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MICROBIAL CO-CULTIVATION: DISCOVERY OF NOVEL SECONDARY METABOLITES WITH DIFFERENT BIOLOGICAL ACTIVITIES

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In recent decades overuse and misuse of antibiotics as well as social and economic factors have accelerated the spread of antibiotic-resistant bacteria, making them a major problem for humanity. One of the most effective approaches to the discovery of new secondary antimicrobial metabolites is co-cultivation of microorganisms, in which the producer of the target products is grown together with competitive microorganisms (inductors), in response to the presence of which silent biosynthetic genes of the producer strain are activated and an increase in the biological activity of the synthesized secondary metabolites and/or even the synthesis of new metabolites is observed. The review summarizes the current literature data on the co-cultivation of antimicrobial substances producers with competitive microorganisms, which results in the synthesis of new metabolites with antimicrobial and cytotoxic activity, not typical for monocultures. During the co-cultivation of fungi, bacteria, and fungi with bacteria, the synthesis of new antimicrobial and anticancer metabolites, which are classified as alkaloids, phenylpropanoids, macrolides, polyketides, cyclopeptides, terpenoids, anthraquinones, and steroids, is observed. These data indicate that the mixed fermentation of microorganisms is a simple, cheap, and quite effective way to obtain new metabolites that are promising for use in medicine.

Key words: co-cultivation; antimicrobial products; anticancer agents.

Nowadays, the number of studies on the development of new antibiotic drugs is decreasing, due to the increasing resistance of pathogenic microorganisms to them due to their excessive use in medicine and agriculture. This situation can lead to dangerous consequences for the world's population, so novel safe natural products are needed [1].

Microorganisms from various terrestrial and marine habitats are a source of new bioactive natural compounds, but one of the problems in the process of discovering new microbial metabolites is the re-isolation of already known compounds. In addition, the biological activity of microbial secondary metabolites depends on the conditions of cultivation of the producers, so the development of approaches that allow to obtain a final product with stable specified properties is a priority in the development of current biotechnology. Recent advances in microbial genomics have clearly demonstrated that the biosynthetic potential of microorganisms as producers of metabolites with unique properties is much higher than expected, because a significant number of microbial gene clusters may remain silent under typical cultivation conditions [2, 3].

At present, both traditional physiological approaches (optimization of cultivation conditions, introduction of exogenous precursors into the culture medium) and methods of genetic and metabolic engineering are being implemented to increase the biosynthetic ability of producers of practically valuable compounds. The application of the above mentioned methods made it possible to effectively activate silent genes as one of the mechanisms for producing new secondary metabolites. An alternative to the chemical

modification of known compounds to increase their antimicrobial activity is the strategy of co-cultivation of microorganisms, which is superior to other approaches in terms of cost and convenience, since it does not require expensive reagents or methods of gene manipulation [4–6]. In addition, the use of co-cultivation methods, in which the producer of the final product is cultivated together with competitive microorganisms (inductors), is a promising approach to increase the activity of existing and/or search for new compounds that are not inherent in axenic cultures (monocultures), metabolites with strong antimicrobial [7, 8], antagonistic [9, 10], and cytotoxic [11] effects.

This review aimed to summarize current literature data on the co-cultivation of antimicrobial compound producers with competitive microorganisms, that results in the synthesis of new biologically active metabolites that are not typical for monocultures.

Novel secondary metabolites with antimicrobial activity

In the works on the co-cultivation of microorganisms published over the past 5–7 years, it has been reported the production of alkaloids [12, 13, 19, 20], phenylpropanoids [14–16], macrolides [7, 12, 27, 28], polyketides [22, 26, 31, 49], cyclopeptides [10], terpenoids [17, 18] and others [21, 23, 24, 29, 30]. It should be noted that these novel synthesized metabolites demonstrate antibacterial and antifungal properties and are not synthesized in monocultures of microorganisms.

The synthesis of new antimicrobial metabolites is reported in the co-cultivation of fungi-fungi [11-18], bacteria-bacteria [26-31, 49], and fungi-bacteria [7, 10, 19-24].

Co-Cultures between Fungal Strains

A new alkaloid, identified as aspergicin, was isolated from a mixed fermentation of *Aspergillus* FSY-01 and *Aspergillus* FSW-02, accompanied by neoaspergic acid and ergosterol [12]. It was found that aspergicin has high antimicrobial activity against bacterial test cultures (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Bacillus dysenteriae*, *Bacillus proteus*, *Escherichia coli*): the minimum inhibitory concentrations (MIC) were 15.62–62.5 μg/ml.

A new nonadride derivative (byssochlamic acid imide) isolated from the co-culture of *Phomopsis* sp. K38 and *Alternaria* sp. E33 was characterized by antifungal activity against *Fusarium graminearum* and *Fusarium oxysporum* with MIC values of 50 and 60 μg/ml, respectively [13].

Phenylpropanoids are a large and structurally diverse group of secondary metabolites characterized by the presence of a C6-C3-phenolic scaffold, that plays a crucial role in a wide spectrum of biological and pharmacological activities. New metabolites of this group were obtained by co-cultivation of fungi of the genus *Penicillium* [14-16]. The metabolites show high antibacterial and antifungal activity. For example, ten citrinine analogs, including a new dimer, secopentitrinol A and pencitrinol L, were isolated from the co-culture of *Aspergillus sydowii* EN-534 and *Penicillium citrinum* EN-535 [14]. The new compounds showed antimicrobial activity against *Vibrio parahaemolyticus* and *Edwardsiella ictaluri*: the minimum inhibitory concentrations were 32-64 μg/ml [14], which is lower than those reported for penixilarins A-C [15]. Penixilarins A-C isolated from the mixed fermentation of *Penicillium crustosum* PRB-2 and *Xylaria* sp. HDN13-24, had antibacterial activity against *Mycobacterium phlei*, *B. subtilis* and *V. parahaemolyticus* (MIC range from 6.25 to 100 μg/ml) [15].

In addition, a new phenylpropanoid, named secopenicillide C, was identified from the co-culture of *Penicillium pinophilum* FKI-5653 and *Trichoderma harzianum* FKI-5655, which was characterized by antimicrobial activity against *E. coli* and *Micrococcus luteus* with MIC values of 16 and 64 μg/ml, respectively [16].

A new terpenoid derivative, asperterrein, was found among the newly synthesized terpenoids by co-cultivation of *Aspergillus terreus* EN-539 and *Paecilomyces lilacinus* EN-531 [17]. The compound showed antibacterial activity against *Alternaria brassicae*, *E. coli*, *Physalospora piricola* and *S. aureus* with MIC values from 4 to 64 μg/ml.

