes. The ways of IAA synthesis in bacteria are the most studied, but only a small amount

of work is devoted to the study of the phytohormone formation in fungi and yeast [55—58].

In [55], the authors noted that the yeast strain *Rhodosporidium paludigenum* DMKURP301, isolated from rice phyllosphere, formed up to 1.33 g/L IAA with the addition of tryptophan. At the same time, in the absence of the precursor in the culture medium, IAA was not detected. Fu et al. have reported [56] that eight isolated from the phyllosphere of sundew strains *Aureobasidium pullulans* synthesized 56—610 mg/L IAA in the presence of 1 g/L tryptophan. The obtained values were almost three times the ones in the absence of the precursor. Strain *Rhodosporidiobolus fluvialis* DMCU-CP293, isolated from maize phylloplan, on a peptone-dextrose medium (YPD) synthesized 1.062 g/L IAA in the presence of tryptophan, while without it auxins were not found [57].

Analyzing the literature, we can conclude that eukaryotes synthesize significantly higher amounts of IAA than bacteria [55, 57, 58]. The exception is the strain *Enterobacter* sp. DMKU-RP206 [45], which forms several g/L IAA, but this can be explained by the presence in the environment of a very high concentration of tryptophan (11 g/L). In our opinion, the supersynthesis of IAA by eukaryotes is related to the morphology of yeast and fungal cells. The presence of a nucleus and a developed synthetic apparatus makes it pos-

Table 2. **The effect of tryptophan on the synthesis of IAA**

Microorganism-producer	Carbon source	Content of tryptophan in the culture medium, g/L	Concentration of IAA, μg/mL		Literature
			Before intensification	After intensification	
Plant-associated microorganisms					
Bacillus cereus So3II b	Glucose	30.0	23.3	70.0	[49]
Strain DPY-05	Peptone Glycerol	0.5	2.5	67.18	[54]
Bacillus subtilis DR2	Trypton	1.2	100.3	168.1	[48]
Enterobacter sp. DMKU-RP206	Lactose	11.0	415	**5560**	[45]
Rhizobium sp.	Mannitol	2.0	0.09	0.116	[53]
Bacillus sp. JH 2-2	Sucrose	0.5	0	5.9	[52]
Microorganisms not associated with plants					
Streptomyces fradiae NKZ-259	Starch	2.0	4.876	82.363	[46]
Pseudomonas syringae pv. *tomato* DC3000	Citrate	0.05	0.03	2.76	[12]
Eukaryotes					
Strains *Aureobasidium pullulans*	Peptone Dextrose	1.0	9—236	56—610	[56]
Rhodosporidiobolus fluvialis DMCU-CP293	Peptone Dextrose	1.0	0	1062	[57]
Rhodosporidium paludigenum DMKURP301	Sucrose	5.0	0	1330	[55]

sible to form more enzymes for the biosynthesis of secondary metabolites, which in turn leads to the formation of high concentrations of auxins.

Generalized data on the intensification of IAA synthesis in microorganisms are given in Table 2. It shows that the highest concentration of IAA (5.56 g/L) is achieved by the strain *Enterobacter* sp. DMKU-RP206 with introduced tryptophan. The obtained results of IAA synthesis make it possible to consider the above microorganisms as promising producers of auxins for the development of respective technology.

Influence of other factors on IAA synthesis in bacteria. Abiotic and biotic stressors, as well as plant signaling molecules that are associated with plant responses to adverse conditions, can affect the growth of plant-associated bacteria and their ability to synthesize IAA [5]. This is primarily due to the fact that one of the IAA functions is its impact on root growth and differentiation, increasing its permeability to nutrients and water, as well as participation in the formation of resistance to adverse conditions, which is an important factor in drought, osmotic, and heat shocks [59].

Plant signaling molecules include abscisic, salicylic, and jasmonic acids, which are synthesized by plants in response mainly to abiotic stress factors (drought, salinity, high temperature, and the presence of heavy metals) [59, 60]. Among the mediators of biotic stress, a large group is one of the protein NPR1 (non-expressor of pathogenesis-related genes1), which is formed in small quantities in phytopathogenically infected plant tissues and triggers the mechanism of synthesis of PR (pathogenesis-related) proteins (chitinases, glucanases, and protease inhibitors) [60].

It is known from the literature that for some rhizospheric bacteria, increased auxin synthesis is observed under adverse environmental conditions (pH, temperature, high salinity), as well as in the presence of IAA antagonists such as salicylic and jasmonic acids [5, 6, 47, 48].

