ISSN 0026-2617, Microbiology, 2010, Vol. 79, No. 3, pp. 277–286. © Pleiades Publishing, Ltd., 2010.
Original Russian Text © V.P. Zhelifonova, T.V. Antipova, A.G. Kozlovsky, 2010, published in Mikrobiologiya, 2010, Vol. 79, No. 3, pp. 291–300.

REVIEW

Secondary Metabolites in Taxonomy of the *Penicillium* Fungi

V. P. Zhelifonova, T. V. Antipova, and A. G. Kozlovsky[1]

Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia

Received June 2, 2009

Abstract—A correlation was established between species specificity and synthesis of specific secondary metabolites by the *Penicillium* fungi. Strains of the subgenus *Aspergilloides* usually synthesize metabolites of polyketide nature. Most strains of the subgenus *Furcatum* produce clavine ergot alkaloids and metabolites of diketopiperazine nature. The only clavine ergot alkaloids and diketopiperazine alkaloids produced by strains of the subgenus *Biverticillium* are rugulovasines and rugulosuvines, respectively. Species designations of the strains of the subgenus *Penicillium* isolated from permafrost soil, the *Mir* orbital complex, and sites undergoing anthropogenic load were refined based on the marker secondary metabolites. Changes in the taxonomic position of some strains in the genus *Penicillium* are suggested.

Key words: microscopic fungi, subgenera *Aspergilloides, Furcatum, Biverticillium*, and *Penicillium*, biosynthesis of secondary metabolites.

DOI: 10.1134/S002626171003001X

INTRODUCTION

Although fungi of the genus *Penicillium* are the most widespread ones, they are also the most difficult objects for species identification by traditional microbiological techniques. The accepted identification based on micro- and macromorphological characteristics [1] often fails to produce unambiguous results, especially in the case of isolates from anthropogenically disturbed or poorly known extreme habitats. Available information on species-specific production of biologically active compounds, including alkaloids, antibiotics, mycotoxins, and allergens [2] suggests importance of reliable species identification of penicillia.

Modern advances in biology provide a basis for new schemes considering a broader range of characteristics. A new polyphasic taxonomy of the *Penicillium* subgenus has been recently suggested, employing the profiles of secondary metabolites together with micro- and macromorphological characteristics [3]. The phenotypic concept of a species implies that each species is homogeneous, with a distinct phenotypic cluster clearly separated from other clusters. The species following this pattern exhibit matching in other species categories, which are based on ecology and phylogeny. Chemotaxonomy is based on empiric observation of the physiological and biochemical characteristics shared by phylogenetically related organisms. Potential and actual production of secondary metabolites is a part of physiologo–biochemical identification [4]. Since constitutive metabolic mechanism has been demonstrated for production of some metabolites, biosynthesis of marker metabolites should be expected of specific taxa [3]. The criteria applied by Samson and Frisvad for taxonomy of the subgenus *Penicillium*, i.e., a combination of micro- and macromorphological characteristics, some physiological characteristics, and the spectrum of secondary metabolites, made it possible to create the taxonomy of these fungi that is presently the most adequate one.

Screening of producers of secondary metabolites (potentially biologically active compounds) among the strains of different subgenera of *Penicillium* [5–24] obtained from the All-Russian Collection of Microorganisms (VKM); the collection of the Soil Biology Department, Faculty of Soil Sciences, Moscow State University (KBP); and the collection of the Institute of Medico-Biological Problems, Russian Academy of Sciences (IMBP) has been recently carried out at the Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences. New producers of both known and new secondary metabolites were discovered, and the characteristics of their biosynthesis were determined. A data bank was created containing about 100 producer strains and tens of compounds. Among the diverse metabolites synthesized by the fungi of the genus *Penicillium*, such biologically active compounds as ergot alkaloids, diketopiperazines, and quinoline alkaloids are of special interest. They are biogenetically related to the shikimate pathway of amino acid biosynthesis, especially to the branch leading to anthranilate and tryptophan.

The goal of the present review was to summarize the previously obtained data on the profiles of nitro-

[1] Corresponding author; e-mail: Kozlovski@ibpm.pushchino.ru

gen-containing and other secondary metabolites in fungi of different subgenera of the genus *Penicillium* and analyze their possible application for specification of the taxonomic position of these fungi.

SECONDARY METABOLITES OF THE FUNGI OF THE GENUS *PENICILLIUM*

The secondary metabolites produced by fungi of the genus *Penicillium* vary in structure and are synthesized via different biosynthetic pathways (table). Among the identified metabolites, nitrogen-containing compounds, mainly indole compounds biogenetically related to tryptophan, form a large group. Structural diversity of these alkaloids is promoted by the nucleophilic nature of the indole ring, with every position in the heterocyclic nucleus subject to electrophilic attacks [25]. *Penicillium* may contain simple tryptophan derivatives, tryptamines and their derivatives (indolyl-3-acetic acid, *N*-acetyltryptamine).

Penicillium fungi produce structurally diverse ergot alkaloids of the clavine line containing a tetracyclic ergoline nucleus with the D ring modified in some metabolites. The ergoline structure is formed from tryptophan and mevalonic acid. Clavine alkaloids were originally described by Abe (1951) in fungi of the genus *Claviceps* [26]. The ability of penicillia to synthesize this group of metabolites was established relatively recently [27]. The diversity of clavine alkaloids results from the number of possibilities for structural modifications of the D ring. These compounds may have a double bond in different positions within the ring and differ in the substituting radicals. They contain several asymmetric carbon atoms and respective isomers, including stereoisomers. Clavine alkaloids synthesized by penicillia may be subdivided into three groups. The first one includes such 6-*N*-methylergoline derivatives as festuclavine, epicostaclavine, costaclavine, fumigaclavines, and isofumigaclavines A and B with completely saturated D ring. The second group includes ergolenes with a double bond in the 8,9 position: agroclavine, agroclavine-I, chanoclavine-I, chanoclavine-III, and isochanoclavine-I; epoxyagroclavine-I may be included into this group as an exceptional case. The third group comprises clavine alkaloids with the modified rings C or D: rugulovasines A and B, aurantioclavine, 6-*N*-ethylaurantioclavine, α-cyclopiazonic acid (CPA), and the α-CPA imine.