The most effective antimicrobial agents of the new compounds synthesized as a result of co-cultivation of *Penicillium bilaiae* MA-267 and *Penicillium chermesinum* EN-480 were two new meroterpenoid derivatives - chermebilenes A and B [18]. The MIC of chermebilene A against *Ceratobasidium cornigerum* and *Edwardsiella tarda* was 0.5 and 0.25 μg/ml, respectively, which makes this compound perspective for use as an antimicrobial agent in clinical practice.

During the co-cultivation of *Penicillium fuscum* (Sopp) Raper & Thom and *Penicillium camembertii/slavigerum* Thom, five new

16-membered macrolides (berkeleylactones A, B, D, E, G) were synthesized, including berkeleylactone A, which demonstrated the most effective antimicrobial effect compared to the known macrolides: MIC against strains of *S. aureus*, *Bacillus anthracis*, *Streptococcus pyogenes*, *Candida albicans* and *Candida glabrata* were 1-2 μg/ml [11].

Co-Cultures between Fungi and Bacteria

The novel alkaloid compound pulicatin H, isolated from the co-culture of the fungus *P. citrinum* and bacterium *Pantoea agglomerans*, was characterized by high antifungal activity. The MIC values for *P. citrinum*, *Aspergillus niger*, and *C. albicans* were 25, 8.4, and 50 μg/ml, respectively [19]. Also, new alkaloids, dihydrolateropyrone and fusatricinones A-D, were identified from the mixed-fermentation of *Streptomyces lividans* and *Fusarium tricinctum*, and were characterized by antibacterial activity against *S. aureus* and *Pseudomonas aeruginosa* [20], but the authors of this article did not provide the antimicrobial activity of these compounds.

As a result of the co-cultivation of *Streptomyces rochei* MB037 and *Rhinocladiella similis* 35, the macrolides borelidin J and K were obtained, which proved to be effective antimicrobial agents against *S. aureus*: minimum inhibitory concentrations were 0.195 and 1.563 μg/ml, respectively [7].

It is known from the literature that only one new antimicrobial steroid metabolite (ergosterol derivative) was synthesized during the co-cultivation of *Bacillus wiedmannii* Com1 and *Pleosporales* sp. F46 [21]. This compound had antimicrobial activity against *S. aureus*: microbial growth inhibition zone and minimum inhibitory concentration were 71 mm and 100 μg/ml, respectively.

Moderate antibacterial activity against *Streptomyces coelicolor* and *S. lividans* (MIC 1000 and 250 μg/ml, respectively) was demonstrated by a new polyketide fumigermin synthesized by the mixed-fermentation of *Aspergillus fumigatus* with the bacteria *Streptomyces iranensis*, *S. coelicolor, S. lividans*, and *Streptomyces rapamycinicus* [22].

Under co-cultivation of *Bacillus amyloliquefaciens* ACCC11060 and *Trichoderma asperellum* GDFS1009, the synthesis of new cyclopeptides BT1 and BT2 was observed [10], which inhibited the growth of *Bacillus cinerea* by 47.86% and 66.86%, respectively.

New antimicrobial compounds (marcocarpone C, 2-(carboxymethylamino) benzoic acid and (-)-citreoisocoumarinol) were obtained from the co-culture of *B. subtilis* 168 trpC2 and *Fusarium tricinctum* [23]. Macrocarpon C and 2-(carboxymethylamino) benzoic acid are characterized by high antimicrobial activity against bacteria *B. subtilis* 168 trpC2, *S. aureus* ATCC 25697, *Streptococcus pneumonia* ATCC 49619, *E. coli* ATCC 25922, *P. aeruginosa* B 63230 with MIC in the range of 2-64 μg/ml.

During the co-culture of *Cladosporium* sp. WUH1 and *B. subtilis* CMCC (B) 63501, a new compound (trihydroxybutyl ester of 4-carboxydiorcinol) with antibacterial activity was synthesized: MIC against *Klebsiella pneumonia*, *B. subtilis*, *E. coli, S. aureus, S. epidermidis* were 16, 64, 64, and 32 μg/ml, respectively [24].

Co-Cultures between Bacterial Strains

As a result of the co-cultivation of *Tsukamurella pulmonis* TP-B0596 and *S. coelicolor* S-552, a new polyketide alchivemycin A was obtained [31]. The minimal inhibitory concentration of this polyketide against *Micrococcus luteus* TP-B100 was 0.06 μg/ml. The new antimicrobial polyketide glycoside gordonic acid was synthesized in the co-culture of *Streptomyces tendae* KMC006 and *Gordonia* sp. KMC005 [49]. At a concentration of gordonic acid of 10 μg/disc, the growth inhibition zones of *M. luteus* KCCM11548 and *Enterococcus hirae* KCCM11768 were 1.5–2.5 mm.

In 2018, two new polyketides (janthinopolyenemycins A and B) were isolated from a co-culture of two strains of *Janthinobacterium* spp. ZZ145 and ZZ148 [26]. Both polyketides exhibited antifungal activity against *C. albicans* (MIC 15.6 μg/ml). It was found that janthinopolyenemycin congeners are active against methicillin-resistant *S. aureus* and *E. coli*.

In recent years, four new lactams have been discovered as a result of co-cultivation of bacteria [27, 28]. One of these compounds is umezawamide A, synthesized during the co-cultivation of *T. pulmonis* TP-B0596 with *Umezawaea* sp. RD066910 [27]. Umezawamide A is characterized by moderate antimicrobial activity against *C. albicans*: at a concentration of 5 μg/disc, the growth inhibition zone was 1.7 mm. Under the co-cultivation of *Actinosynnema mirum* NBRC 14064 with *T. pulmonis* TP-B0596, antimicrobial mirilactams C, D, E were synthesized [28], and

they exhibited antimicrobial activity against *C. albicans, Bacillus cereus, S. aureus* MSSA (activity parameters are not given).

An effective antimicrobial metabolite is keyicin, synthesized as a result of co-cultivation of *Micromonospora* sp. WMMB-235 and *Rhodococcus* sp. [29], the minimum inhibitory concentrations of keyicin against *Mycobacterium* sp., *Rhodococcus* sp., *B. subtilis, S. aureus* were 2-8 μg/ml.

During the co-cultivation of *T. pulmonis* TP-B0596 with *Streptomyces nigrescens* HEK616, a new compound spirohemiaminal was obtained, which was characterized by antimicrobial activity against the test cultures *B. subtilis, E. coli, S. aureus*: at a concentration of 100 μg/disc, the growth inhibition zones were 2-10 mm [30].

Table 1 summarizes the data on the synthesis of new antimicrobial metabolites during the co-cultivation of fungi, bacteria, and fungi with bacteria. These data indicate that the co-cultivation of microorganisms is a simple, cheap, and quite effective way to obtain new metabolites with significant antimicrobial activity.

