UDC 579.64:581.1

https://doi.org/10.15407/biotech11.01.005

MICROBIAL SYNTHESIS OF PHYTOHORMONES

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Received 27.12.2017

The aim of the review was to analyze current literature data and the results of own studies on the synthesis of auxins, cytokinins, and gibberellins by plant-associated microorganisms (living in rhizosphere, endophytic, nitrogen-fixing, and phytopathogenic), and by those not involved in symbiotic interactions. Many microorganisms can generate phytohormones, and microbial synthesis of indole-3-acetic acid can be enhanced which can be used in producing it instead of extracting it from plants or by chemical synthesis. Recent progress in intensifying the synthesis of gibberellic acid in deep and solid-phase producer cultivation allows substantially reducing the prime cost of biotechnological production of that phytohormone. The ability of microorganisms to simultaneously synthesize phytohormones and other biologically active compounds with antimicrobial, nematocidal, and other various effects enables creating complex polyfunctional microbial preparations with various biological properties for use in crop production to stimulate plant growth and pest control.

Key words: phytohormones, microbial synthesis, complex microbial preparations.

Plant growth regulators (PGR) attract a lot of attention in the agro-industrial complexes of economically developed countries. Using them allows optimizing the plant metabolism in order to increase the yield and improve the quality of crops.

By origin, growth regulators are divided into the following groups [1]: endogenous compounds synthesized by plants (phytohormones); products of microbial synthesis; synthetic compounds. Because growth regulators of microbial origin are similar to compounds synthesized by plants (auxins, cytokinins, gibberellins, abscisic acid), they are also called phytohormones.

In agriculture growth regulators are used in stimulating seeds germination, activating vegetative growth of plants, accelerating their flowering and maturing, increasing yields, protecting against certain diseases, etc. Using those in agriculture production can significantly reduce the use of chemical plant protectors [1].

In 2015, the global market for plant growth regulators was estimated at $ 1.6 billion. From 2015 to 2020, its growth is forecasted to be 3.6% (to $ 1.91 billion) [2]. The leading PGR producers are FMC (Food Machinery Corporation), Dow (USA), Syngenta AG (Switzerland), BASF SE (Germany) and Nufarm Limited (Australia). The most marketed plant growth regulators are gibberellins, consumed in about 60 tons per year [2].

Increasing the efficiency of microbial synthesis of gibberellins [3–6] and understanding of the plant-microbial interaction mechanisms [7–9] stimulated studying the ability of various physiological and taxonomic microorganism groups to produce phytohormones [10–19], as well as the development of microbial technologies to obtain several of them [6, 20, 21].

The production of phytohormones by microorganisms was previously reviewed in 2013 [22]. This work summarizes the results of studies mainly by Ukrainian scientists, focusing on the bacterial synthesis of phytohormones included in preparations for crop production, and the methods for determining these compounds are analyzed.

The purpose of this review is to summarize the current literary data on the phytohormone synthesis by microorganisms either plant-associated or not, and description of approaches to intensify the corresponding technologies of microbial phytohormone's synthesis.

The general characteristics of phytohormones

The term "hormone" was first proposed by animal physiologists Bayliss and Starling in 1904 (cited in [6]). At that time, a chemical compound was considered a hormone if during the migration with blood from one part of the body to another it caused a behavioral change. A few years later, in 1910, this term was introduced in the physiology of plants. In 1948, after a lengthy discussion, the term "plant hormones" or "phytohormones" was established. By Thimann's definition (cited in [6]) phytohormone is an organic substance which is synthesized in trace amounts in certain parts of the plant and can be transported to other parts for the implementation of specific physiological functions.

Now, the term "phytoregulators" describes both synthetic and natural organic compounds that affect plant life processes, but are not nutritional [1].

Currently, a substance is considered a phytohormone if it has the following properties (cited in [22, 23]):

- causes a specific physiological response;
- is synthesized in a plant by one group of cells, and causes a response in another group (different places of synthesis and action);
- has almost no significance in the main cell metabolism, and is used only for signal regulation;
- acts in low concentration: 10^{-5}–10^{-12} mol/l.

About five thousand compounds of plant and microbial origin, as well as artificially synthesized are known to have a regulatory effect on plants. However, no more than 50 are used in production [1].

Until recently, five types of phytohormones have been universally recognized: gibberellins, auxins, cytokinins, abscisic acid (ABA) and ethylene [1, 22–24]. There also are hormone-like compounds of double auxin-cytokinin action, such as brassinosteroids and fusicoccin. Fusicoccin is synthesized by fungus *Fusicoccum amygdali* (parasitizing mainly on peach and almonds), and also is isolated from flowering plants. Steroid hormones brassinosteroids characterized by high biological activity, were isolated in 1979 from rapeseed pollen by American scientists. Today, more than 40 brassinosteroids have been identified, but the most physiologically effective are three of them: brassinolide, 24-epibrassinolide and homobrassinolide [1].

Phytohormones of microbial origin

The fundamental difference between plant and microbial phytohormones is that microorganisms do not need phytohormones to exist. These compounds are secondary metabolites, which are synthesized irregularly and often have undetermined physiological functions [23].

Synthesis of phytohormones are integral in the interaction between plants and plant-associated microorganisms (symbionts, epiphytes, inhabitants of rhizosphere and rhizoplane) [8, 9, 13, 24–29]. Thus, for example, *Azotobacter* spp., *Rhizobium* spp., *Rhodospirillum rubrum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Paenibacillus polymyxa* synthesize cytokinins and most representatives of the genus *Rhizobium* produce indole 3-acetic acid (IAA) [30–37]. In addition, microorganism's synthesis of hormones-stimulators and inhibitors can be considered pathogenic, since phytopathogens can produce these compounds in ultrahigh quantities, which leads to a disruption of the plant's hormonal status and causes some diseases [30–36]. The phytopathogenic bacteria *Pantoea agglomerans* is known to synthesize significant amounts of IAA [37]. The study [10] is one of the first to research the role and biosynthesis pathways of auxins (IAA) in gram-positive phytopathogenic bacteria *Rhodococcus fascians*. In addition to auxins, *R. fascians* also produces cytokinins [30].

The ability to synthesize phytohormones (auxins, abscisic acid, cytokinins, and gibberellins) is also found in many microalgae [12, 14]. Although the functional role of endogenous phytohormones in microalgae remains unknown, studies conducted on *Nannochloropsis oceanica* suggest that it is similar to that of plants [14].

Not only phytopathogenic, endophytic, epiphytic, symbiotic bacteria synthesize phytohormones. Phytohormones produce also microorganisms that not directly associated with plants [15, 16, 18]. The synthesis of phytohormones in phytopathogenic or plant growth promoting bacteria (PGRB) can be explained by their interaction with plants [24–38], but physiological role of such compounds in metanotrophs, yeasts, non-pathogenic micromycetes is often unclear [15–17, 39].

Microbial synthesis of auxins

As the most common plant hormone, auxin is well studied not only as a factor of the growth and development of vascular plants, but also as a metabolite of cyanobacteria [12, 14], bacteria [11, 20, 24, 25, 29] and fungi [28, 33].

Most publications are devoted to the synthesis of auxins by *Rhizobacteria*. As early as in 1990's it has been shown that 80% of the bacteria isolated from the rhizosphere synthesize IAA [40].