Cyclic peptides consisting of two amino acid residues and mevalonic acid belong to another group of secondary metabolites synthesized by *Penicillium*. These compounds are characterized by the presence of a diketopiperazine nucleus. The main precursors of these alkaloids are tryptophan, other amino acids, and mevalonic acid (the source of five-carbon units). Condensation of tryptophan, histidine, and mevalonic acid results in biosynthesis of the roquefortine group of alkaloids (roquefortine, 3,12-dihydroroquefortine, glandicolines A and B, meleagrin, and oxaline). Tryptophan and mevalonic acid are also precursors for diketopiperazine alkaloids, fellutanines and isofellutanines. Similarly, brevianamides A and B and new alkaloids piscarinines A and B are formed from tryptophan, proline, and one or two molecules of mevalonic acid. Leucyltryptophanyl diketopiperazine and verrucosine are diketopiperazine alkaloids with tryptophan and leucine as precursors. Compounds formed from tryptophan and phenylalanine residues are such alkaloids as rugulosuvine, isorugulosuvine, puberuline (= rugulosuvine A), and puberuline A (= rugulosuvine B). If anthranilic acid replaces tryptophan at the beginning of the biosynthetic chain, benzodiazepine alkaloids (cyclopeptine, cyclopenine, and cyclopenol) and quinolinic compounds (viridicatine, viridicatole, and quinocitrinines A and B) are formed.

Compounds of polyketide nature (griseofulvin, ochratoxins A and B, mycophenolic acid, patulin, citrinin, and cyclocitrinol) were detected among the metabolites of investigated *Penicillium* strains.

SUBGENUS *ASPERGILLOIDES*

In 12 strains of *P. decumbens, P. glabrum*, and *P. restrictum* isolated from anthropogenically disturbed and permafrost soils, compounds were revealed that were not stained with the Dragendorff and Ehrlich reagents and thus did not contain nitrogen in their structures [5]. Since only production of metabolites of polyketide and isoprenoid nature is known for these species [27–29], the absence of synthesis of nitrogen-containing metabolites by investigated strains is in agreement with the literature data.

For the species *P. spinulosum*, production of indole-containing metabolites of diketopiperazine structure (dioxopyrasine indole, gliotoxins, and penitrem A) is known, as well as of various polyketides, including citrinins [27–29]. In strain KBP no. 15 from an anthropogenically damaged environment, synthesis of other indole-containing compounds was revealed: fellutanine A (tryptophanyl–tryptophanyl–diketopiperazine), roquefortine, and cyclopenine (table). This metabolite spectrum is a marker for the *Penicillium* subgenus, supporting changes in the taxonomic position of strain KBP no. 15.

SUBGENUS *FURCATUM*

The spectra of secondary metabolites were studied for 43 strains of the species *P. citrinum, P. piscarium, P. canescens, P. melinii, P. jensenii, P. janczewskii, P. fellutanum*, and *P. waksmanii* of the subgenus *Furcatum* [6–16].

For *P. citrinum*, production of various polyketides is known, including citrinin and the clavine alkaloid pyroclavine [27–30]. Among the nine strains of this species isolated from various environments, only VKM F-253, VKM F-1079, VKM F-3013, and VKM F-3053

Species distribution of secondary metabolites in penicillia of different subgenera

Metabolites	Biosynthetic precursors	Subgenera			
		Aspergilloides Dierckx	*Furcatum* Pitt	*Biverticillium* Dierckx	*Penicillium* [32]
N-acetyltryptamine	Tryptophan	–*	–	–	*P. chrysogenum* IMBP 1-3, 1-4; *P. polonicum* IMBP 2-6
Indolyl-3-acetic acid	Tryptophan	–	–	*P. vulpinum* VKM F-258 [19]	–
Chanoclavine-I, costaclavine, epicostaclavine	Tryptophan, mevalonic acid	–	*P. citrinum* VKM F-1069, F-1079 [7]; *P. melinii* VKM F-1070 [15]; *P. janczewskii* VKM F-685, F-2377 [16]	–	–
Festuclavine, fumigaclavines A and B	″	–	–	–	*P. palitans* VKM FW-657, FW-667, FW-690, FW-704, FW-747, FW-794; KBP no. 4
Chanoclavine-III, isochanoclavine-I, agroclavine-I, epoxyagroclavine-I	″	–	*P. citrinum* VKM FW-800 [8]; *P. corylophillum* VKM F-152 [9]; *P. fellutanum* (= *P. sizovae*) VKM F-1073 [10]	–	–
Rugulovasines A and B, chlororugulovasines A and B	Tryptophan, mevalonic acid, chlorine	–	–	*P. variabile* VKM FW-655, FW-806, FW-811, FW-816, FW-818, F-2075, F-2528, FW-2531, FW-2701, FW-2758 [18]	–
Aurantioclavine, 6-*N*-ethylaurantioclavine	Tryptophan, mevalonic acid	–	*P. janczewskii* VKM FW-685 [16]	–	–
α- and β-CPA, imine-CPA	Tryptophan, mevalonic acid, acetoacetyl	–	–	*P. vulpinum* VKM F-260, F-2360; KBP no. 113 [19]	–
Brevianamides A and B	Tryptophan, proline, mevalonic acid	–	*P. waksmanii* VKM F-682, F-1022 [15]	–	–
Piscarinines A and B	″	–	*P. simplicissimum* (= *P. piscarium*) VKM F-691 [14]	–	–
Roquefortine, dihydroroquefortine, meleagrin, oxaline, glandicoline A and B	Tryptophan, histidine, mevalonic acid	*P. spinulosum* KBP no. 15 [5]	*P. citrinum* VKM F-1290; *P. jensenii* VKM F-292, F-293 [15]; *P. janczewskii* VKM F-2489 [16]	*P. vulpinum* VKM F-256, F-258, F-259, F-1255; KBP no. 16 [19]	*P. melanoconidium* VKM FW-738, FW-741, FW-766; *P. chrysogenum* VKM F-227, F-692, F-1078, F-1987; KBP no. 105; IMBP 1-3, 1-4, 2-4, 2-5

Table. (Contd.)