In [47], the authors found the effect of pH change on the synthesis of IAA in PGPR strains (CA1001, CA2003, and CA2004) isolated from the rhizosphere of stevia honey. The concentration of IAA synthesized by strain CA1001 increased from 65 μg/mL to 91.7 μg/mL with changing pH of the culture medium from 7 to 9, respectively.

Hasuty et al. [48] found that the strain *Serratia marcescens* subsp. *marcescens* KB05 synthesizes the maximum concentration of IAA under stress (pH 9.0, t = 40 °C) in the presence of tryptophan.

Table 3. **Increased auxin synthesis under stress**

Microorganism-producer	Carbon source	Cultivation conditions	Concentration of IAA, μg/mL		Literature
			No stress	Under stress	
Strain CA1001	Peptone	pH 9.0	65	91.7	[47]
Serratia marcescens subsp. *marcescens* KB05	Tryptone	pH 9.0, t=40 °C	7	56.6	[48]
Azospirillum brasilense Sp245 (pFAJ64)	Malate	0.02 g/L of abscisic acid	4.71	5.29	[5]
		0.01% chitosan	5.65	6.34	
Azospirillum brasilense Az39 (pFAJ64)	Malate	0.02 g/L abscisic acid	4.87	5.47	[5]
		4% supernatant of *F. oxysporum*	5.98	6.21	
		0.01% chitosan	5.98	6.208	
Serratia sp. ZM	Glucose	0.2 g/L $CO(NH_2)_2$	n/d*	82.0	[6]

*n/d — the IAA concentration was not determined.

The results obtained were 8 times higher than those under conditions of growing this strain at pH 7.0 and a temperature of 30 °C.

In [5], scientists investigated the effect of abiotic (salinity, high temperature 45 °C, the presence in the culture medium of chitosan and abscisic acid) and biotic factors (supernatant of phytopathogenic microorganisms *Pseudomonas savastanoi* and *Fusarium oxysporum*, different concentrations of salicylic and methyl jasmonic acid) on the synthesis of IAA by strains *Azospirillum brasilense* Sp245 (pFAJ64) and *A. brasilense* Az39 (pFAJ64). When studying the action of abiotic factors, it was found that the presence of 0.1 mM of abscisic acid contributed to an increase in the concentration of IAA in both strains (+ 12.1% and 11.2%, respectively). The level of expression of the *ipdC* gene was 10.7% and 17.2% higher than in the control variant. Among the biotic factors for the Sp245 strain, the best synthesis was observed when 0.01% chitosan was added to the culture medium: the IAA concentration increased by 1.12 times relative to the control. In strain Az39, IAA synthesis was equally affected by both chitosan and *F. oxysporum* supernatant (4% by volume): auxin concentration increased by 4%.

Under conditions of abiotic stress (low nitrogen content in the culture medium — 0.2 g/L $CO(NH_2)_2$), the concentration of IAA synthesized by *Serratia* sp. ZM isolated from the *Populus euphratica* rhizosphere reached 82 μg/mL. In the absence of stress factors, when the nitrogen concentration increased to 1.2 g/L, the accumulation of biomass improved, but the concentration of auxins was lower. Unfortunately, the authors did not indicate the initial concentration of IAA under normal conditions [6].

The generalized data on the influence of cultivation conditions on the synthesis of IAA in bacteria are given in Table 3.

Thus, the authors found that increasing the temperature and/or pH can increase the concentration of IAA. However, it should be noted that this method of intensifying the synthesis of auxins does not allow one to obtain high initial concentrations of IAA and is selected experimentally for each individual strain.

***

Analysis of the literature data show that various groups of microorganisms are capable of IAA synthesis but the largest number of works is devoted to the study of the biosynthesis of IAA by plant-associated bacteria.

Note that understanding the ways of synthesis of the target product opens up new perspectives and approaches for researchers to intensify the synthesis of IAA. First, the use as a precursor of the synthesis of not only tryptophan but also other intermediates of the pathway functioning in this microorganism (IP — IP pathway, IAM — IAM pathway, IAN — IAN pathway). Second, knowledge of the genetic sequences responsible for the synthesis of key enzymes will increase the expression of the required genes [10] and create recombinant strains of IAA supersynthetics [16, 23]. In addition, there is a third approach to increase IAA synthesis — the effect of stress factors on PGPR bacteria. However, this method of intensification of synthesis has a number of disadvantages compared to the others: low efficiency, individual approach to each strain, and the complexity of the technological organization of large-scale production.

Currently, the most recognized and widespread way to increase the synthesis of IAA is to introduce a precursor into the culture medium. One of the promising producers of IAA, which uses tryptophan as a synthesis precursor, is *Enterobacter* sp. DMKU-RP206 [45]: it synthesizes up to 5.56 g/L IAA.