Synthesis of auxins by rhizobacteria. In [24], rhizobacterial proximity to the roots is described as follows: (1) rhizosphere: the microbes exist in the soil near the roots; (2) rhizoplane: the bacteria colonize the surface of the root; (3) endophytes live in the root tissue; (4) symbiotic nitrogen fixing bacteria include two groups: rhizobia (in symbiosis with leguminous plants) and representatives of the genus *Frankia* (symbionts of alder). Rhizobacteria can stimulate plant growth either directly (as a result of nitrogen fixation, phosphate solubilizing, iron ion chelating and phytohormones synthesis) or indirectly (inhibition of phytopathogens, induction of resistance to phytopathogens and abiotic stress conditions). That is why they are also called PGPR (plant growth promoting rhizobacteria) [24, 41, 42]. Vessey [43] suggested calling the representatives of the first three groups of *Rhizobacterium* extracellular (extracellular PGPR, ePGPR), and the fourth — intracellular (intracellular PGPR, iPGPR). Extracellular rhizobacteria include representatives of the genera *Bacillus*, *Pseudomonas*, *Erwinia*, *Caulobacter*, *Serratia*, *Arthrobacter*, *Micrococcus*, *Flavobacterium*, *Chromobacterium*, *Agrobacterium*, *Hyphomycrobium*. Intracellular rhizobacteria belong to the genera *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium*, *Mesorhizobium* and *Allorhizobium* [24]. In [44], it is proposed to divide rhizobacteria into two groups: symbiotic and free-living.

In early 1990's it was found that the auxin synthesis in rhizobia increases in the presence of flavonoids secreted by the plant to start the processes of nodules formation [45]. This supports the theory of the interaction between symbiotic microorganisms and plants through the excretion of phytohormones in natural conditions.

The major auxin phytohormone synthesized by most of rhizobacteria is IAA [24, 25, 42, 46–49]. *Pseudomonas aurantiaca* and *Pseudomonas extremorientalis* [50], as well as *Klebsiella oxytoca* Rs-5 [25] under salt stress synthesize IAA, stimulating seeds germination in wheat and cotton growth, respectively.

The ability to synthesize IAA is found in *Klebsiella pneumoniae* strains, isolated from the rhizosphere of wheat [51]. Studies conducted with *Klebsiella oxytoca* isolated from the rhizosphere of *Aspidosperma polyneuron* showed that immobilized on inorganic matrices microorganisms retain or even increase the ability to synthesize IAA, while free cells gradually lose it [52].

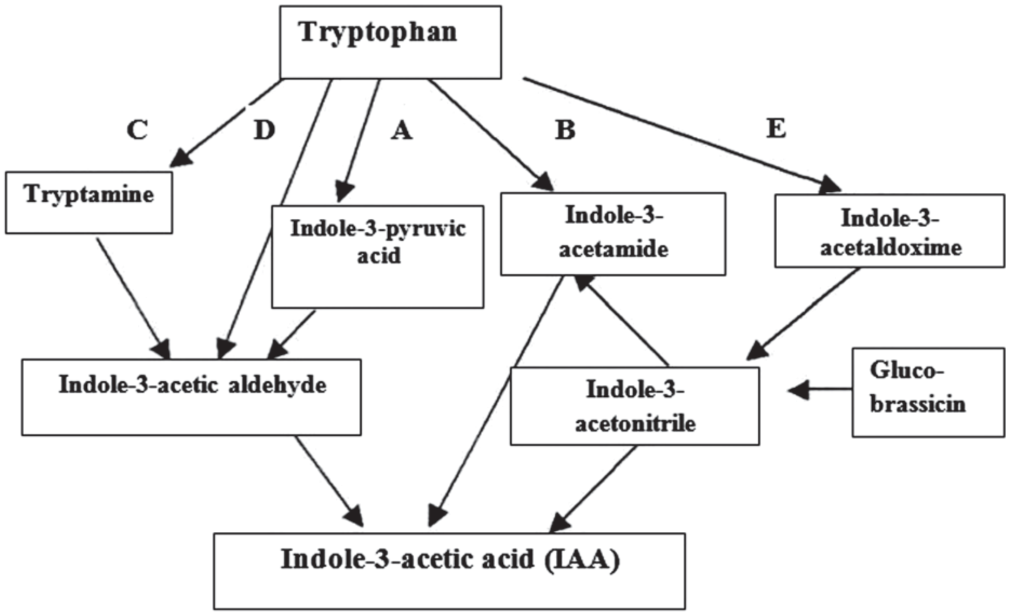
Symbiotic and non-symbiotic nitrogen fixing bacteria of the genera *Agrobacterium*, *Paenibacillus*, *Rhizobium* and *Azotobacter* [53] are also capable of synthesizing IAA. Maximum synthesis (up to 5.23 μg/mg of biomass) was observed in *Rhizobium* and *Paenibacillus*, and if the representatives of these genera were grown together, their auxinogenic ability increased compared to that found for monocultures.

Our study [54] on the auxin's synthesis by highly effective strains of soybean rhizobia *Bradyrhizobium japonicum* UCM B-6023, *B. japonicum* UCM B-6036 and by an ineffective strain *B. japonicum* 604k showed that this ability does not correlate with their symbiotic activity. The strain *B. japonicum* 604k forms a large number of nodules with almost totally absent nitrogenase activity and synthesizes high amounts of auxins (indole-3-carboxylic acid, indole-3-carbinol and indole-3-acetic acid hydrazide) but does not form IAA which would be physiologically active in plants.

It was first established in [55] that rhizospheric bacteria of the phylum *Acidobacteria* (representatives of the genera *Granulicella* and *Acidicapsa*) through the synthesis of IAA and iron chelating, stimulate the growth of *Arabidopsis thaliana*, and therefore can be considered representatives of PGPR-microbiota.

Bacteria of the genera *Sphingomonas*, *Microbacterium*, *Mycobacterium*, *Bacillus*, *Rhizobium*, *Rhodococcus*, *Cellulomonas*, *Pseudomonas* and *Micrococcus*, isolated from the rhizosphere of orchids *Dendrobium moschatum* and *Acampe papillosa*, exhibited auxinogenic ability at levels up to 90 μg/ml if exogenous tryptophan (200 μg/ml) was added into the the cultivation medium [39].

The synthesis of IAA in the presence of tryptophan is intensified because in microorganisms this amino acid is a precursor in the biosynthesis of IAA [33, 39, 49]. Tryptophan can be transformed into IAA in three ways (Figure):



Biosynthesis pathways of from tryptophan in bacteria:
A — with indole-3-pyruvate; *B* — with indole-3-acetamide; *C* — with tryptamine; *D* — tryptophan pathway; *E* — with indole-3-acetonitrile

– synthesis via indole-3-pyruvic acid and indole-3-acetic aldehyde. This is the main route, typical for mushrooms and bacteria;

– transformation of tryptophan to indole-3-acetic aldehyde may include an alternative pathway with the synthesis of tryptamine. This path is found in mycorrhiza fungi and cyanobacteria;

– IAA formation through indole-3-acetamide. It is characteristic for phytopathogenic bacteria and fungi.