Metabolites	Biosynthetic precursors	Subgenera			
		Aspergilloides Dierckx	*Furcatum* Pitt	*Biverticillium* Dierckx	*Penicillium* [32]
Fellutanines A–E	Tryptophan, mevalonic acid	*P. spinulosum* KBP no. 15 [5]	*P. canescens* VKM F-1148, F-1287 [15]; *P. fellutanum* VKM F-3020 [12]; *P. simplicissimum* (= *P. piscarium*) VKM F-325, F-1823 [13]; *P. waksmanii* VKM F-1027 [15]	–	–
Isofellutanines B and C	Tryptophan, mevalonic acid	–	*P. canescens* VKM F-3108 [15]; *P. fellutanum* VKM F-3020, F-1292 [12]	–	–
Rugulosuvines A (puberuline A) and B (puberuline)	Tryptophan, phenylalanine, acetate, mevalonic acid	–	*P. simplicissimum* (= *P. piscarium*) VKM F-325 [15]	*P. rugulosum* VKM F-352, F-2369 [17]	*P. polonicum* IMBP 2-2, 2-3
Isorugulosuvine	Tryptophan, phenylalanine	–	*P. canescens* VKM F-240, F-1148, F-1287 [15]; *P. melinii* VKM F-311 [15]; *P. simplicissimum* (= *P. piscarium*) VKM F-325, F-1823 [13]	–	*P. chrysogenum* VKM F-1078; *P. polonicum* IMBP 2-2, 2-3, 2-6, 2-7
Prolyltryptophanyldiketopiperazine	Tryptophan, proline	–	*P. simplicissimum* (= *P. piscarium*) VKM F-691, F-325, 1823 [13], [14]	–	–
Verrucosine	Tryptophan, leucine, mevalonic acid, acetate	–	*P. simplicissimum* (= *P. piscarium*) VKM F-325 [13]	–	–
Cyclopeptine, cyclopenine, cyclopenol	Anthranilate, phenylalnine, O_2	*P. spinulosum* KBP no. 15 [5]	–	*P. vulpinum* VKM F-259, F-2360 [19]	–
Viridicatin, viridicatol	"	–	–	–	*P. polonicum* IMBP 2-2
Quinocitrinins A and B	Anthranilate, isoleucine	–	*P. citrinum* VKM FW-800 [11]	–	–
Questiomycin A	Anthranilate	–	–	–	*P. chrysogenum* IMBP 1-5, 1-6; *P. polonicum* IMBP 2-7
Xanthocillin X	Shikimate pathway	–	–	–	*P. chrysogenum* IMBP 1-5, 1-6; *P. polonicum* IMBP 2-7

Table. (Contd.)

Metabolites	Biosynthetic precursors	Subgenera			
		Aspergilloides Dierckx	*Furcatum* Pitt	*Biverticillium* Dierckx	*Penicillium* [32]
Griseofulvin, decholro-griseofulvin	Heptaketide, chlorine	–	*P. janczewskii* VKM F-312, F-2191, F-2378, F-3023; KBP no. 2 [5, 16]	*P. vulpinum* VKM F-257 [19]	–
Mycophenolic acid	Tetraketide, farnesyl	–	–	–	*P. bialowiezense* VKM FW-725, 791
Ochratoxin A and B	Pentaketide, phenylalanine, chlorine	–	–	–	*P. verrucosum* VKM FW-875, FW-877, FW-878, FW-907, FW-908
Patulin	Tetraketide	–	*P. janczewskii* VKM F-312 [16]	*P. vulpinum* VKM F-257, F-1255 [19]	–
Citrinin, cyclocitrinol	Pentaketide, 2 methyl groups	–	*P. citrinum* VKM F-253, F-3013, F-3053, F-1079 [6]	–	–

*Not detected.

synthesized citrinin. Three of them (VKM F-253, VKM F-3013, and VKM F-3053) produced cyclocitrinol, a previously unknown metabolite of polyketide nature [6]. According to [31], within a *Penicillium* species the ratio of isolates synthesizing citrinin may vary from 10 to 97% of investigated strains. Clavine ergot alkaloids were synthesized by three strains. Chanoclavine-I, isochanoclavine-I, costaclavine, and epicostaclavine were revealed in strain VKM F-1079 [7]. Epicostaclavine was also identified in strain VKM F-1069 [7]. Strain VKM FW-800 isolated from permafrost sediments synthesized ergot alkaloids epoxyagroclavine-I and agroclavine-I, which are stereochemically different from the typical *Claviceps* clavine alkaloids [8]. Production of agroclavine-I and epoxyagroclavine-I, which are not contaminated with respective isomers, may be explained only by high stereospecificity of the enzymes responsible for the key stages of biosynthesis of these metabolites. Epoxyagroclavine-I and agroclavine-I have been previously revealed only in two strains of the subgenus *Furcatum, P. corylophillum* VKM F-152 [9] and *P. fellutanum* (= *P. sizovae*) VKM F-1073 [10]. Strain VKM FW-800 also synthesized quinoline alkaloids, quinocitrinins A and B [11]. Both branches of biosynthesis of quinoline and ergoline compounds are biogenetically related by the shikimate pathway of biosynthesis of their precursors, tryptophan and anthranilic acid. Identification of roquefortine and meleagrin, compounds unknown for *P. citrinum*, in strain VKM F-1290 casts doubt on its classification within this species.

Thus, *P. citrinum* strains are able to synthesize both polyketide metabolites (citrinin and cyclocitrinol) and various clavine ergot alkaloids. Quinocitrinins A and B, quinoline alkaloids of unique structure, are presently known to occur only in one *P. citrinum* strain isolated from permafrost soil 1.8–3.0 Ma old.