Figure 1 illustrated the classes of new antimicrobial compounds synthesized during the co-culture of microorganisms. Metabolites based on co-cultures of bacteria and fungi were identified as alkaloids, anthraquinones, macrolides, phenylpropanoids, polyketides, cyclopeptides, terpenoids, with the largest proportion being macrolides, alkaloids, phenylpropanoids, and polyketides.

Antimicrobial compounds, such as phenylpropanoids and terpenoids, were identified only on the basis of co-cultures of fungi. At the same time, metabolites characterized as alkaloids were synthesized as a result of co-cultivation of both bacterial and fungal cultures.

Novel secondary metabolites with cytotoxic and antimicrobial activity

Studies on the co-cultivation of microorganisms published in the last 5–7 years have reported the production of new alkaloid compounds [32, 33, 39, 42–45], phenylpropanoids [40, 41], macrolides [11], polyketides [35, 48], cyclopeptides [36, 41, 46, 47], terpenoids [34], and compounds of others [11, 34, 37, 38, 41–43]. It should be noted that some of the new metabolites exhibit both cytotoxic and antimicrobial activity [11, 36, 41, 44, 45], and some — only cytotoxic activity [32–35, 37–40, 42, 43, 46–48].

The synthesis of new metabolites with both cytotoxic and antimicrobial activity is reported in the co-culture of fungi-fungi [11, 32–38], bacteria-bacteria [44–48], and fungi with bacteria [39–43].

Co-Cultures between Fungal Strains

Five new prenylated indole alkaloids were isolated from the mixed fermentation of *Aspergillus sulphureus* KMM 4640 and *Isaria felina* KMM 4639: 17-hydroxynotoamide D, 17-O-

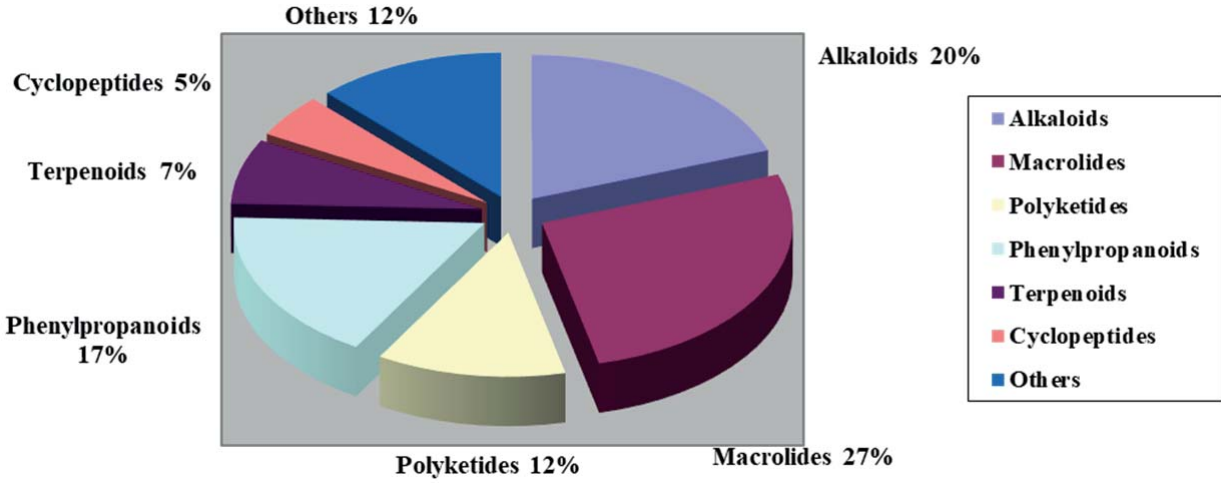

Fig. 1. **The number of new metabolites with antimicrobial activity synthesized as a result of co-cultivation of microorganisms [7, 10–24, 26–31, 49]**

Table 1

Characterization of new antimicrobial compounds synthesized during mixed cultivation of microorganisms

Microorganisms	Carbon source	Novel secondary metabolites		Test-cultures for determining antimicrobial activity	Minimum inhibitory concentration, μg/ml	References
		Compounds	Classes			
Co-cultivation of fungal strains						
Aspergillus FSY-01 + *Aspergillus* FSW-02	Glucose (GYP- broth)	Aspergicin	Alkaloid compound $CH_3COOC_2H_5$	*Staphylococcus aureus*	62.5	[12]
				Staphylococcus epidermidis	31.25	
				Bacillus subtilis	15.62	
				Bacillus dysenteriae	15.62	
				Bacillus proteus	62.5	
				Escherichia coli	31.25	
Penicillium pinophilum FKI-5653 + *Trichoderma harzianum* FKI-5655	Brown rice, glycerin, yeast extract, potato-dextrose broth	Secopenicillin C	Secotype of the penicillin compound $C_{20}H_{22}O_6$	*Escherichia coli*	16	[16]
				Micrococcus luteus	64	
Penicillium fuscum (Sopp) Raper & Thom + *Penicillium camembertii/ clavigerum* Thom	Potato dextrose medium (dextrose, potato broth)	Berkeleylactone A Berkeleylactone B Berkeleylactone D Berkeleylactone E Berkeleylactone G	Macrolide $C_{23}H_{36}O_{10}S$ Macrolide $C_{20}H_{30}O_8$ Macrolide $C_{20}H_{32}O_7$ Macrolide $C_{20}H_{33}O_8$	*Staphylococcus aureus* 13709	4/13/45/24	[11]
				Streptococcus pyogene	119/50/>90/>50	
				Candida glabrata	31/>400/>90/ >50	
				Bacillus subtilis	31/100/ >90/>50	
				Candida albicans	>119/>400/ >90/>50	
				Bacillus anthracis	8/26/>90/24* * — MIC Berkeleylactone B/ Berkeleylactone D/ Berkeleylactone E/ Berkeleylactone G	
Aspergillus sydowii EN-534 + *Penicillium citrinum* EN-535	Rice medium (Rice, corn flour, peptone, monosodium glutamate)	Secopenicitrinol A Penicitrinol L	Citrine dimer Penicillin derivatives $C_{23}H_{26}O_6$ Citrine monomer Penicillin derivatives $C_{14}H_{18}O_5$	*Edwardsiella ictaluri*	64/64*	[14]
				Vibrio parahaemolyticus	32/64* * — MIC Secopenicitrinol A/ penicitrinol L	

Table 1 (Continued)