In [17, 42, 56, 57] it is noted that the synthesis of IAA by rhizobacteria significantly increased in the presence of tryptophan in the cultivation medium. Table 1 shows indicators of the synthesis of IAA by a number of rhizobacteria depending on the presence of tryptophan in the cultivation medium. Thus, in order to establish the ability of bacteria to form IAA, virtually all researchers introduced the precursor of biosynthesis of this phytohormone to cultivation medium.

Formation of IAA and antimicrobial compounds complex by rhizobacteria. Recently, the rhizobacterial production of biologically active substances complex, in particular, phytohormones and metabolites with antimicrobial, nematocidal, etc. effects [27, 29, 57–67] has been actively studied. Actinobacteria (in particular, *Streptomyces*) [58–67, 68–77], as well as representatives of the genera *Bacillus* and *Paenibacillus* [29, 57, 67, 68] (Table 2) are the most active producers of the complex of such compounds with diverse biological activity.

It should be noted that the antimicrobial compounds of *Streptomyces* bacteria are mainly antibiotics, in particular geldanamycin [65, 72], avermectins [77], blasticidin S, kasugamycin, oligomycin A, paramycin, and pyrroles [73]. Our studies [75–77] showed that soil streptomycetes *Streptomyces netropsis* IMV Ac-5025 and *Streptomyces violaceus* IMV Ac-5027 also synthesize a complex of metabolites (including IAA) with phytoprotective, growth-stimulating, adaptogenic and antistress properties. These bacteria have an insecto-acari-nematocidal contact effect against crop pests.

Synthesis of auxins by bacteria which are not associated with plants. Though phytohormone synthesis is one of the main factors of plant-microbe interaction, the plant-associated microbes are not necessarily the only producers of auxins. There is a lot of data supporting the auxinogenic activity of PGPR-microbiota; however, many microbes not associated with plants also can synthesize IAA.

From twelve samples of sea water, sediment, and shrimp collected in the Egyptian coastal areas, 112 isolates belonging to the genus *Streptomyces* were isolated [15]. The level of IAA synthesis by six most active strains was in the range of 5–50 μg/ml in the presence of tryptophan in starch-casein medium. Marine *Streptomyces*, in addition to

Table 1. **Effect of exogenous tryptophan on the indole-3-acetic acid synthesis by rhizobacteria**

Strain	Main components of cultivation medium	IAA concentration		Source
		With added tryptofan	Without tryptofan	
Klebsiella oxytoca Rs-5	Clucose, glucuronic acid, citrate	42.14 µg/ml	N.d.	[25]
Stenotrophomonas rhizophila ARS3	Malate	72.32 µg/ml	62.45 µg/ml	[42]
Acetobacter pasteurianus ARS2	Malate	7.56 µg/ml	0.12 µg/ml	[42]
Bacillus sp. ARS4	Malate	1.88 µg/ml	0	[42]
Bacillus amyloliquefaciens SQR9-E	Peptone, yeast extract	39 mg/l	N.d.	[46]
Enterobacter lignolyticus TG1	Murashige and Skoog medium with saccarose	90 µg/ml	N.d.	[47]
Bacillus pseudomycoides SN29	same	85 µg/ml	N.d.	[47]
Burkholderia sp. TT6	same	60 µg/ml	N.d.	[47]
Pseudomonas aeruginosa KH45	same	43 µg/ml	N.d.	[47]
Bacillus sp. BM24	Tryptone, soy peptone	21.07 µg/ml	N.d.	[48]
Bacillus amyloliquefaciens SQR9	LB medium	0.4 ng/ml	0.3 ng/ml	[49]
Bacillus subtilis LK14	LB medium	8.7 µM	N.d.	[56]
Paenibacillus polymyxa CR1	Dextrose, soy tryptone	64.2 µg/ml	4.4 µg/ml	[57]
Paenibacillus polymyxa CR1	Mannitol, sodium glutamate	67.1 µg/ml	0.9 µg/ml	[57]

Note: N.d. — not determined.

phytohormones, synthesized metabolites with antibacterial and antifungal activity, and 28 strains showed nematocidal activity.

Synthesis of IAA by fungi and yeasts. Many phytopathogenic cecidia-inducing fungi, as well as mycorrhizal fungi, are capable of synthesizing IAA. Those include representatives of the genera *Taphrina*, *Phytophthora*, *Ustilago*, *Colletotrichum*, *Laccaria*, *Pisolithus*, *Amanita*, *Rhizopogon*, *Paxillus*. Among the micromycetes, auxins are produced by fungi of the genera *Fusarium*, *Rhizoctonia*, *Rhizopus*, *Absidia*, *Aspergillus*, and *Penicillium* [39]. It was found that *Aspergillus niger* synthesizes up to 128.3 mg/l of this auxin, and the production of phytohormones and fungal growth are positively influenced by the presence of gibberellin in the cultivation medium [78].

It was shown that the foliar treatment of *Agrostis* leaves with cells of *Pythium aphanidermatum* capable of auxin synthesis was accompanied by 200 times increased IAA content in leaves (up to 9760 ng/g of raw mass) and started an infectious process [79]. However, the infectious process and phytohormonal activity are not always interconnected. For example, *Ustilago maydis* damages corn causing the formation of tumors with increased IAA concentration due to the fungal phytohormonal ability. However, mutants that do not generate this auxin still cause the development of tumors, so the infectious process is not related to IAA synthesized by the fungus [80].

Yeasts are typical inhabitants of the phyllosphere, but until in the last decade their phytohormonal activity has not been studied. The first works on the synthesis of phytohormones in yeasts (2004-2006) appeared immediately after their ability for endophytic development had been discovered [81, 82]. At present, the literature has information on mainly auxin synthesis by yeasts.

For example, *Cyberlindnera* (*Williopsis*) *saturnus*, isolated from the roots of maize, produce IAA [81]. These yeasts were selected from 24 endophytes' species and artificially inoculated in studied corn plants. L-tryptophan, a precursor of auxin, was introduced in the soil on which the plants inoculated with *C. saturnus* grew. In one of the versions, L-tryptophan was not added in soil. It was established that plants inoculated with yeast grew faster than non-inoculated plants and the best growth was observed in soil with tryptophan.

Table 2. **Synthesis of metabolites with various biological properties by rhizobacteria**

Strain	IAA concentration	Effect				Source
		antibacterial	fungicidal	nematocidal	against oomycetes	
Paenibacillus polymyxa CR1	67.1 μg/ml	*Pseudomonas syringae, Xanthomonas campestris*	*Rhizoctonia solani, Cylindrocarpon destructans*	–	*Phytophthora sojae*	[57]
Bacillus sp. RMB7	8 mg/l	–	*Aspergillus niger, Aspergillus flavus, Colletotrichum gloeosporioides, Colletotrichum falcatum, Fusarium oxysporum*	–	*Pythium ultimatum*	[68]
Bacillus sp.ZB2	3 μg/ml	–	*Fusarium oxysporum, Sclerotinia sclerotiorum*	*Meloidogyne incognita*	–	[67]
Serratia marcescens TTD7	73 μg/ml	–	*Nigrospora sphaerica, Pestalotiopsis theae, Curvularia eragrostidis, Glomerella cingulata, Rhizoctonia solani*	–	–	[69]
Streptomyces hydrogenans DH16	30 μg/ml	–	*Colletotrichum acutatum, Cladosporium herbarum, Alternaria brassicicola, Exserohilum* sp., *Alternaria mali, Colletotrichum gleospoiroides, Alternaria alternata*	–	–	[59]
Streptomyces cameroonensis sp. nov. JJY4T	+	*Agrobacterium tumefaciens, Streptomyces scabiei*	*Aspergillus niger, Botrytis cinerea, Fusarium oxysporum*	–	*Phytophthora megakarya, Phytophthora erythroseptica, Pythium myriotylum*	[65]
Streptomyces olivaceus BPSAC77	52.3 μg/ml	*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli*	*Fusarium proliferatum, Fusarium oxysporum, Candida albicans*	–	–	[70]
Streptomyces sp. 9p	+	–	*Alternaria brassiceae, Collectotrichum gleosporioides, Rhizoctonia solani*	–	*Phytophthora capsici*	[71]

Notes: «–» – no data; «+» – IAA concentration is not given.