For *P. fellutanum*, production of various polyketides, including citrinin, is known, as well as of indole-containing diketopiperazine alkaloids gliotoxins [27–30]. Six strains of this species isolated from soils of different geographical location exhibited no citrinin production [12]. Ergot alkaloids agroclavine-I and epoxyagroclavine-I were synthesized by *P. fellutanum* (= *P. sizovae*) VKM F-1073. Diketopiperazine alkaloids fellutanines A–E and isofellutanines B and C were found in strain VKM F-3020, while strain VKM F-1292 produced only isofellutanine B. Strain VKM F-690 synthesized an indole compound of undetermined structure. Fellutanines were not found in strains VKM F-248 and VKM F-2817 [12]. Thus, four out of six investigated *P. fellutanum* strains are able to synthesize indole-containing compounds, fellutanines and ergot alkaloids, for which tryptophan and mevalonic acid are the main precursors.

P. piscarium, which has long been considered a separate species, was classified by Pitt as a synonym to *P. simplicissimum* (Oudem.) Thom [1]. None of the three investigated strains of *P. piscarium* [13] synthesized both the metabolites known for *P. piscarium* (janthitrem B) and for *P. simplicissimum* (penicillic acid and indole-containing diketopiperazine alkaloids fumitremorgen B, verruculogen, and paraherqua-

mides) [27–30]. Tryptophan and proline are biogenetic precursors of these alkaloids, as well as of prolyltryptophanyldiketopiperazine found in strains VKM F-325 and VKM F-1823 [13]. Moreover, alkaloids were identified in these strains with their diketopiperazine ring formed by condensation of tryptophan with other amino acids: leucine (verrucosine), isoleucine (piscarinines A and B), phenylalanine (isorugulosuvine, puberuline), or tryptophan (fellutanine A). Verrucosine, prolyltryptophanyldiketopiperazine, puberuline A, isorugulosuvine, and fellutanine A were found in strain VKM F-325. Strain VKM F-1823 synthesized only isorugulosuvine and fellutanine A. Production of prolyltryptophanyldiketopiperazine and piscarinines A and B was revealed in strain VKM F-691 [14].

Strains classified as *P. canescens* and isolated from soils of various geographical zones synthesized two types of diketopiperazine alkaloids, fellutanines and/or isorugulovasine [15]. Strain VKM F-240 produced isorugulovasine and strain VKM F-3108 produced isofellutanine B, while both isorugulovasine and fellutanine A were detected in strains VKM F-1148 and VKM F-1287. None of the investigated strains produced griseofulvin, a known metabolite of *P. canescens* [27–30].

Metabolite profiles of *P. melinii* differed at the strain level [15]. Strain VKM F-311 produced isorugulosuvine, while strain VKM F-1070 produced clavine alkaloids chanoclavine-I, costaclavine, and epicostaclavine. Patulin and griseofulvin, which are known for this species [27–30], were not found in the investigated strains.

Strains VKM F-292 and VKM F-293, classified as *P. jensenii*, synthesized roquefortine, 3,12-dihydroroquefortine, and meleagrin [15] and did not synthesize griseofulvin known for the species [27–30]. No significant production of secondary metabolites was detected in other investigated strains, VKM F-294, VKM F-1147, and VKM F-1295 [15].

The spectrum of metabolites identified in eight *P. janczewskii* strains was diverse [5, 16]. Griseofulvin, which is known for the species [27–30], was identified in five strains isolated from different environments of various geographical zones, VKM F-312, VKM F-2191, VKM F-2378, VKM F-3023, and KBP no. 2. Strain VKM F-312 also synthesized dechlorogriseofulvin and patulin. Clavine alkaloids with a complete (epicostaclavine) and modified D ring of the ergoline nucleus (aurantioclavine and 6-*N*-ethylaurantioclavine) were synthesized by strains VKM F-2377 and VKM F-685, respectively. Unlike other investigated *P. janczewskii* strains, strain VKM F-2489, isolated from Antarctic ice, produced diketopiperazine alkaloids of the roquefortine family—(E)-3-(1H-imidazole-4-ilmethien)-6-(1H-indole-3-ilmethyl)-2,5-piperazineindole, roquefortine, 3,12-dihydroroquefortine, 16-*N*-ethylroquefortine, meleagrin, and glandicoline B [16]. Penitrem A, nigrifortin, verruculogen, and penicillic acid, which are known for *P. janczewskii* [27–30], were not found in investigated strains.

For *P. waksmanii*, strain differences were found in production of secondary metabolites. Brevianamide A, which is not known for the fungi of subgenus *Furcatum*, was synthesized by strains VKM F-682 and VKM F-1022 [15]. Fellutanine A, which had been isolated from other species of this subgenus, was identified in strain VKM F-1027.

Thus, most of the investigated strains of the subgenus *Furcatum* are characterized by production of diketopiperazine alkaloids, fellutanines, and puberulines, as well as of clavine ergot alkaloids of diverse structure (table). The production of diketopiperazine alkaloids revealed in strains *P. citrinum* VKM F-1290, *P. jensenii* VKM F-292 and VKM F-293, and *P. janczewskii* VKM F-2489 may suggest their classification within the subgenus *Penicillium*.

SUBGENUS *BIVERTICILLIUM*

Unlike anomorphous penicillia of other subgenera, which are associated with the teleomorphous genus *Eupenicillium* (class *Ascomycetes*, order *Eurotiales*), anamorphous penicillia of the subgenus *Biverticillium* are associated with teleomorphous fungi of the genus *Talaromyces* (class *Ascomycetes*, order *Eurotiales*) [1]. Penicillia of the subgenus *Biverticillium*, except for *P. vulpinum*, synthesize metabolites structurally different from those produced by other subgenera [3, 27–29]. Moreover, they are not known to produce diketopiperazine alkaloids other than rugulovasines [3].