Microorganisms	Carbon source	Novel secondary metabolites		Test-cultures for determining antimicrobial activity	Minimum inhibitory concentration, μg/ml	References
		Compounds	Classes			
1	2	3	4	5	6	7
Penicillium crustosum PRB-2 + *Xylaria* sp. HDN13-249	Starch, maltose, sucrose, yeast extract	Penicillarin A Penicillarin B Penicillarin C	Alkyl aromatic compounds Penicillin derivatives $C_{33}H_{49}O_6$ $C_{33}H_{49}O_6S$ $C_{32}H_{39}O_5$	*Mycobacterium phlei*	>200/>200/6.25*	[15]
				Bacillus subtilis	>200/100/>200*	
				Vibrio parahemolyticus	>200/>200/12.5* * — MIC of penicillars A/B/C	
Aspergillus terreus EN-539 + *Paecilomyces lilacinus* EN-531	Not given	Asperterrein	A derivative of terrein Cycloalkane $C_9H_{14}O_2$	*Escherichia coli*	32	[17]
				Staphylococcus aureus	32	
Phomopsis sp. K38 + *Alternaria* sp. E33	Glucose, Yeast extract, Pepton	Imide (−)-bys-sochlamic acid	Nonadrenaline derivative Alkaloid $C_{18}H_{21}O_5N$	*Fusarium graminearum*	50	[13]
				Fusarium oxysporum	60	
Penicillium bilaiae MA-267 + *Penicillium chermesinum* EN-480	Rise, Pepton, Corn syrup	Chermebilene A Chermebilen B	Derivatives Meroterpenoids $C_{35}H_{56}O_4Na$ $C_{25}H_{40}O_9N$	*Edwardsiella tarda*	0.25	[18]
				Ceratobasidium cornigerum	0.5	
Co-cultivation of fungi and bacteria						
Fusarium tricinctum + *Bacillus subtilis* 168 trpC2	Rice medium	Macrocarpon C 2-(carboxy-methylamino) benzoic acid	Heterocyclic compound $C_{13}H_{12}O_4$ Derivative of benzoic acid $C_9H_9NO_4$	*Bacillus subtilis* 168 trpC2	8–16*	[23]
				Staphylococcus aureus ATCC 25697	2–8*	
				Staphylococcus aureus ATCC 29213	2–8*	
				Streptococcus pneumonia ATCC 49619	2–8*	
				Escherichia coli ATCC 25922	2–8*	
				Enterococcus faecalis UW 2689	2–8*	

Table 1 (Continued)

1	2	3	4	5	6	7
				Pseudomonas aeruginosa B 63230	> 64* * — macrocarpone C and 2-carboxy-methylamino-benzoic acid exhibit the same antimicrobial activity against the given test-cultures	
Trichoderma asperellum GDFS1009 + *Bacillus amyloliquefaciens* ACCC11060	Meat extract, Pepton	Complex BT1 and BT2	BT1: 4-hydroxybenzoic acid, apigenin, glycine betaine, malic acid and nicotinic acid BT2: indole-3-acetic acid, indole-3-carboxylic acid, phenacillamine, trans-3-coumaric acid and transcinnamic acid	*Botrytis cinerea*	Growth inhibition under the action of BT1 47.86%, under the influence of BT2 — 66.86%	[10]
Pleosporales sp. F46 + *Bacillus wiedmannii* Com1	Rice medium	Not given	Steroid compound	*Staphylococcus aureus*	100	[21]
Rhinocladiella similis 35 + *Streptomyces rochei* MB037	Malt extract, dextrose	Borelidin J Borelidin K	Macrolides $C_{28}H_{45}NO_7$ $C_{29}H_{46}NO_7$	Methicillin-resistant *Staphylococcus aureus* strain	0.195 1.563	[7]
Fusarium tricinctum + *Streptomyces lividans* TK24	Not given	Fusatricinones A-D Dihydrolatero-pyrone	Petroquinone dimers: Fusacitron A $C_{31}H_{24}O_{16}$ Fusacitron B $C_{30}H_{22}O_{16}$ Fusacitron C $C_{32}H_{26}O_{16}$ Fusacitron D $C_{32}H_{26}O_{16}$ A derivative of lateropinone $C_{15}H_{12}O_8$	*Staphylococcus aureus* *Pseudomonas aeruginosa*	all compounds exhibit antimicrobial activity against the test cultures given, activity parameters are not given	[20]

Table 1 (Continued)

1	2	3	4	5	6	7
Cladosporium sp. WUH1 + *Bacillus subtilis* CMCC(B) 63,501	Potato broth, dextrose, yeast extract, peptone	Trihydroxybutyl ester of 4-carboxydiorcinol	Ester $C_4H_8O_3$	*Bacillus subtilis*	32	[24]
				Escherichia coli	64	
				Staphylococcus aureus	64	
				Staphylococcus epidermidis	32	
				Klebsiella pneumoniae	16	
				Pseudomonas aeruginosa	>64	
Penicillium citrinum + *Pantoea aggolomerans*	Potato broth, dextrose, yeast extract, peptone	Pulicatin H Pulicatin F	Siderophore derivatives Alkaloid $C_{13}H_{13}NO_3S$ $C_{10}H_8O_2N_2S$	*Penicillium citrinum*	25/53*	[19]
				Pantoea aggolomerans	>200/>200*	
				Aspergillus niger	8.4/22.6*	
				Candida albicans	>50/>50* * — MIC pulicatin H/ pulicatin F	
Aspergillus fumigatus + *Streptomyces rapamycinicus* + *Streptomyces iranensis* + *Streptomyces coelicolor* + *Streptomyces lividans*	Lactose, Glucose, arginine	Fumigermin	Microbial α-pyrone (polyketide) $C_{11}H_{15}O_3$	*Streptomyces coelicolor*	1000	[22]
				Streptomyces lividans	250	
Co-cultivation of bacterial strains						
Streptomyces endus S-552 + *Tsukamurella pulmonis* TP-B0596	Starch, glycerin, glucose, yeast extract	Alchivemycin A	Polyketide $C_{35}H_{53}NO_{10}$	*Bacillus subtilis* ATCC 6633	40	[31]
				Escherichia coli NIHJ JC-2	>50	
				Staphylococcus aureus 209P JC-1	>50	
				Micrococcus luteus TP-B100	0,06	
				Candida albicans TP-F0176	>50	
				Saccharomyces cerevisiae TP-F0176	>50	

Table 1 (End)