In 2009, strains of *Rhodotorula graminis* and *Rhodotorula mucilaginosa*, which synthesized up to 40 mg/g biomass of IAA acid, were isolated from the apexes of poplar trees [83]. In 2012, scientists have researched the ability to synthesize auxins in 114 strains of yeast isolated from leaves of tropical plants [84]. Thirty nine strains were found to produce phytohormones, although in different quantities (27–234 μg/ml). In 2014, the same authors isolated 158 yeast strains from sugar cane and found that 69 of them synthesized IAA. The maximum concentration (565.1 mg/l) was observed in *Rhodosporidium fluviale*. In these studies, tryptophan (1 g/l) was added into the cultivation medium [85].

The work [17] researched IAA synthesis by strains of *Saccharomyces cerevisiae* and

Saccharomyces paradoxus, isolated from different ecosystems. All 24 studied yeast strains synthesized from 10 to 120 μg/ml of IAA in the presence of tryptophan in the medium. Growing on tryptophan-less medium, only three strains were able to synthesize IAA.

In 2017, 147 strains of 46 yeast species were studied. Most of them were isolated from phyllosphere, rhizosphere, leaf bedding, soil and entomophilic flowers [23]. The ability to synthetize IAA was found in 92% of the researched strains. *Metschnikowia pulcherrima* KBP Y-6020 and *Saitozyma podzolica* KBP Y-4614 synthesized the highest amount of this phytohormone (18693.3 and 22206.7 μg/ml, respectively). The levels of IAA synthesis by *Rhodotorula mucilaginosa* KBP Y-5419 and *Candida trypodendroni* KBP Y-5475 ranged 7593.3-5033.3 μg/ml [23].

Nutaratat et al. [86] researched the synthesis of IAA by yeasts isolated from rice leaves and sugar cane. Of more than 1000 tested strains, only 13 produced this hormone in a concentration of 1.2 to 29.3 mg/g of biomass. The highest amount of IAA was synthesized by the strain *Rhodosporidium paludigenum* DMKURP301.

Ways of intensification of IAA synthesis in microorganisms. The IAA synthesis path and mechanisms of its genetic and biochemical regulation were studied in *Pseudomonas mendocina* BKMB 1299 [20]. It was established that the synthesis of this hormone occurs by indole-3-pyruvic acid path (Figure) involving three enzymes: tryptophan-aminotransferase, indole-3-pyruvate decarboxylase and indole-3-acetaldehyde dehydrogenase.

The shikimate pathway in the studied bacteria was shown to be regulated by retro-inhibition of the key enzyme 3-deoxy-*D*-arabinoheptulosonate 7-phosphate (DAHP) synthase with two amino acids, tyrosine and tryptophan. The synthesis of this enzyme in *P. mendocina* BKMB 1299 was not repressed. The synthesis of tryptophan is controlled by the repression of *trpE*-, *trpD*- and *trpC*-genes by tryptophan, and by retro-inhibition of anthranilate synthase with the same amino acid. On the contrary, the synthesis of tryptophan-aminotransferase and indole-3-pyruvate decarboxylase is activated by tryptophan. In addition, the synthesis of tryptophan-aminotransferase is repressed by anthranilate. Using nitrosoguanidine mutagenesis and further selection of clones resistant to 5-Fluoro-dl-tryptophan (which is the toxic analogue of tryptophan) produced regulatory mutants capable of over-synthesis of IAA. The production of the hormone in the *P. mendocina* mutant strain 9–40 was 10 times higher than that of *P. mendocina* BKMB 1299. IAA over-synthesis correlated with an increase in the synthesis of key enzymes of the aromatic pathway, DAHP synthase and tryptophan synthase (twice), tryptophan-aminotransferase and indole-3-pyruvate decarboxylase (approximately eight and 80 times, respectively) [20].

Then, the strains producing indole-3-pyruvate decarboxylase, an enzyme involved in IAA synthesis, were genetically engineered. In order to create such IAA producing strain of *P. mendocina*, the *ipdC* gene (encoding the synthesis of indole-3-pyruvate decarboxylase) was cloned in *Escherichia coli* DH5α strains using pUC18 and pXcmKn12 vectors. As a result, a hybrid plasmid pTVN4 (4.5 kb) was obtained with the inserted *ipdC* gene of 1.7 kb. To study the expression of the *ipdC* gene in *P. mendocina*, a plasmid pAYSCD1.7 was constructed. It was stably inherited in the bacterial cells.

The presence of a plasmid with an integrated *ipdC* gene increased the level of synthesis of indole-3-pyruvate decarboxylase (in 5.3 times) and IAA. Thus, the *P. mendocina* 9–40 regulatory mutant and the recombinant strain carrying the plasmid pAYC1.7 with the integrated *ipdC* gene are promising for use in crop production [20].

The study [21] is a continuation of research on IAA synthesis by *R. paludigenum* DMKURP301 [86]. This is one of the few publications in which the authors optimized the process of biosynthesis of this phytohormone. Mathematical planning of the experiment allowed increasing the concentration of the target product to 1,624 g/l. The maximum IAA synthesis was achieved under conditions of growth of DMKURP301 strain on sucrose (1%) as carbon source, corn extract (0.1%) as nitrogen source, yeast extract (1%) as growth factors, and tryptophan (0.4%) as precursor of biosynthesis. The optimum temperature was 30 °C, pH 7.0, the duration of cultivation under agitation (200 rpm) was 9 days. The process was scaled: the IAA concentration was 1.627 g/l in fermenter of 2 liters [21].

However, the authors of [21] failed to exceed the levels of IAA synthesis by the bacterial strain *Pantoea agglomerans* PVM [87]. The optimization of the cultivation conditions (in particular, the composition of the nutrient medium) increased the concentration of IAA to 2.191 g/l. The medium contained sucrose (1%) as carbon source, meats extract (8 g/l) as nitrogen source, and tryptophan (1 g/l) as the precursor of IAA biosynthesis.

Thus, the production of auxins (in particular, their physiologically active form, IAA) is characteristic of microorganisms that interact with plants, as well as of many bacteria, fungi and yeast that are non-associated with plants. The synthesis of IAA *in vitro* is usually enhanced by the presence of tryptophan, a precursor of this phytohormone biosynthesis, in the cultivation medium. Representatives of the genera *Streptomyces*, *Bacillus* and *Paenibacillus* simultaneously with phytohormones synthesize metabolites that can be used for pest control in crop production. The progress achieved so far in increasing IAA synthesis by *P. agglomerans* PVM and *R. paludigenum* DMKURP301 means that biotechnological production of IAA is possible.