A total of 39 strains belonging to six species of the subgenus *Biverticillium, P. funiculosum, P. minioluteum, P. purpurogenum, P. rugulosum, P. variabile*, and *P. vulpinum* were studied [17–19]. No nitrogen-containing metabolites were found in the investigated strains of *P. funiculosum, P. minioluteum*, and *P. purpurogenum*. These species synthesize compounds of polyketide nature, which are not found in other subgenera—mitorubrin, rubratoxin, islandicin, etc. [3, 27–29].

Strains of the species *P. rugulosum* differ in production of secondary metabolites. While strains VKM F-352 and VKM F-2369 synthesized diketopiperazine alkaloids rugulosuvines A and B, which are known for the species [17], strains VKM FW-665, VKM FW-717, VKM FW-733, and VKM FW-769 isolated from permafrost soils did not synthesize nitrogen-containing metabolites. Production of rugulovasines A and B is known for the species *P. rugulosum* [27–29].

Half of the investigated *P. variabile* strains isolated both from modern environments (VKM F-2075, VKM FW-2528, VKM FW-2531, VKM FW-2701, and VKM FW-2758) and from permafrost soils (VKM FW-655, VKM FW-806, VKM FW-811, VKM FW-816, and VKM FW-818) produced rugulovasines A and B [18]. This was the first detection of rugulovasines in this species. Rugulovasines were identified

in members of subgenera *Biverticillium* (*P. biforme, P. islandicum, P. purpurogenum*, and *P. rugulosum*) [27–29] and *Penicillium* (*P. atramentosum, P. caseifulvum*, and *P. commune*) [3, 27–30]. *P. variabile* isolated from modern environments are known as producers of polyketide mycotoxins ochratoxin A and rugulosin [27–30].

Nine *P. vulpinum* strains synthesized diketopiperazine alkaloids of the roquefortine family, α-CPA, imine α-CPA, cyclopenines, and patulin [5, 19]. In spite of the differences in the spectra of secondary metabolites in individual strains, biosynthesis of these metabolites is typical of the subgenus *Penicillium*. Similar results have been obtained previously, suggesting classification of *P. vulpinum* within the subgenus *Penicillium* [3].

Thus, metabolite production by members of the subgenus *Biverticillium* exhibited pronounced species specificity (table). *P. rugulosum* strains synthesized diketopiperazine alkaloids rugulosuvines A and B, while *P. variabile* synthesized clavine ergot alkaloids rugulovasines. These metabolites are known for the members of *Biverticillium*, confirming classification of the investigated strains within this subgenus according to micro- and macromorphological characteristics.

SUBGENUS *PENICILLIUM*

To specify the taxonomic position of 44 strains of the subgenus *Penicillium* isolated from various novel and poorly studied environments, polyphasic taxonomy was used based on the profiles of marker metabolites [32].

The spectrum of secondary metabolites produced by three *P. aurantiogriseum* strains isolated from permafrost [20] consisted of metabolites of the roquefortine family, roquefortine and dihydroroquefortine. In modern *P. aurantiogriseum* isolates, benzodiazepine alkaloids anacine and aurantine are unequivocal chemotaxonomic markers [3]. Production of alkaloids of the roquefortine family is typical of other species of the subgenus *Penicillium* [3]. The discrepancy between the species position of the relic strains and produced metabolites may result from the difficulties of species identification of the strains isolated from permafrost sediments. Among the species producing metabolites of the roquefortine family, the morphologically closest one to *P. aurantiogriseum* is the new species *P. melanoconidium* (Frisvad) Frisvad & Samson comb. nov. (2004), which previously has been considered a variant of *P. aurantiogriseum, P. aurantiogriseum* var. *melanoconidium* Frisvad (1989) [3]. Based on the spectrum of secondary metabolites, classification of strains VKM FW-738, VKM FW-741, and VKM FW-766 within the species *P. melanoconidium* was proposed [32]. The secondary metabolite of *P. aurantiogriseum* KBP no. 3 isolated from an urban environment also did not correspond to the species markers [5]. The strain synthesized fellutanine A, which has been previously detected only in members of the subgenus *Furcatum*. Since, according to [3], this metabolite is not a marker for any of the species within the subgenus *Penicillium*, this strain should be reclassified.

Strains VKM FW-725 and VKM FW-791 isolated from permafrost soils and classified as *P. brevicompactum* Dierckx based on their micro- and macromorphological characteristics were found to produce only mycophenolic acid [32]. Biosynthesis of this metabolite is typical of one species, *P. bialowiezense* Zaleski [3]. Brevianamide A (*P. brevicompactum*), dihydroroquefortine, patulin, isofumigaclavines A and B, penitrem A (*P. carneum* Frisvad 1996), and dihydroroquefortine and PR-toxin (*P. roqueforti*) are additional chemotaxonomic markers for other producers of mycophenolic acid [3]. Differentiation between the species *P. brevicompactum* and *P. bialowiezense* based on their morphological characteristics is difficult. These strains may therefore be assigned to *P. bialowiezense* [32].

Strains VKM FW-657, VKM FW-667, VKM FW-690, VKM FW-704, VKM FW-747, and VKM FW-794 isolated from ancient permafrost sediments synthesized clavine ergot alkaloids with complete D ring, belonging to the fumigaclavine family: fumigaclavines A and B and festuclavine [21]. Identification of these strains yielded different species names: *P. verrucosum* Dierckx, *P. puberellum, P. commune, P. granulatum*, and *P. aurantiogriseum*. Strain KBP no. 4 isolated from an anthropogenically damaged ecosystem and classified as *P. chrysogenum* based on its morphological characteristics had a similar metabolite composition [5]. Fumigaclavines A and B are chemotaxonomic markers for the species *P. palitans* Westling [3]. The classification of producers of these compounds within other species is doubted by the authors of polyphasic taxonomy of the subgenus *Penicillium* [3]. Based on production of fumigaclavines A and B, these strains were classified as *P. palitans* Westling, in spite of their uncertain identification by morphological criteria [21].