1	2	3	4	5	6	7
Umezawaea sp. RD066910 + *Tsukamurella pulmonis* TP-B0596	Starch, glycerin, glucose, yeast extract	Umezawamide A	Polycyclic tetramate macrolactam $C_{29}H_{40}N_2O_6$	*Candida albicans*	Growth inhibition zone of 1.7 mm (5 μg/disc)	[27]
Actinosynnema mirum NBRC 14064 + *Tsukamurella pulmonis* TP-B0596	Starch, glycerin, glucose	Mirilactam C Mirilactam D Mirilactam E	Monocyclic polyene macrolactams $C_{27}H_{37}NO_6$	*Candida albicans*	all compounds exhibit antimicrobial activity against the test cultures given, activity parameters are not given	[28]
				Bacillus cereus		
				Staphylococcus aureus MSSA		
Streptomyces tendae KMC006 + *Gordonia* sp. KMC005	Malt extract	Gordonic acid	Polyketide glycoside $C_{24}H_{36}NO_6$	*Micrococcus luteus* KCCM11548	Growth inhibition zone of 2.5 mm (10 μg/disc)	[49]
				Enterococcus hirae KCCM11768	Growth inhibition zone of 2.5 mm (10 μg/disc)	
Streptomyces nigrescens HEK616 + *Tsukamurella pulmonis* TP-B0596	Not given	Spirohemiaminal	Lipid [5,5]-spiroge mimetics $C_{18}H_{34}NO_2$	*Bacillus subtilis*	Growth inhibition zone of 12 mm (30 μg/disc)	[30]
				Escherichia coli	Growth inhibition zone of 12 mm (30 μg/disc)	
				Staphylococcus aureus	Growth inhibition zone of 12 mm (30 μg/disc)	
Janthinobacterium spp. ZZ145 + *Janthinobacterium* spp. ZZ148	Rice medium	Janthino-polyenemycin A Janthino-polyenemycin B	Polyketides $C_{26}H_{36}O_3$	*Candida albicans*	15.6* * — compounds exhibit the equal antimicrobial activity against the test-culture	[26]
Micromonospora sp. WMMB-235 + *Rhodococcus* sp. WMMA185	Yeast extract, malt extract, dextrose	Keyicin	Nitroglycosylated anthracycline Anthraquinone $C_{75}H_{108}N_4O_{34}$	*Mycobacterium* sp.	activity parameters are not given	[29]
				Rhodococcus sp.		
				Bacillus subtilis	8	
				Staphylococcus aureus MSSA	2	

ethylnotoamide M, 10-O-acetylsclerotiamide, 10-O-ethylsclerotiamide, and 10-O-ethylnotoamide R [32]. The compound 17-O-ethylnotoamide M inhibited the growth of human prostate cancer cells 22Rv1 at concentration of 10 μM. The first natural 1,2,4-oxadiazin-6-one (sclerotiorumin C) and aluminiumneohydroxyaspergillin were isolated from the co-culture of fungi *Aspergillus sclerotiorum* and *P. citrinum* [33]. Aluminiumneohydroxyaspergillin exhibited high cytotoxicity against human histiocytic lymphoma U937 cell line (IC_{50} = 4.2 μm) and strong toxicity towards brine shrimp (LC_{50} = 6.1 μm).

New macrolides were synthesized after co-cultivation of *P. fuscum* (Sopp) Raper & Thom and *P. camembertii/slavigerum* Thom, including berkeleylactones A, C, F and A26771B [11]. The compounds exhibited cytotoxic activity against K-562, RPMI-8226, and CCRF-CEM leukemia cells with IC_{50} values of 10 μM and drastically reduced the viability of cancer cells by 38-85%.

Eight newly induced secondary metabolites were isolated during the co-cultivation of *Armillaria* sp. with *Epicoccum* sp. YUD 17002, including five protoiludane-type sesquiterpenoids and three aryl esters [34]. One of aryl ester exhibited moderate cytotoxicity against five human cancer cell lines (HL-60, A549, MCF-7, SMMC-7721, and SW480) with IC_{50} values ranging from 15.80 to 23.03 μM [34]. The newly synthesized polyketides, in particular, nafuredin B, identified from a co-culture of *Talaromyces aculeatus* and *Penicillium variabile*, exhibited higher activity against human tumor cell lines [35]. Nafuredin B demonstrated high cytotoxicity against human tumor HeLa, K562, HCT-116, HL-60, A549, and MCF-7 cell lines with IC_{50} values in the range of 1.2–9.8 μM, respectively. At the same time, a new cyclopeptide, lateritin, was identified after co-cultivation of *Ovadendron sulphureoochraceum* MIC 5759, *Ascochyta pisi* MIC 5620, *Emericellopsis minima* MIC 5835, *Cylindrocarpon destructans* MIC 5638, *F. oxysporum* MIC 5789, were characterized by cytotoxic activity against human tumor cell lines (BXPC-3, MCF-7, CNS SF268, NSC H460, KM20L2 and DU-145) with half maximal inhibitory concentration in the range of 1.7-2.0 μg/ml [36]. In addition to human tumor cell lines, the compound inhibited mouse leukemia P388 cells (IC_{50} = 1.8 μg/mL).

High cytotoxic activity is typical for the compound diorcinol J, synthesized as a result of the co-cultivation of *Aspergillus sulphureus* KMM 4640 and *Isaria felina* KMM 4639 [37]. The IC_{50} value for mouse Ehrlich carcinoma cells was 37.6 μM. It was found that diorcinol J is able to affect the expression of the heat shock protein Hsp70 in Ehrlich ascites carcinoma cells. It is well known that the heat shock protein 70 (HSP70) was frequently overexpressed in tumor cell lines as an ATP-dependent molecular chaperone and played a significant role in refolding misfolded proteins and promoting cell survival under stress. Thus, compounds that could inhibit HSP70 had great potential in tumor therapy [37].

Under the co-cultivation of *Aspergillus fischeri* NRRL 181 and *Xylaria flabelliformis* G536, a new compound wheldone was obtained [38], that was characterized by cytotoxic activity against breast cancer cells MDA-MB-231, ovarian cancer cells OVCAR-3, human melanoma cells MDA-MB-435 with IC_{50} values of 7.6, 3.8 and 2.4 μM, respectively.

Co-Cultures between Fungi and Bacteria

As a result of co-cultivation of *Saccharomonospora* sp. UR22 and *Dietzia* sp. UR66 obtained a new compound saccharomonosporin A with cytotoxic activity against human colon adenocarcinoma NT-29 and human promyelocytic leukemia HL-60: IC_{50} values of 3.6 and 2.8 μM, respectively [39].

During the co-cultivation of *Trichoderma* sp. 307 and *Acinetobacter johnsonii* B2, one new depsidone, botryorodin H, was synthesized together with three known analogues (botryorodins C, D, and G) [40]. Botryorodins H, C, D showed α-glucosidase inhibitory activity with IC_{50} ranging from 8.1 to 11.2 μM, and botryorodin H exhibited potent cytotoxicity against rat prolactinoma MMQ and rat pituitary adenoma GH3 cell lines (IC_{50} = 3.09 and 3.64 μM).