Microbial synthesis of cytokinins. Symbiotic nitrogen-fixing bacteria. Unlike auxins, there is much less data on formation of cytokinin by microorganisms, although the ability of *Rhizobium leguminosarum* bacteria to synthesize these phytohormones *in vitro* has been reported for the first time in 1970s [88]. Recently, the study of cytokinin synthesis by symbiotic nitrogen-fixing bacteria of the genera *Sinorhizobium*, *Mesorhizobium* and *Bradyrhizobium* is associated with finding out the role of these phytohormones in nodulation [8, 26, 89, 90]. It was found in [26] that 9 strains of *Sinorhizobium meliloti*, *Sinorhizobium fredii*, *Sinorhizobium medicae* and *Mesorhizobium loti* synthesize 25 forms of cytokinins, some of which are methylated. The strain *Bradyrhizobium* sp. ORS285 also synthesizes mainly 3-methyl-thiol derivatives of *trans*-zeatin and N^6-(2-isopentenyl) adenine in concentrations three orders of magnitude higher than non-methylated analogs (1000–5000 and 5–60 pmol/l, respectively) [89]. However, it is noted [26, 89, 90] that the bacterial synthesis of cytokinins is not a prerequisite for the symbiotic relationship with a plant, and the decisive role in this process belongs to plant cytokinins [90]. At the same time, our research [54] found a direct correlation between the symbiotic efficiency of rhizobial strains and the level of cytokinins synthesis. For example, highly effective strains of soybean symbionts *B. japonicum* UCM B-6023 and *B. japonicum* UCM B-6036 produced a wide range of cytokinins, mostly zeatin and *trans*-zeatin-riboside. Ineffective strain *B. japonicum* 604k synthesized these phytohormones in significantly smaller amounts comparable to the highly effective strains.

Other rhizobacteria. Inoculating wheat rhizosphere by strains of *Bacillus* sp., capable of synthesizing cytokinins, resulted in an increased content of zeatin-riboside in roots and then in stems. Strains of *Bacillus licheniformis*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, isolated from the rhizosphere of different plants, synthesized cytokinins. The maximum concentration of phytohormones (1091.9 g/ml *trans*-zeatin and 521 ng/ml zeatin-riboside) was achieved in the stationary phase of *Bacillus licheniformis* growth, which is typical of secondary microbial metabolites [91].

The endophytic strain of *Bacillus amyloliquefaciens* IMV B-7100, isolated from cotton, produced cytokinins in the concentration of 141 µg/g of biomass, mostly zeatin (113 µg/g biomass) [92]. *B. amyloliquefaciens* subsp. *plantarum* UCM B-5113 synthesized 152.4 pcmol/l of cytokinins in 120 hours of growth in liquid LB medium [93], and in the presence of *Arabidopsis thaliana* root extractors under similar conditions of growth, the concentration of phytohormones increased to 295.4 pcmol/l. The authors suppose that cytokinins can stimulate synthesis of SHY2, the key regulator of growth and development of plant meristem. *Pseudomonas fluorescens* 6-8 improves the growth of cauliflower roots under gnotobiotic conditions [94]. Investigated strain is characterized by high ability to colonize the surface of the roots due to the synthesis of cytokinins. A fundamentally new role of cytokinins of *Pseudomonas fluorescens* G20-18 as intermediaries in the biocontrol of phytopathogenic bacteria *Pseudomonas syringae* was established in [18].

Phytopathogens. The role of cytokinins of the fungi *Magnaporthe oryzae*, *Ustilago maydis*, *Claviceps purpurea*, *Colletotrichum graminicolainfected* in the pathogenesis is established in [35, 36, 95–98]. However, *C. graminicolainfected*, unlike other fungi, was incapable of synthesizing cytokinins *in vitro* [98]. The levels of synthesis of N^6-(2-isopentenyl)adenine, *trans*-, *cis*- and dihydrozeatin in *Leptosphaeria maculans* JN3 at the ninth day of cultivation were 13, 8, 25 and 3 pmol/g biomass respectively [36]. Our studies [54] showed that the level of cytokinins' synthesis by phytopathogenic bacteria for soybean varied widely (from 75 to 1914 µg/g of biomass).

There are two main ways of synthesizing cytokinins in microorganisms [39]: *de novo* synthesis of isopentenyl pyrophosphate and adenosine-5′-monophosphate (characteristic of phytopathogenic bacteria) and the destruction of tRNA resulting in *cis*-zeatin produced by tRNA-isopenthenyltransferase. This path is found in phytopathogenic fungi. The process of cytokinins formation was interrupted in the mutants of *Magnaporthe oryzae* which lacked the gene responsible for the synthesis of tRNA-isopenthenyltransferase and they were characterized by reduced virulence [35].

The strain of phytopathogenic bacteria *Rhodococcus fascians* D188 synthesizes N^6-(2-isopentenyl)adenine, *cis*-zeatin, *trans*-zeatin, 2-methylthio derivatives of N^6-(2-isopentenyl) adenine, 2-methylthio derivatives of *cis*-zeatin at a concentration of 3, 2.5, 0.03, 0.4 and 4.5 nM respectively [30]. Synthesis of cytokinins is encoded by six genes that form the *fas* operon on pFiD188 plasmid. Analysis of various *fas* mutants, defective in one or more genes, showed that the formation of cytokinins is only one of the mechanisms of pathogenicity in this strain.

Other microorganisms. If the role of cytokinins in phytopathogenic microorganisms is clear, then the discovery of these phytohormones in the tuberculosis pathogen *Mycobacterium tuberculosis* in 2015 was a real surprise [16]. The authors [16] suggested that cytokinins can contribute to infecting cells and serve as a kind of communicative molecule between mycobacteria to control the development of infection.

In 1980's it was found that bacteria isolated from the sea and sea sediments synthesized cytokinins in concentrations of 0.05–0.30 μg/l [99]. Moreover, 45–55% of bacteria isolated from the sediments were capable of synthesizing phytohormones, compared to 5–15% isolated from water. The role of bacterial cytokinins in marine ecosystems remains a controversial issue, but it is assumed that they can be associated with algal blooms of water.

Thus, the ability to synthesize cytokinins and auxins is detected in a wide range of microorganisms, not necessarily associated with plants. Until recently there were not so many publications about the production of these phytohormones, and researchers mainly studied the *cis*-, *trans*-zeatin and zeatin-riboside, since these phytohormones are the most widespread in nature. However, the development of analytical methods [100, 101] offered new opportunities for detecting new forms of these phytohormones [26, 30].

Microbial synthesis of gibberellins. Many micromycetes are capable of synthesizing gibberellins, not only the representatives of the genus *Fusarium* (*Gibberella*), which are industrial producers of gibberellic acid [5, 6, 102, 103]. Table 3 shows data on the synthesis of gibberellins by endophytic fungi isolated from various plants [103]. The level of synthesis of gibberellins in these fungi is low, but there are fungi that can synthesize from 6 to 600 ng/ml of A_4. Synthesis of gibberellins by endophytic fungi of the genus *Penicillium* is one of the mechanisms that allow plants to survive under salt stress [104]. Concerning the relationship between pathogenicity and phytohormonal activity, the study of growth stimulating and pathogenic strains of *Fusarium culmorum* showed that the latter synthesized four times less gibberellin [105]. Many plant-associated phytopathogens [9, 24, 34, 41] and freely existent [12, 15] bacteria also synthesize gibberellins. Ten wild and mutant (*nod*↓, *fix*↓) strains of *Rhizobium phaseoli* were study in 1980s and showed that gibberellins were also synthesize by mutants unable to form nodules and fix nitrogen. So, nitrogen-fixing ability is not related to phytohormonal activity [106].