Given the current capabilities of biotechnology and genetic engineering, one can hope that in the near future researchers will regulate the synthesis of IAA in microorganisms and create effective producers of IAA, which in turn will facilitate the microbial synthesis of auxins on an industrial scale.

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Т.П. Пирог[1,2], *Д.В. П'ятецька[1], Н.О.Клименко[1], Г.О.* Іутинська[2]

[1] Національний університет харчових технологій,
вул. Володимирська, 68, Київ, 01601, Україна
[2] Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України,
вул. Академіка Заболотного, 154, Київ, 03143, Україна

ШЛЯХИ БІОСИНТЕЗУ АУКСИНІВ У МІКРООРГАНІЗМІВ

Серед рослинних гормонів найбільш вивченими та дослідженими є ауксини, зокрема індол-3-оцтова кислота (ІОК). Утворювати ауксини здатні майже всі групи ґрунтових мікроорганізмів, як асоційовані, так і не асоційовані з рослинами бактерії, гриби, а також фітопатогенні мікроорганізми. Створення препаратів для рослинництва безпосередньо пов'язано з отриманням штамів бактерій з високим ауксин-синтезувальним потенціалом, що можливе тільки при повному розумінні шляхів регуляції і синтезу ауксинів у бактерій. Синтез ауксинів у мікроорганізмів може проходити двома шляхами: постадійним перетворенням в ІОК триптофану (триптофан-залежний шлях) або з використанням інших інтермедіатів (триптофан-незалежний шлях). Останній шлях синтезу ауксинів є маловивченим, і в доступній на сьогоднішній день літературі є лише поодинокі відомості щодо його функціонування в мікроорганізмах. В огляді наведено дані літератури про шляхи біосинтезу ауксинів у різних груп мікроорганізмів, а також про підходи до інтенсифікації синтезу ІОК. Утворення ІОК з триптофану може здійснюватися такими шляхами: індол-3-піруватний, індол-3-ацетамідний та індол-3-ацетонітрильний. Переважна більшість публікацій стосується асиміляції триптофану через утворення індол-3-пірувату, оскільки цей шлях є найбільш поширеним серед мікроорганізмів. Так, він функціонує у ризосферних, симбіотичних, ендофітних та вільноіснуючих бактеріях. Серед природних штамів концентрація синтезованої ІОК перебуває в межах від 260 до 1130 мкг/мл. Мікроорганізми, у яких функціонує індол-3-ацетамідний шлях, характеризуються нижчою ауксин-синтезувальною здатністю порівняно з тими, що асимілюють триптофан через індол-3-піруват. До них належать бактерії родів *Streptomyces*, *Pseudomonas, Bradyrhizobium* та гриби роду *Fusarium.* Рівень синтезу ІОК у таких мікроорганізмів становить від $1{,}17 \cdot 10^{-4}$ до 255,6 мкг/мл. У доступній літературі описано лише два штами, які асимілюють триптофан через індол-3-ацетонітрильний шлях і утворюють до 31,5 мкг/мл ІОК. Для інтенсифікації синтезу ІОК дослідники використовують два основні підходи: перший — внесення у середовище культивування екзогенних попередників біосинтезу (найчастіше триптофану, рідше — індол-3-пірувату, індол-3-ацетаміду та індол-3-ацетонітрилу); другий — підвищення рівня експресії відповідних генів і створення рекомбінантних штамів-надсинтетиків ІОК. Найбільша кількість публікацій присвячено підвищенню синтезу ІОК за наявності у середовищі попередників біосинтезу. Залежно від виду бактерій, складу поживного середовища та кількості екзогенно внесеного попередника, синтез цільового продукту вдалося підвищити в 1,2—27 разів порівняно з таким до інтенсифікації. Так, за наявності 11 г/л триптофану *Enterobacter* sp. DMKU-RP206 синтезував 5,56 г/л ІОК, у той час як на середовищі без попередника —всього 0,45 г/л. Рекомбінантні штами бактерій *Corynebacterium glutamicum* ATCC 13032 та *Escherichia coli* MG165 у разі внесення в середовище культивування триптофану (10 г/л) утворювали відповідно 7,1 та 7,3 г/л ІОК . Рівень синтезу ауксинів у мікроорганізмів може підвищуватися в стресових умовах (температура, pH, фактори біотичного та абіотичного стресу), однак у цьому разі концентрація їх не перевищує 100 мг/л, у зв'язку з чим такий спосіб інтенсифікації не може конкурувати з іншими наведеними вище.

Ключові слова: *триптофан, індол-3-піруват, індол-3-ацетамід, індол-3-ацетонітрил, індол-3-оцтова кислота, інтенсифікація синтезу фітогормонів.*