Strains VKM FW-875, VKM FW-877, VKM FW-878, VKM FW-907, and VKM FW-908 isolated from ancient permafrost sediments produced ochratoxins A and B [21]. These metabolites are synthesized by *P. verrucosum* Dierckx and *P. nordicum* Dragoni, Cantoni ex Ramirez, species that are similar in micro- and most macromorphological characteristics. Some *P. verrucosum* isolates, apart from ochratoxin A, synthesize citrinin and verrucolone as well, while, in *P. nordicum*, apart from ochratoxin A and verrucolone, anacine, an indole-containing compound, is a chemotaxonomic marker [3]. The reddish-brown reverse on agarized YES medium and the absence of biosynthesis of indole-containing metabolites by these strains undoubtedly indicate their affiliation with *P. verrucosum* [21].

Penicillins, roquefortine, and chrysogine are the chemotaxonomic markers for *P. chrysogenum*. Pro-

duction of meleagrin, another metabolite of the roquefortine family is also possible, as well as of PR-toxin and xanthocillin [3]. While biosynthesis of penicillins is an unequivocal evidence that the isolates belong to *P. chrysogenum* (section *Chrysogena*), it should be noted that identification of penicillins was not carried out. The *P. chrysogenum* strains VKM F-227, VKM F-692, VKM F-1078, and VKM F-1987 isolated from soils of different climatic zones synthesized only metabolites of the roquefortine family: roquefortine, dihydroroquefortine, meleagrin, and glandicolines A and B [22]. No production of other chemotaxonomic species markers was detected. *P. chrysogenum* strains isolated from the living quarters of the *Mir* station [23] synthesized the species marker metabolites. Strains IMBP 1-3 and IMBP 1-4 produced metabolites of the roquefortine family (roquefortine and meleagrin) and N-acetyltryptamine, while strains IMBP 1-5 and IMBP 1-6 synthesized simultaneously xanthocillin X and questiomycin A. Strains *P. chrysogenum* VKM FW-653, VKM FW-659, VKM FW-679, VKM FW-684, VKM FW-694, VKM FW-720, VKM FW-721, VKM FW-778, and VKM FW-799 isolated from ancient permafrost sediments did not exhibit production of known marker metabolites of *P. chrysogenum* [32]. This may indicate that these isolates belong to other species of the *Chrysogena* section, *P. dipodomyis* Frisvald, Filtenborg, Wicklow or *P. nalgiovense* Laxa [3].

Confirmation of the species position *P. expansum* Link of six strains isolated from the *Mir* orbital station is difficult since the spectra of their marker metabolites do not correspond to the species markers. For *P. expansum*, the set of marker metabolites includes roquefortine, patulin, and citrinin [3]. Patulin and citrinin production was not detected in *P. expansum* strains from the *Mir* station [23, 24]. Only strains IMBP 2-4 and IMBP 2-5 were able to synthesize roquefortine. They also produced other metabolites of the roquefortine family, dihydroroquefortine and meleagrin. Since this set of metabolites is typical of *P. chrysogenum*, these strains should be classified within this species [3]. Xanthocillin X and questiomycin A revealed in strain IMBP 2-7 are chemotaxonomic markers of *P. chrysogenum, P. flavigenum*, or *P. italicum*, but not of *P. expansum* [3]. Interestingly, after several transfers on agar media, this strain switched to production of isorugulosuvine alone. The capacity for synthesis of xanthocillin X and questiomycin A by the strain adapted to artificial maintenance conditions was restored by supplementing the Abe medium with zinc ions [24]. Strains IMBP 2-2, IMBP 2-3, and IMBP 2-6 synthesized isorugulosuvine and rugulosuvine D; strain IMBP 2-2 synthesized also viridicatine and viridicatol, metabolites of the cyclopenine family [23]. Simultaneous synthesis of rugulosuvines and viridicatines is typical of *P. polonicum* Zaleski and possible in *P. tricolor* [3].

Thus, most of the strains of the subgenus *Penicillium* isolated from permafrost soils, *Mir* orbital complex, and sites subject to anthropogenic load exhibited discrepancy between their species identification based on micro- and macromorphological characteristics and the marker secondary metabolites known for type cultures. This discrepancy may be explained, first of all, by the difficulty of identification based on morphological characteristics for the isolates obtained from conditions different from the type ones. The morphological differences between the isolates and type strains are of adaptive nature and are probably phenotypic. These differences result from development of fungi under specific conditions, resulting in changes of the morphological phenotype. The variability of morphological characteristics in different isolates of the same species is a significant problem in taxonomy of these fungi [1]. All species within the subgenus have numerous synonyms and variants [1, 3].

Thus, biosynthesis of metabolites of a specific structure (table) is characteristic of each subgenus of penicillia; the profiles of secondary metabolites may be used for taxonomic purposes. The fungi of the subgenus *Aspergilloides* usually synthesize polyketide metabolites. For strains of the subgenus *Furcatum*, the spectrum of secondary metabolites, including clavine ergot alkaloids and diketopiperazine metabolites, was enlarged. Costaclavine, epicostaclavine, agroclavine-I, epoxyagroclavine-I, isorugulosuvine, fellutanines, and isofellutanines. Production of isorugulosuvine is also characteristic of some fungi of the subgenus *Penicillium* [3]. Fungi of the subgenus *Biverticillium* synthesize only rugulovasines of clavine ergot alkaloids and rugulosuvines (puberulines) of diketopiperazine alkaloids. These metabolites are also specific for some species of the subgenus *Penicillium* [3]. In the investigated isolates of the subgenus *Penicillium* isolated from permafrost soils, *Mir* orbital complex, and sites subject to anthropogenic load, marker metabolites were found, which are known for type cultures of the species of the subgenus *Penicillium* [3]. These are alkaloids of the roquefortine and viridicatine families, rugulosuvines, festuclavine, fumigaclavines A and B, and mycophenolic acid. Based on the spectrum of marker metabolites, species affiliation of the strains of the subgenus *Penicillium* were refined. Biosynthesis of metabolites of the roquefortine family by strains of the subgenus *Aspergilloides* (*P. spinulosum* KBP no. 15) and of the subgenus *Furcatum* (*P. citrinum* VKM F-1290, *P. jensenii* VKM F-292 and VKM F-293, and *P. janczewskii* VKM F-2489) may indicate that they belong to the subgenus *Penicillium*.