Since the gibberellic acid (A_3) is the first phytohormon produced by microbial synthesis, and this technology has been developing for more than 50 years, we will now consider recent approaches to improve it.

Intensification of synthesis of microbial gibberellins

Famous industrial producers of gibberellic acid are *Gibberella fujikuroi* and *Fusarium monilforme*. This phytohormone is mainly obtained by submerged fermentation [3, 5, 6, 102, 103, 107, 108], but recently, these compounds were produce in conditions of solid state fermentation [4, 109].

Optimization of cultivation conditions for producers of gibberellic acid. Under optimal conditions for the cultivation of *F. moniliforme* (Egyptian local isolate), the synthesis of gibberellic acid increased by 4.3 times (up to 1.4 g/l). Such conditions are: the concentration of fructose 6%, ammonium sulfate 0.6 g/l, magnesium sulfate 1.5 g/l, potassium dihydrogen phosphate 1.0 g/l, temperature 30 °C, initial pH 5.0 [101].

Optimizing the cultivation conditions of *F. moniliforme* M104 strain (temperature 30 °C, initial pH 5.5, cultivation duration 8 days, glucose concentration in the medium 30 g/l, ammonium chloride 3 g/l) led to 4.775 g/l

Table 3. **Production of gibberellins by endophytic fungi [103]**

Plant	Endophyte	Giberellins (A, ng/ml)
Glycine max L.	*Aspergillus fumigatus* sp. LH02	**A_4 (8.38)**, A_9 (2.16), A_{12} (1.56)
	Cladosporium sphaerospermum	A_1 (0.24), A_3 (8.9), A_4 (2.58), A_7 (1.37), A_5 (1.2), A_{15} (1.1), A_{19} (2.1), A_{24} (1.8)
	Phoma herbarum	A_1 (0.11), A_3 (2.91), A_4 (3.21), A_7 (1.4), A_9 (0.05), A_{12} (0.23), A_{15} (0.42), A_{19} (0.53), A_{20} (0.06)
	Chrysosporium pseudomerdarium	A_1 (0.24), A_3 (8.5), A_4 (2.58), A_9 (1.39), A_{15} (1.2), A_{19} (1.4), A_{20} (2.1)
	Penicillium minioluteum LHL09	**A_4 (12.84)**, A_7 (48.91)
	Scolecobasidium tshawytschae	A_1 (0.3), A_3 (17.84), **A_4 (18.58)**, A_7 (8.95), A_{15} (0.45), A_{24} (1.07)
Monochoria vaginalis Cucumis sativus	*Aspergillus* sp. i *Penicillium* sp.	A_3 (2.8), A_4 (2.6), A_7 (6.68), A_9 (1.61), A_{24} (0.18)
	Cladosporium sp. MH-6	A_1 (0.81), A_3 (4.34), **A_4 (9.31)**, A_9 (0.74),
	Phoma sp. AH7	A_{15} (0.97), A_{19} (1.67), A_{20} (0.46)
	Phoma glomerata LWL2, *Penicillium* sp. LWL3	A_1 (8.720), A_3 (2.420), A_4 (0.220), A_7 (4.2)
	Exophiala sp. LHL08	A_{12} (1.4), A_{20} (2.2), A_{24} (13.6), A_1 (3.546), A_3 (3.98), **A_4(121.50)**, A_5 (1.50), A_7 (133.47), A_9 (2.12), A_{12} (27.81), A_{20} (4.12)
Elymus mollis	*Gliomastix murorum* KACC43902	A_1(0.32), A_3(5.76), A_4 (0.82), A_7(0.1), A_5(0.59), A_{20}(0.25), A_{24}(2.03)
Sesamum indicum	*Penicillium commune* KNU5379	A_1 (71.69), **A_3 (252.4), A_4 (612.0)**, A_7 (259.0), A_9 (202.69)
Capsicum annuum	*Chaetomium globosum* LK4	A_1 (0.67), **A_4 (21.8)**, A_9 (0.51), A_{12} (13.4), A_{20} (1.11)

concentration of synthesized gibberellic acid, which is more than 5.5 times higher with indicators before optimization [102].

Cultivating *F. moniliforme* NCIM 1100 strain for eight days on a Caspec-Dax liquid medium with sucrose as a carbon source at 30 °C and an initial pH of 7.0 was accompanied by synthesis of almost 15 g/l of gibberellic acid. At present, this is the highest level of microbial synthesis of A_3 gibberellin [5].

Improvement of strains producing gibberellic acid. To enhance the synthesis ability, *F. moniliforme* strain was subjected to γ-irradiation (^{60}Co γ-radiation, sublethal dose of 6.5 kgy). Of the 28 obtained mutants, *F. moniliforme* γ-14 strain synthesized twice more gibberellic acid compared to the nonirradiated original strain [107].

In other studies, *F. moniliforme* (Egyptian local isolate) after γ-irradiation (^{60}Co γ-radiation, 0.5 kg) synthesized 2.36 g/l gibberellic acid, which is 1.4 times more than the original strain under similar cultivation conditions [101].

The initial pigmented strain *G. fujikuroi* NCIM 1019, characterized by the presence of intracellular carotenoids, was exposed to ultraviolet irradiation, resulting in a non-pigmented intermediate mutant Car-1 [3]. After the UV irradiation of the Car-1 strain, the mutant Mor-1, capable of increased synthesis of gibberellic acid, was obtained. As a result of further irradiation of the Mor-1 strain, the strain Mor-25 was isolated. It was characterized by the presence of short, heavily branched hyphae. While growing in a liquid medium, the Car-1 strain formed a high-tensile culture liquid, unlike the Mor-25 strain. The concentration of gibberellic acid synthesized by the Mor-25 strain was twice higher than that generated by the Car-1 strain. The strain Mor-25 synthesized only gibberellic acid, while Car-1 also produced fusaric acid. These results are quite significant, since fusaric acid is toxic for animals and plants [3].

Improvement of producers of gibberellins A_4 and A_7. The gibberellin A_4 is notable for its high biological activity and promotes the formation and growth of fruits like apples and grapes, and vegetables (tomatoes, peas) [108]. Despite the high biological activity of gibberellin A_4, its application in crop production is limited due to the high costs. The spectrum of action of gibberellin A_7 is wider, and its biological activity in many cases is higher compared with such A_3 and A_4. Most well-known producers synthesize a mixture of gibberellins A_4 and A_7, in which the A_4/A_7 ratio varies greatly. In addition, isolating individual preparations of A_4 and A_7 from the mixture is complicated because of their very close polarity.

In 1997, the strain *F. moniliforme* VKPM F-446 was created. It is the first superproducer of gibberellin A_7 which forms gibberellins A_3 and A_4 in insignificant quantities. The superproducing strain was obtained by fusing protoplasts of a strain isolated from the affected rice, followed by UV irradiation treatment. The strain synthesized 400–700 mg/l of gibberellin A_7 and only 20–80 mg/l of A_4 on medium with sunflower oil (60 g/l), corn extract (35 g/l) and ammonium acetate (0.57 g/l) [110].