Under co-cultivation of *Aspergillus versicolor* and *B. subtilis*, the synthesis of the cyclic pentapeptide cotteslosin C, aphaquinolone, 22-epi-aflaquinolone B, two anthraquinones and the known isoversicolorin B and O-demethylsterigmatocystin, sterigmatocystin, sterigmatine was observed [41]. O-demethylsterigmatocystin, sterigmatocystin, sterigmatin inhibited rat lymphoma cell lines L5178Y, with IC_{50} values ranging from 2.2 to 5.8 μM.

The new compounds, ochraspergillic acids A and B, and the known viomellein and ochratoxin B were obtained from the co-culture of *Aspergillus ochraceus* and *B. subtilis* [42]. Viomellein and ochratoxin are characterized by high cytotoxic activity against the A2780

human ovarian carcinoma cells with IC_{50} values of 5.0 and 3.0 μM, respectively.

As a result of co-cultivation of *Bionectria* sp. and *S. lividans* TK24, a new alkaloid, 1,2-dihydrophenopyrrozin, was obtained together with five known analogues, including penicolinate A. Penicolinate A exhibited potent cytotoxic activity against the human ovarian cancer cell line A2780 with an IC_{50} value of 4.1μM [43].

Co-Cultures between Bacterial Strains

In recent years, two new alkaloid compounds with cytotoxic activity have been identified as a result of co-cultivation of bacteria [44, 45]. One of these compounds is the alkaloid BE-13793C, synthesized by co-cultivation of *Streptomyces* sp. MA37 and *Pseudomonas* sp. [44]. BE-13793C exhibited strong antiproliferative activity against human colon carcinoma HT-29 cells (ATCC HTB-38), with an IC_{50} value of 3.16 μM, but did not cause toxic effects on normal lung cells (ATCC CCL-171). During the cultivation of *Actinokineospora* sp. EG49 with *Nocardiopsis* sp. RV163, 1,6-dihydroxyphenazine was synthesized, which, in addition to cytotoxic (IC_{50} against human parasite Trypanosoma brucei TC 221 was 19 μM), also showed antimicrobial activity (growth inhibition zones of *Bacillus* sp. P25, *Actinokineospora* sp. EG49 were 11-15 mm) [45].

An effective anti-cancer compound was a novel cyclic peptide, dentigerumycin E, synthesized as a result of co-cultivation of *Streptomyces* sp. JB5 and *Bacillus* sp. GN1 [46]. Experiments have shown that dentigerumycin E demonstrated antimetastatic activity against cancer cells. Thus, the moderate cytotoxicity against cancer cell lines A549 (lung cancer), HCT116 (colorectal cancer), MDA-MB-231 (breast cancer), SK-HEP-1 (liver cancer) and SNU638 (gastric cancer) with half maximal inhibitory concentration (IC_{50}) in the range of 27–39 μM.

Two new isomers of heterocyclic peptides (catenulobactins A and B) were synthesized under cultivation of *Catenuloplanes* sp. RD067331 with *T. pulmonis* TP-B0596 [47]. Catenulobactin B exhibited Fe(III)-chelating activity and moderate cytotoxicity against P388 murine leukemia cells (IC_{50} = 22.4 μM). New metabolites (chojalactones A and B) identified after co-cultivation of *Streptomyces* sp. CJ5 and *T. pulmonis* TP-B0596 also had cytotoxic activity against P338 murine leukemia cells [48]. Thus, the IC_{50} values of chojalactone A stereoisomers were 28–37, and those of chojalactone B stereoisomers were 17–18 μM.

Table 2 summarizes the data on the synthesis of new metabolites with antimicrobial and cytotoxic activity during the co-cultivation of fungi, bacteria, and fungi with bacteria. These data indicate that the largest number of new metabolites with potent cytotoxic activity was identified as a result of the co-cultivation of fungi.

Figure 2 illustrated the classes of new metabolites with anticancer activity synthesized during the co-cultivation of

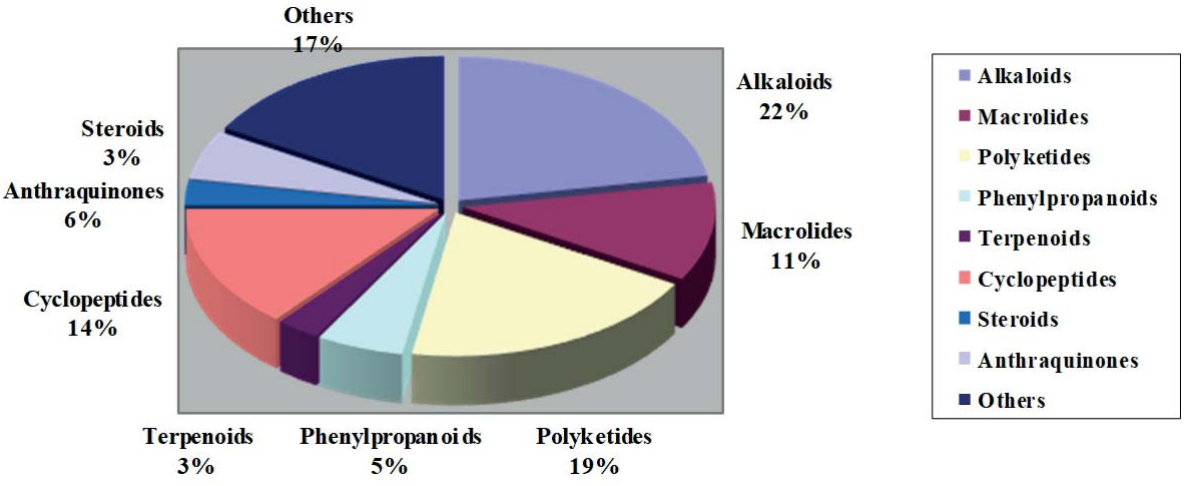

Fig. 2. **Number of new metabolites with cytotoxic activity synthesized as a result of co-cultivation of microorganisms [11, 32–48]**

Table 2

Characterization of new compounds with antimicrobial and cytotoxic activity, formed as a result of combined cultivation of microorganisms