REFERENCES

1. Pitt, J.I., *The Genus Penicillium and Its Teleomorphic States Eupenicillium and Talaromyces*, London: Academic Press, 1979.

2. Tutel'yan, V.A., Kravchenko, L.V., and Sergeev, A.Yu., Mycotoxins, in *Mikologiya segodnya* (Mycology Today), vol. 1, D'yakov, Yu.T., and Sergeev, Yu.V., Eds., Moscow: Natsional'naya akademiya mikologii, 2007, pp. 283–304.
3. Samson, R.A. and Frisvad, J.C., *Penicillium* subgenus *Penicillium*: New Taxonomic Schemes, Mycotoxins and Other Extrolites, *Studies in Mycology*, 2004, no. 49, pp. 1–174.
4. Dreyfuss, M.M and Chapela, I.H, Potential of Fungi in the Discovery of Novel, Low-Molecular Weight Pharmaceuticals, in *The Discovery of Natural Products Therapeutic Potential*, Gullo, V.P., Ed., Boston-London: Buttererworth-Heinemann Press, 1994, pp. 49–79.
5. Kozlovsky, A.G., Marfenina, O.E., Vinokurova, N.G., Zhelifonova, V.P., and Adanin, V.M., Mycotoxins of *Penicillium* Strains Isolated from Soils of Undisturbed and Anthropogenically Disturbed Ecosystems, *Mikrobiologiya*, 1997, vol. 66, no. 2, pp. 206–210 [*Microbiology* (Engl. Transl.), vol. 66, no. 2, pp. 169–172].
6. Kozlovsky, A.G., Zhelifonova, V.P., Adanin, V.M., Ozerskaya, S.M., and Grafe, U., Cyclocitrinol, a New Metabolite from *Penicillium citrinum, Pharmazie*, 2000, vol. 55, no. 6, pp. 470–471.
7. Kozlovsky, A.G., Vinokurova, N.G., Zhelifonova, V.P., Adanin, V.M., and Ozerskaya, S.M., Alkaloids Synthesized by Penicillia Isolated from Syrian Soils, *Mikrobiologiya*, 1997, vol. 66, no. 5, pp. 600–604 [*Microbiology* (Engl. Transl.), vol. 66, no. 5, pp. 501–504].
8. Kozlovsky, A.G., Zhelifonova, V.P., Adanin, V.M., Antipova, T.V., Ozerskaya, S.M., Kochkina, G.A., and Grefe, U., The Fungus *Penicillium citrinum* Thom 1910 VKM FW-800 Isolated from Ancient Permafrost Sediments As a Producer of the Ergot Alkaloids Agroclavine-I and Epoxyagroclavine-I, *Mikrobiologiya*, 2003, vol. 72, no. 6, pp. 816–821 [*Microbiology* (Engl. Transl.), vol. 72, no. 6, pp. 723–727].
9. Kozlovsky, A.G., Solov'eva, T.F., Sakharovskii, V.G., and Adanin, V.M., Ergot Alkaloids Agroclavine-I and Epoxyagroclavine-I, Metabolites of *Penicillium corylophilum, Prikl. Biokhim. Mikrobiol.*, 1982, vol. 18, no. 4, pp. 525–541.
10. Kozlovsky, A.G., Vepritskaya, I.G., and Gayazova, N.B., Alkaloid Production by the Fungus *Penicillium sizovae, Prikl. Biokhim. Mikrobiol.*, 1986, vol. 22, no. 2, pp. 205–210.
11. Kozlovsky, A.G., Zhelifonova, V.P., Antipova, T.V., Adanin, V.M., Ozerskaya, S.M., Kochkina, G.A., Schlegel, B., Dahse, H.M., Gollmick, F.A., and Grafe, U., Quinocitrinines A and B, New Quinoline Alkaloids from *Penicillium citrinum* VKM FW-800, a Permafrost Fungus, *J. Antibiot.,* 2003, vol. 56, no. 5, pp. 488–491.
12. Kozlovsky, A.G., Vinokurova, N.G., Adanin, V.M, and Sedmera, P., Secondary Metabolites of Various Strains of *Penicillium fellutanum, Prikl. Biokhim. Mikrobiol.,* 1997, vol. 33, no. 4, pp. 408–414 [*Appl. Biochem. Microbiol.* (Engl. Transl.), vol. 33, no. 4, pp. 364–369].
13. Kozlovsky, A.G., Vinokurova, N.G., and Adanin, V.M., Diketopiperazine Alkaloids from the Fungus *Penicillium piscarium* Westling, *Prikl. Biokhim. Mikrobiol.*, 2000, vol. 36, no. 3, pp. 317–321 [*Appl. Biochem. Microbiol.* (Engl. Transl.), vol. 36, no. 3, pp. 271–275].
14. Kozlovsky, A.G., Vinokurova, N.G., Adanin, V.M., and Grafe, U., Piscarinines, New Polycyclic Diketopiperazine Alkaloids from *Penicillium piscarium* VKM F-691, *Natural Products Lett.*, 2000, vol. 14, no. 5, pp. 333–340.
15. Kozlovsky, A.G., Vinokurova, N.G., Zhelifonova, V.P., and Ozerskaya, S.M., Alkaloid Formation by Penicillia of the Series *Fellutana* and *Canescentia, Mikrobiologiya*, 1997, vol. 66, no. 4, pp. 514–519 [*Microbiology* (Engl. Transl.), vol. 66, no. 4, pp. 429–433].
16. Kozlovsky, A.G., Vinokurova, N.G., Zhelifonova, V.P., and Adanin, M.M., Secondary Metabolites of Fungi Belonging to the Species *Penicillium janczewskii, Prikl. Biokhim. Mikrobiol.*, 1997, vol. 33, no. 1, pp. 70–74 [*Appl. Biochem. Microbiol.* (Engl. Transl.), vol. 33, no. 1, pp. 61–64].
17. Kozlovsky, A.G., Adanin, V.M., Daze, Kh.M., and Grefe, U., Rugulosuvines A and B, Diketopiperazine Alkaloids of *Penicillium rugulosum* and *Penicillium piscarium* Fungi, *Prikl. Biokhim. Mikrobiol.*, 2001, vol. 37, no. 3, pp. 292–296 [*Appl. Biochem. Microbiol.* (Engl. Transl.), vol. 37, no. 3, pp. 253–256].
18. Antipova, T.V., Zhelifonova, V.P., Kochkina, G.A., and Kozlovsky, A.G., Growth and Biosynthesis of Rugulovasines in *Penicillium variabile* Sopp 1912, *Mikrobiologiya*, 2008, vol. 77, no. 4, pp. 502–507 [*Microbiology* (Engl. Transl.), vol. 77, no. 4, pp. 446–450].
19. Kozlovsky, A.G., Vinokurova, N.G., and Zhelifonova, V.P., Mycotoxin Production Profiles of *Penicillium vulpinum* (Cooke & Massee) Seifert & Samson Strains, *Mikrobiologiya*, 2000, vol. 69, no. 1, pp. 45–48 [*Microbiology* (Engl. Transl.), vol. 69, no. 1, pp. 36–39].
20. Kozlovsky, A.G., Zhelifonova, V.P., Adanin, V.M., Antipova, T.V., Ozepskaya, S.M., Kochkina, G.A., and Grefe, U., *Penicillium aurantiogriseum* Dierckx 1901: Producer of Diketopiperazine Alkaloids (Roquefortine and 3,12-Dihydroroquefortine), Isolated from Permafrost, *Prikl. Biokhim. Mikrobiol.*, 2003, vol. 39, no. 4, pp. 393–397 [*Appl. Biochem. Microbiol.* (Engl. Transl.), vol. 39, no. 4, pp. 393–397].
21. Zhelifonova, V.P., Antipova, T.V., Ozerskaya, S.M., Kochkina, G.A., and Kozlovsky, A.G., Secondary Metabolites of *Penicillium* Fungi Isolated from Permafrost Deposits as Chemotaxonomic Markers, *Mikrobiologiya*, 2009, vol. 78, no. 3, pp. 393–398 [*Microbiology* (Engl. Transl.), vol. 78, no. 3, pp. 350–354].
22. Kozlovsky, A.G., Vinokurova, N.G., and Ozerskaya, S.M., Peculiarities of Alkaloid Formation in *Penicillium chrysogenum* Strains Isolated from Soils of Different Climatic Zones, *Mikrobiologiya*, 1998, vol. 67, no. 4, pp. 483–487 [*Microbiology* (Engl. Transl.), vol. 67, no. 4, pp. 397–400].
23. Kozlovsky, A.G., Zhelifonova, V.P., Adanin, V.M., Antipova, T.V., Shnyreva, A.V., and Viktorov, A.I., The Biosynthesis of Low-Molecular-Weight Nitrogen-Containing Secondary Metabolites—Alkaloids—by the Resident Strains of *Penicillium chrysogenum* and *Penicillium expansum* Isolated on Board the *Mir* Space Station, *Mikrobiologiya*, 2002, vol. 71, no. 6, pp. 773–777 [*Microbiology* (Engl. Transl.), vol. 71, no. 6, pp. 666–669].
24. Kozlovsky, A.G., Zhelifonova, V.P., Antipova, T.V., Adanin, V.M., Novikova, N.D., Deshevaya, E.A., Shlegel', B., Daze, Kh.M., Gollmik, F., and Grefe, U.,