In other studies [108], the strain *G. fujikuroi* 1019 was subjected to combined mutagenesis using UV- irradiation and pravastatin (250 mg/l), which inhibits the activity of HMG-CoA reductase, involved in the formation of mevalonic acid (an intermediate of biosynthesis of gibberellins). Thus, the mutant Mor-189 was obtained, capable of synthesizing gibberellins A_3 and A_4. That mutant synthesized mostly A_4 at pH levels of 5.5 during cultivation using glucose and wheat gluten as sources of carbon and nitrogen, respectively. The A_4 synthesis increased with glucose supplementation during the cultivation of the strain. Under such conditions, the concentration of gibberellin A_4 reached 600 mg/l, which was 84% of the total amount of A_4 and A_3 (713 mg/l) [108].

Immobilization of producer cells. F. moniliforme γ-14 was immobilized by adsorption on sponge disks (2–4 mm in diameter and 18–20 mm in diameter) cut from dried *Luffa* fruits [107]. Cultivating immobilized cells increased the concentration of A_3 to 1.9 g/l, and at initial pH 5.0 of the medium (milk permeate), up to 2.25 g/l.

Subsequent experiments showed the possibility of repeated reuse of immobilized *F. moniliforme* γ-14 cells on *Luffa* disks. Thus, a one-time replacement of the nutrient medium was accompanied by an increase in the concentration of gibberellic acid to 2.4 g/l on the eighth day of cultivation.

The main advantages of this technology are [107]: immobilization of cells, which allows them to live and be active for a long time; immobilization via adsorption (as opposed to inclusion in gel or covalent binding) avoids the cost of purchasing expensive gels and prevents cell release due to weak binding to carriers; the use of a non-toxic cheap and affordable natural matrix, *Luffa* sponge with a lot of free pores for new cells which provides stable contact surface in prolonged re-use; using whey as a substrate, which is a cheap by-product of the dairy industry.

Cultivating the immobilized in Ca-polygalacturonate *G. fujikuroi* cells in a fluidized bed reactor in a medium containing glucose and ammonium chloride (carbon/nitrogen ratio 38.6), rice flour (2 g/l) at pH 5.0 and 30 °C was accompanied by a synthesis of 3.9 g/l of gibberellic acid, which is three times higher compared to the values established for the suspension culture under similar conditions of cultivation [111].

Immobilized on sponge cubes *F. moniliforme* (Egyptian local isolate) cells in a medium based on milk permeate synthesized 1.93 g/l gibberellic acid, while free cells produced only 1.6 g/l. One-time replacement of the nutrient medium after six days of immobilized cell cultivation was accompanied by an increase in the concentration of gibberellic acid to 2.2 g/l [101].

Synthesis of gibberellic acid on industrial waste. Industrial wastes as substrates for the production of gibberellic acid are used predominantly in solid-phase cultivation.

Table 4 shows data on biosynthesis of gibberellic acid on different substrates in solid phase cultivation. According to Table 4, the highest rates of synthesis of gibberellic acid (105 g/kg) were achieved using *Jatropha* press cake as a substrate [5]. In these studies, *F. moniliforme* NCIM 1100 was used as a producing strain. *Jatropha* press cake is a biodiesel production waste; the seed oil of this plant is transetherified into biodiesel.

Press cakes are relatively useless lignocellulosic substrate containing 15% cellulose and 30% lignin. In addition, these wastes are toxic because of the presence of phorbol ethers and require detoxification before use as animal feed. It should be noted that the level of synthesis of gibberellic acid by the strain *F. moniliforme* NCIM 1100 is the highest presently for solid-phase cultivation of producers [5].

In other studies [4], strains *G. fujikuroi* LPB 02, LPB 05, LPB 06, LPB Bca and

Table 4. **Production of gibberellic acid under conditions of solid-phase cultivation**

Substrate	Intensification approaches	Cultivation conditions	Synthesis indexes	Source
Wheat flour	Additional nutrition	50 l fermenter	3 g/kg	[112]
Coffee husks and manioc pulp	Optimized cultivation conditions	Flasks	492.5 mg/kg	[113]
Wheat flour and starch	Optimized cultivation conditions	Flasks	4.5–5 g/kg	[114]
Citrus pulp	Method of inoculum preparation	Flasks	5.9 g/kg	[4]
Jatropha seed press cake	Optimized cultivation conditions	Flasks	105 g/kg	[5]
Shea nut shells	Optimized cultivation conditions	Flasks	1.8 mg/ml	[109]
Citrus pulp	Levels of aeration	Column reactor	7.34 g/kg	[115]

F. moniliforme LPB 03 were used as producers of gibberellic acid in solid-phase cultivation on such industrial waste as citrus pulp, soybean bran, cane pulp, soybean and coffee bean husks, and manioc pulp. The cultivation of strains producing gibberellic acid was carried out on both mono- and mixed industrial waste. In the mixed substrates, the ratio of mono substrates was 1: 1.

The highest concentration of gibberellic acid was observed under cultivation of all strains on citrus pulp (3.1–5.7 g/kg), as well as on a mixture of citrus pulp and coffee husks (about 3 g/kg). For further research, the strain *F. moniliforme* LPB 03 was selected because it was characterized by the highest level of synthesis of the final product. The following experiments showed that *F. moniliforme* LPB 03 inoculum cultivated on citrus pulp extract with the addition of 35 g/l sucrose, synthesized 5.9 g gibberellic acid per kg of citrus pulp at the third day of cultivation [4]. Subsequently [115], the same authors found that the level of synthesis of gibberellic acid on a citrus pulp depends on the level of aeration: during the cultivation of *F. moniliforme* LPB 03 in a column reactor, the amount of the target product increased to 7.34 g/kg.

So, the methodology of microbial synthesis of gibberellic acid has recently developed much. If in the first technologies of submerged cultivation A_3 concentration did not exceed 0.3–0.5 g/l, now it reaches 5–15 g/l. A significant advantage of solid-phase cultivation compared to the submerged fermentation is the possibility of bioconversion of industrial waste into economically valuable phytohormones. In addition, there are other advantages that make the process of solid phase cultivation commercially viable: high output of the final product, lower energy consumption, and lesser environmental impact. At the same time, the final product yield is sufficient to compensate for higher allocation costs, thereby reducing the cost of gibberellic acid.

Synthesis of phytohormones by the producers of surfactantas. Acinetobacter calcoaceticus IMV B-7241, Rhodococcus erythropolis IMV Ac-5017 *and Nocardia vaccinii IMV B-7405.* In recent years, there has been evidence that some microorganisms synthesize other metabolites (enzymes, bacteriocins, polysaccharides, polyhydroxyalkanoates) simultaneously with surfactants under certain conditions of cultivation [116–118]. The ability of strains to synthesize a complex of metabolites with a variety of biological properties greatly extends the scope of their practical application.

Our studies have shown that *Rhodococcus erythropolis* IMV Ac-5017 *Acinetobacter calcoaceticus* IMV B-7241 and *Nocardia vaccinii* IMV B-7405 have antimicrobial properties against a number of microorganisms, including phytopathogenic bacteria of genera *Pseudomonas* and *Xanthomonas* [119]. Moreover, the water phase remaining after the extraction of surfactant from the supernatant of the culture liquid activated the cell growth of several phytopathogenic bacteria. Such unexpected results allowed us to assume that the producers of surfactants synthesize also other biologically active substances, in particular, phytohormones.