Microorganisms	Carbon source	Compounds	Cytotoxic and antitumor activity	Test-cultures for determining antimicrobial activity	Minimum inhibitory concentration, μg/ml	References
1	2	3	4	5	6	7
Co-cultivation of fungal strains						
Ovadendron sulphureoochraceum MIC 5759 + *Ascochyta pisi* MIC 5620 + *Emericellopsis minima* MIC 5835 + *Cylindrocarpon destructans* MIC 5638 + *Fusarium oxysporum* MIC 5789	Malt extract, Maltose, Dextrose, Yeast autolysate	Lateritin N-methylated peptide	Cytotoxic activity against human tumor cell lines (BXPC-3, MCF-7, CNS SF268, NSC H460, KM20L2 and DU-145) with IC_{50} in the range of 1.7-2.0 μg/mL and against mouse leukemia P388 cells 1.8 μg/mL	*Candida albicans* ATCC 90028	4–8	[36]
				Micrococcus luteus Presque Isle 456	2–4	
				Staphylococcus aureus ATCC 29213	4–8	
				Enterococcus faecalis ATCC 29212	8	
				Streptococcus pneumoniae ATCC 6303	8-16	
Penicillium fuscum (Sopp) Raper & Thom + *Penicillium camembertii/ clavigerum* Thom	Potato-dextrose medium	Berkeleylactone A Monocyclic macrolide $C_{19}H_{32}O_7S$ Berkeleylactone A26771B macrolide $C_{20}H_{30}O_7$ Berkeleylactone C macrolide $C_{20}H_{30}O_8$ Berkeleylactone F macrolide $C_{16}H_{28}O_5$	The IC_{50} value for the leukemia cell line K-562 was 10 μM, causing inhibition (85%) and lethality (2.4%) of the cells The IC_{50} value of RPMI-8226 leukemia cells was 10 μM, and caused inhibition of cells (48%) The IC_{50}value of CCRF-CEM leukemia cells was 10 μM, causing (48%) of cells, as well as inhibition (46%) of K-562 leukemia cells The IC_{50} value of CCRF-CEM leukemia cells was 10 μM, causing inhibition (38%) of cells	*Staphylococcus aureus* 13709	1/3/6/19	[11]
				Streptococcus pyogene	3/48/26/150	
				Candida glabrata	6/48/26/>300	
				Bacillus subtilis	13/12/26/>300	
				Candida albicans	26/96/50/>300	
				Bacillus anthracis	3/6/6/75 * — MIC Berkeleylactone A / A26771B / C / F	

Table 2 (Continued)

1	2	3	4	5	6	7
Aspergillus fischeri NRRL 181 + *Xylaria flabelliformis* G536	Oatmeal medium	Wheldone macrolide $C_{25}H_{34}O_6$	Cytotoxic activity against breast cancer cells MDA-MB-231, ovarian cancer cells OVCAR-3, human melanoma cells MDA-MB-435 with IC_{50} values of 7.6, 3.8 and 2.4 μM	Does not exhibit antimicrobial activity		[38]
Aspergillus sulphureus KMM4640 + *Isaria felina* KMM4639	Rice medium	Diorcinol J Diphenyl ether $C_{19}H_{22}O_4$	The IC_{50} value for mouse Ehrlich carcinoma cells was 37.6 μM	Does not exhibit antimicrobial activity		[37]
Aspergillus sulphureus KMM4640 + *Isaria felina* KMM4639	Rice medium	17-O-ethylno-toamide M Alkaloid $C_{28}H_{35}N_3O_5$	The IC_{50} value for human prostate cancer cells 22Rv1 was 10 μM	Does not exhibit antimicrobial activity		[32]
Epicoccum sp. YUD 17002 + *Armillaria* sp.	Potato dextrose medium	Epicoterpene A-E Sesqui-terpenoids Armilliphatic A Aryl ester $C_{23}H_{28}O_5Cl$	Armilliphatic A had cytotoxicity against five human cancer cell lines (HL-60, A549, MCF-7, SMMC-7721, and SW480) with IC_{50} values ranging from 15.80 to 23.03 μM	Does not exhibit antimicrobial activity		[34]
Talaromyces aculeatus + *Penicillium variabile*	Maltose medium	Penitalarins A–C Polyketides Nafuredin B Polyketide $C_{22}H_{32}O_3Na$	The IC_{50} values of six human cancer cell lines (HeLa, K562, HCT-116, HL-6.0 A549, MCF-7) were in the range of 1.2 — 9.8 μM for nafuredin B	Do not exhibit antimicrobial activity		[35]
Aspergillus sclerotiorum SCSGAF 0053 + *Penicillium citrinum* SCSGAF 0052	Starch, Glucose, Peptone	Sclerotiorumin A Alkaloid $C_{14}H_{14}O_5$ Aluminium-neohydroxy-aspergillin $C_{36}H_{57}AlN_6O_9$	The IC_{50} value of human histiocytic lymphoma U937 cell line was 4.2 μM for aluminiumneohydroxyaspergillin	Do not exhibit antimicrobial activity		[33]

Table 2 (Continued)

1	2	3	4	5	6	7
Co-cultivation of bacterial strains						
Actinokineospora sp. EG49 + *Nocardiopsis* sp. RV163	ISP 2 medium	1,6-dihydroxy-phenazine Alkaloid $C_{12}H_8N_2O_2$	The IC_{50} value of human parasites Trypanosoma brucei TC 221 was 19 μM	*Bacillus* sp. P25	Growth inhibition zone of 11 mm (10 μg/disc)	[45]
				Actinokineo-spora sp. EG49	Growth inhibition zone of 15 mm (10 μg/disc)	
Streptomyces sp. CJ5 + *Tsukamurella pulmonis* TP-B0596	Not given	Chojalactone A Contains 2-hydroxy-3-methyl-γ-butyrolactone fragment $C_{13}H_{16}O_4$ Chojalactone B Contains 2-hydroxy-3-methyl-γ-butyrolactone $C_{13}H_{14}O_4$	IC_{50} values for murine leukemia cells P338 of two stereoisomers of choyalactone A was 37 and 28 μM IC_{50} values for murine leukemia cells P338 of two stereoisomers of choyalactone B was 18 and 17 μM	Do not exhibit antimicrobial activity		[48]
Streptomyces sp. MA37 + *Pseudomonas* sp.	Yeast extract, malt extract, glucose	Indole carbazole alkaloid BE-13793C $C_{20}H_{11}N_3O_2$	Antiproliferative activity against human colon carcinoma HT-29 cells (ATCC HTB-38), with an IC_{50} value of 3.16 μM	*Enterococcus faecalis* ATCC 29,212	>140	[44]
				Staphylococcus aureus ATCC 25,923	>140	
				Streptococcus B. ATCC 12,386	>140	
				Escherichia coli ATCC 25,922	>140	
				Pseudomonas aeruginosa ATCC 27,853	>140	

Table 2 (Continued)