Penicillium expansum, a Resident Fungal Strain of the Orbital Complex *Mir*, Producing Xanthocyllin X and Questiomycin A, *Prikl. Biokhim. Mikrobiol.*, 2004, vol. 40, no. 3, pp. 344–349 [*Appl. Biochem. Microbiol.* (Engl. Transl.), vol. 40, no. 3, pp. 291–295].

25. Williams, R.M., Stocking, E.M., and Sans-Cervera, J.F., Biosynthesis of Prenylated Alkaloids Derived from Tryptophan, *Topics in Curr. Chem.*, 2000, vol. 209, pp. 98–171.
26. Abe, M., Researches on Ergot Fungus, *Annual Reports Takeda Research Labs.*, 1951, vol. 10, p. 73.
27. Mantle, P.G, Secondary Metabolites of *Penicillium* and *Acremonium*, in *Biotechnology Handbooks*, Atkinson, T. and Sherwood, R.F., Eds., New York: Plenum Press, 1987, vol. 1, pp. 161–243.
28. Cole, R.J. and Cox, R.H., *Handbook of Toxic Fungal Metabolites*, New York: Academic Press, 1981.
29. Turner, W.B. and Aldridge, D., *Fungal Metabolites II*, London: Academic Press, 1983.
30. Frisvad, J.C. and Filtenborg, O., Revision of *Penicillium* subgenus *Furcatum* Based on Secondary Metabolites and Conventional Characters, in *Modern Concepts in Penicillium and Aspergillus classification*, Samson, R.A. and Pitt, J.I., Eds., New York: Plenum Press, 1990, pp. 159–170.
31. Frisvad, J.C, Expressions of Secondary Metabolism as Fundamental Characters in *Penicillium* Taxonomy, in *Toxigenic Fungi*, Kurata, H. and Yeno, Y., Eds., Amsterdam: Elsevier, 1984, pp. 98–106.
32. Kozlovsky, A.G., Zhelifonova, V.P., and Antipova, T.V., Secondary Metabolites in the Taxonomy of Fungi of the Subgenus *Penicillium, Mikrobiologiya*, 2009, vol. 78, no. 5, pp. 684–689 [*Microbiology* (Engl. Transl.), vol. 78, no. 5, pp. 618–623].