Table 5 shows the data on the synthesis of phytohormones by *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV B-7405 cultivated on various carbon substrates, including processed sunflower oil.

Presently, there are many publications about the synthesis of phytohormones by microorganisms. The cultivation media,

Table 5. **Influence of cultivation conditions of *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV B-7405 on the synthesis of phytohormones**

Substrate	Strain	Concentration (μg/l)		
		auxins	cytokinins	abscisic acid
Ethanol	IMV B-7241	104.2	3.5	1.3
	IMV Ac-5017	84.3	–	3.6
Glycerol	IMV B-7241	122.0	363.9	0.9
	IMV B-7405	139.9	–	3.1
N-hexadecan	IMV Ac-5017	44.8	21.4	3.2
Refined sunflower oil	IMV B-7241	39.6	75.1	–
	IMV B-7405	770.4	348.0	12.6
	IMV Ac-5017	19.4	17.1	1.5
Sunflower oil waste after frying meat	IMV B-7241	83.2	43.6	2.3
	IMV B-7405	23.3	53.9	–
	IMV Ac-5017	91.3	37.8	8.8
Sunflower oil waste after frying potatoes	IMV B-7405	84.7	15.9	–

Note: « – » — not found

however, contain glucose, sucrose, dextrose, glucuronic acid, peptone, tryptone, mannitol as a source of carbon and exogenously introduced tryptophan as a precursor of auxins biosynthesis (Table 1). Our studies have shown for the first time the possibility of producing phytohormones in cheap media using toxic industrial waste as substrates (in particular, waste oil) without the addition of tryptophan. There are also some reports on the simultaneous synthesis of phytohormones and metabolites with antimicrobial properties in the literature (Table 2), but these antimicrobial metabolites are mostly antifungal (rarely nematocidal). If antibacterial they are antibiotics and consequently, in this case, the resistant forms of microorganisms may rapidly appear. The mechanism of antimicrobial activity of surfactants, unlike antibiotics, prevents the emergence of bacteria resistant to them.

We have also for the time established [120] the ability of surfactant producers to synthesize phytohormones. The formation of indole-3-acetic acid by bacteria (mainly by representatives of the genus *Rhodococcus*), isolated from soils contaminated with hydrocarbons and heavy metals was reported only in 2016 [121]. However, the ability to synthesize surfactants was determined by the emulsification index and decrease in surface tension, which turned out to be insignificant — up to 60–65 mN/m (compared with 30–35 mN/m by the surfactant producers).

Ability of *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N. vaccinii* IMV B-7405 to simultaneously synthesize surfactants and phytohormones when cultivated on different substrates, including cheap industrial waste, allows developing economically profitable non-waste technology for obtaining complex microbial preparations promising for use in plant growing.

Thus, review of the literature on the microbial synthesis of phytohormones confirms the general conclusions drawn in [23]:

– many microorganisms are capable of synthesizing phytohormones of the three main groups of hormonal stimulants: auxins, cytokinins and gibberellins. Moreover, representatives of the same genus and even species are capable of synthesizing several hormones at once;

– microorganisms, capable of synthesizing phytohormones, also stimulate the growth of higher plants, which is confirmed in many studies;

– there is no confirmed association of phytohormonal activity with the pathogenicity of microorganisms or their epiphytic (endophytic) lifestyle;

– the ability to synthesize phytohormones differs greatly not only within the same genus, but even within a species;

– microorganisms synthesize phytohormones as secondary metabolites.

In addition, there are a few reports on simultaneous synthesis of phytohormones and specific final products. This does not support the generally accepted in the biotechnological research concept of "one producer — one product" which focuses only on increasing the synthesis of the main product.

Individual literary data and our own results show the promise in creating multifunctional microbial preparations with diverse biological properties. These preparations would include a complex of biologically active substances, among them phytohormones of different chemical nature, synthesized together. A few of the preparations we have developed at the present time are available in Ukraine (Ecovital, Ecophosphoryn, Azotobacteryn-K, Rizobin, Ecoryz, Averkom-nova) [1, 77].

Increasing IAA synthesis to 1.6–2 g/l by yeast *R. paludigenum* DMKURP301 and bacteria *P. agglomerans* PVM is a reason to hope that this phytohormone and gibberellic acid will be obtained by microbial synthesis in the near future.

Recent significant progress in increasing the production of gibberellic acid in both submerged and solid-phase cultivation on various substrates, including low-cost industrial waste, significantly reduce the cost of the final product. Isolated reports on the study of microbial synthesis of gibberellins A_4 and A_7 indicate a potential opportunity for implementing technologies for the production of these biologically active gibberellins on an industrial scale.

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Метою огляду було проаналізувати сучасні дані літератури і результати власних досліджень синтезу ауксинів, цитокінінів, гіберелінів як асоційованими з рослинами мікроорганізмами (ризосферними, ендофітними, азотфіксувальними, фітопатогенними), так і тими, які не беруть участі у такій взаємодії. Виявлена у широкого кола мікроорганізмів здатність до утворення фітогормонів, а також успіхи у підвищенні ефективності мікробного синтезу індоліл-3-оцтової кислоти свідчать про можливість такого способу її одержання замість екстракції з рослин або хімічного синтезу. Досягнення останнього десятиліття щодо інтенсифікації синтезу гіберелінової кислоти за умов глибинного і твердофазного культивування продуцентів дають змогу суттєво знизити собівартість цього фітогормону, одержуваного біотехнологічним способом.

Здатність мікроорганізмів до одночасного синтезу фітогормонів та інших біологічно активних сполук з антимікробною, нематоцидною та ін. активністю підтверджує можливість створення комплексних поліфункціональних мікробних препаратів з різноманітними біологічними властивостями з метою використання у рослинництві для стимуляції росту рослин і контролю чисельності шкідників.

Ключові слова: фітогормони, мікробний синтез, комплексні мікробні препарати.

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Целью обзора было проанализировать современные данные литературы и результаты собственных исследований синтеза ауксинов, цитокининов, гиббереллинов как ассоциированными с растениями микроорганизмами (ризосферными, эндофитными, азотфиксирующими, фитопатогенными), так и не принимающими участия в таком взаимодействии. Обнаруженная у широкого круга микроорганизмов способность к образованию фитогормонов, а также успехи в повышении эффективности микробного синтеза индолил-3-уксусной кислоты свидетельствуют о возможности такого способа ее получения вместо экстракции из растений или химического синтеза. Достижения последнего десятилетия по интенсификации синтеза гиббереллиновой кислоты в условиях глубинного и твердофазного культивирования продуцентов позволяют существенно снизить себестоимость этого фитогормона, получаемого биотехнологическим способом.

Способность микроорганизмов к одновременному синтезу фитогормонов и других биологически активных соединений с антимикробной, нематоцидной и др. активностью подтверждает возможность создания комплексных полифункциональных микробных препаратов с различными биологическими свойствами с целью использования в растениеводстве для стимуляции роста растений и контроля численности вредителей.

Ключевые слова: фитогормоны, микробный синтез, комплексные микробные препараты.