1	2	3	4	5	6	7
Streptomyces sp. JB5 + *Bacillus* sp. GN1	Yeast extract, malt extract, glucose	Dentigerumycin E cyclic peptide $C_{39}H_{63}N_9O_{16}$	IC_{50} values of cancer cell lines, A549 (lung cancer), HCT116 (colorectal cancer), MDA-MB-231 (breast cancer), SK-HEP-1 (liver cancer) and SNU638 (gastric cancer) were 38, 28, 28, 27, 39 μM, respectively	Does not exhibit antimicrobial activity		[46]
Catenuloplanes sp. RD067331 + *Tsukamurella pulmonis* TP-B0596	Starch, Soybean flour, Yeast extract	Catenulobactin A Heterocyclic Peptide $C_{27}H_{31}N_4O_9$ Catenulobactin B $C_{27}H_{31}N_4O_9$	The IC_{50} value of P388 mouse leukemia cells was 22.4 μM	Do not exhibit antimicrobial activity		[47]
Co-cultivation of fungi and bacteria						
Dietzia sp. UR66 + *Saccharomonospora* sp. UR22	Malt extract, dextrose, yeast extract	Saccharo-monosporin A Brominated oxoindole alkaloid $C_{19}H_{15}O_2N_2Br$	Antiproliferative activity against human colon adenocarcinoma NT-29 and human promyelocytic leukemia HL-60 (IC_{50} = 3.6 and 2.8 μM, respectively)	Does not exhibit antimicrobial activity		[39]
Trichoderma sp. 307 + *Acinetobacter johnsonii* B2	Malt extract, dextrose, yeast extract	Botryorodin H Depsidone Phenylpropanoid $C_{22}H_{18}O_6$	IC_{50} values of rat prolactinoma cell lines MMQ and rat pituitary adenoma GH3 were 3.09 and 64 μM, respectively	Does not exhibit antimicrobial activity		[40]
Aspergillus versicolor + *Bacillus subtilis*	Rice medium	Coteslosin C Cyclopeptide 22-epi-afla-chinolone B Phenylpropanoid Versicolorin B Anthraquinones 6,8-O-dimethyl-bipolarin Anthraquinones Diorcinol D Diorcinol G Diorcinol I	The IC_{50} value of rat lymphoma cell lines L5178Y was 5.8, 2.2, 2.3 μM for O-demethylsterigmatocystin, sterigmatocystin, sterigmatin	*Staphylococcus aureus* ATCC 29213	25/12.5/50*	[41]
				Enterococcus faecalis ATCC 29212	50/12.5/50*	
				Enterococcus faecalis ATCC 51299	50/12.5/50*	
				Enterococcus faecalis ATCC 35667	12.5/12.5/25* * - MIC Diorcinol D/ G/ I	

Table 2 (End)

1	2	3	4	5	6	7
Aspergillus ochraceus + *Bacillus subtilis*	Rice medium	Ochraspergillic acids A, B Viomellein Ochratoxin B	The IC_{50} value of human ovarian carcinoma A2780 cells was 5.0 and 3.0 μM for viomellein and ochratoxin B, respectively	Do not exhibit antimicrobial activity		[42]
Bionectria sp. + *Streptomyces lividans* TK24	Rice medium	1,2-dihydro-pheno-pyrazine Alkaloid $C_{13}H_{16}NO_2$ Penicolinate A Steroid $C_{24}H_{32}O_4N_2$	The IC_{50} of human ovarian cancer cell line A2780 4.1 μM for penicolinate A	Does not exhibit antimicrobial activity		[43]

microorganisms. Metabolites synthesized from co-cultures of bacteria and fungi were identified as alkaloids, cyclopeptides, phenylpropanoids, polyketides, macrolides, steroids, terpenoids, anthraquinones, with alkaloids, cyclopeptides, and polyketides taking the largest part.

As a result of the co-cultivation of microorganisms, a large number of new biologically active secondary metabolites have been obtained. In particular, 77 new metabolites that are not typical for monocultures have been identified (see Tables 1, 2). 29 compounds were isolated as a result of co-cultivation of fungi; 31 compounds were isolated from co-culture of fungi and bacteria, and a total of 17 compounds were isolated from co-culture of bacteria. The largest group (41% of all metabolites) was compounds identified after co-cultivation of fungi and bacteria. The largest number of novel metabolites was found as alkaloids (≥42%), and the smallest (<3%) as steroids. Most of the new compounds of different chemical structures were found as a result of co-cultivation of *Aspergillus* spp. and *Penicillium* spp. fungi with various bacterial strains.

The synthesis of most of the novel compounds is based on a protective mechanism, which results in the activation of silent genes for their biosynthesis. At the same time, it is impossible to predict which clusters of biosynthetic genes will be expressed or what types of molecules will be synthesized during co-cultivation of microorganisms.

The methods of co-cultivation of fungi and bacteria mentioned in this review certainly limit the variety of novel compounds synthesized. Therefore, increasing the diversity of microorganisms used, for example, by using amoebas or phages for co-cultivation, may be a promising step in future research. In addition, in order to understand the complex regulation of secondary metabolism and to determine the possibilities of genetic engineering to induce or enhance the synthesis of target secondary metabolites, it is necessary to establish all the mechanisms that ensure the formation of new compounds during co-cultivation of microorganisms.

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КОМБІНОВАНЕ КУЛЬТИВУВАННЯ МІКРООРГАНІЗМІВ: УТВОРЕННЯ НОВИХ МЕТАБОЛІТІВ З РІЗНОЮ БІОЛОГІЧНОЮ АКТИВНІСТЮ

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Останнім часом через надмірне та необґрунтоване використання антибіотиків антибіотикорезистентність стала найгострішою проблемою людства. Одним з ефективних підходів до відкриття нових вторинних антимікробних метаболітів є спільне культивування мікроорганізмів, за якого продуцент цільового продукту вирощується разом із конкурентними мікроорганізмами (індукторами), у відповідь на наявність яких відбувається активація мовчазних біосинтетичних генів штаму-продуцента і спостерігається підвищення біологічної активності синтезованих вторинних метаболітів та/або навіть утворення нових сполук. В огляді наведено сучасні дані літератури щодо спільного культивування продуцентів антимікробних сполук з конкурентними мікроорганізмами, результатом якого є синтез нових, не характерних для монокультур, метаболітів з антимікробною та цитотоксичною активністю. Під час спільного культивування грибів, бактерій, а також грибів з бактеріями спостерігається утворення нових антимікробних та протипухлинних метаболітів, які належать до алкалоїдів, фенілпропаноїдів, макролідів, полікетидів, циклопептидів, терпеноїдів, антрахінонів, стероїдів. Наведені дані свідчать про те, що комбіноване культивування мікроорганізмів є простим, дешевим та достатньо ефективним способом отримання нових метаболітів, перспективних для використання у медицині.

Ключові слова: спільне культивування; антимікробні препарати; протипухлинні агенти.