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EXPERIMENTAL ARTICLES

The *Penicillium commune* Thom and *Penicillium clavigerum* Demelius Fungi Producing Fumigaclavines A and B

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Abstract—The type strains *Penicillium clavigerum* VKM F-447 and *P. commune* VKM F-3233 are found to produce fumigaclavines A and B. Of the seven other strains of these species, only two strains, *P. commune* VKM F-3088 and F-3491, possess the ability to synthesize these alkaloids. It is suggested that the five other strains under study either lost such an ability or require very specific conditions for the synthesis of these alkaloids.

Key words: fungi, *Penicillium commune*, *Penicillium clavigerum*, metabolites.

Due to their biological activity, ergot alkaloids occupy an important place among the low-molecular-weight secondary metabolites of microscopic fungi [1]. There are two major structural groups of ergot alkaloids, peptide ergot alkaloids (derivatives of lysergic acid) and clavine alkaloids. Most ergot and clavine alkaloids are produced by *Claviceps* species, although some clavine alkaloids are produced by fungi of the genera *Penicillium* and *Aspergillus* [1, 2]. Fumigaclavines A and B are clavine alkaloids with a complete 6-methyl ergoline ring system. These alkaloids are produced by a small number of fungi: *A. fumigatus* [3], *P. oxalicum* VKM F-478 [4], *P. chrysogenum* 4 [5], *P. commune* VKM F-3088 (*P. palitans*) [4], and three strains of *P. crustosum* belonging to one chemotype [6]. There is some evidence that clavine alkaloids of this group are also produced by the fungi *P. clavigerum* and *P. commune* [7]. Isomeric fumigaclavines, isofumigaclavine A (or roquefortine A) and isofumigaclavine B (roquefortine B), have thus far been found only in the fungus *P. roquefortii* and, hence, can be considered as chemotaxonomic markers of this *Penicillium* species [8, 9].

The aim of this work was to investigate the ability of two microscopic fungi, *P. clavigerum* and *P. commune*, to synthesize fumigaclavines A and B.

MATERIALS AND METHODS

The strains used in this work, *Penicillium commune* VKM F-262, F-687, F-689, F-3086, F-3088, F-3233^T, and F-3491, and *Penicillium clavigerum* VKM F-447^T and F-1293, were obtained from the All-Russia Collection of Microorganisms (VKM).

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The strains were grown at 24 ± 1°C in 750-ml Erlenmeyer flasks with 150 ml of one of the following media: synthetic Abe medium (medium 1); glucose–peptone medium with soybean meal (medium 2); and Czapek–Dox medium with 0.5% yeast extract (medium 3) [10]. Submerged cultivations were carried out in medium 1 on a shaker (220 rpm), whereas surface cultivations were performed on media 2 and 3 without shaking. Secondary metabolites were isolated from filtrates of the culture liquids taken in the phase of active growth and in the stationary phase. The filtrates were brought to pH 8–9 with aqueous ammonia and extracted thrice with chloroform. The extracts were pooled, dehydrated with anhydrous Na_2SO_4, filtered, and completely dried in a vacuum rotary evaporator. The extracted metabolites were purified and analyzed by thin-layer chromatography (TLC) on Silufol UV-254 plates (Kavalier, Czech Republic) or Silica gel 60 F-254 plates (Merck, Germany) developed in an ethylacetate–methanol–25% NH_4OH (85 : 15 : 10) mixture (system I), in a chloroform–methanol–25% NH_4OH (90 : 10 : 0.1) mixture (system II), in a toluene–ethylacetate–formic acid (5 : 10 : 1) mixture (system III), or in a chloroform–acetone (9 : 1) mixture (system IV). Systems I and II were used for the isolation of clavine and diketopiperazine alkaloids and α-cyclopiazonic acid (α-CPA), system III was used for the isolation of patulin, and system IV, for the isolation of griseofulvin. The spots were visualized by color reaction with the Ehrlich reagent or through their absorption or fluorescence under UV light. Secondary metabolites were identified on the basis of their UV and mass spectra and relative chromatographic mobility. The UV spectra of metabolites dissolved in methanol were recorded using a Shimadzu UV-160A spectrophotometer (Japan). Mass spectra were obtained using a Finnigan MAT 8430 high-resolution mass spectrometer (Germany).

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Table 1. Some physicochemical characteristics of fumigaclavines

Alkaloid	Relative mobility ($R_f \times 100$) on Merck plates in solvent systems		Melting point, °C
	I	II	
Fumigaclavine A	83	81	80–82
Isofumigaclavine A	76	79	238–240
Fumigaclavine B	72	51	194–195
Isofumigaclavine B	61	36	282–284

Table 2. The secondary metabolites produced by various strains of *P. commune*

Strain	Secondary metabolites produced
F-3233^T	α-CPA, α-CPA imine, fumigaclavines A and B, pyroclavine
F-3086	α-CPA
F-3088	α-CPA, fumigaclavines A and B, pyroclavine, festuclavine, agroclavine, and chanoclavine 1
F-3491	α-CPA, fumigaclavines A and B, pyroclavine
F-262	Roquefortine, 3,12-digidroroquefortine
F-687	Roquefortine and meleagrin
F-689	Brevianamides A and B

RESULTS AND DISCUSSION

Strains of the soil fungus *P. clavigerum* are known as producers of penitrem A, roquefortine A, and phenols [7]. In physiological and morphological characteristics, *P. clavigerum* is most close to the species *P. glandicola* and *P. vulpinum*, but differs from them in the profile of volatile metabolites [11]. Larsen and Frisvad oppose Pitt's view that *P. clavigerum* and *P. duclauxii* are synonyms [12], since the secondary metabolite profiles of these species are different. When using a secondary metabolite profile as a taxonomic criterion, one should take into account that the synthesis of secondary metabolites depends, to a certain degree, on the cultivation conditions of the producing fungus [10, 13]. Bearing this in mind, we studied the production of metabolites by the fungi *P. clavigerum* and *P. commune* under different conditions, i.e., during cultivation on synthetic and complex media in submerged and surface modes. Furthermore, fungal cultures were analyzed for secondary metabolites in two growth phases: the phase of active growth (which corresponded to 5–7 days of cultivation in the submerged mode and 10–12 days of cultivation in the surface mode) and the stationary growth phase (which occurred after 12–15 days of cultivation in the submerged mode and 18–20 days of cultivation in the surface mode).

During surface cultivation, the strain type *P. clavigerum* F-447 did not produce the secondary metabolites described for this species [7, 14], although it was found to produce a small amount of fumigaclavine B during submerged cultivation. In contrast, the other studied strain of this species, F-1293, was found to produce a range of structurally different metabolites: the diketopiperazine alkaloid roquefortine C, the arbitrary clavine alkaloid α-CPA and its imine, and the oxygen-containing heterocyclic mycotoxins patulin, griseofulvin, and dechlorogriseofulvin. During cultivation on medium 1, the major metabolite was patulin; whereas α-CPA and roquefortine were the major metabolites produced on medium 2. Griseofulvin was produced on media 2 and 3. We failed to detect clavine alkaloids in the culture liquid of strain F-1293.

Representatives of the species *P. commune* are widely spread in nature, being frequently isolated from foods and plant rhizospheres. The comprehensive analysis of a great number of fungal strains from various collections and their own isolates allowed Frisvad and Filtenborg to divide these strains (on the basis of some macromorphological characteristics and the secondary metabolite profiles) into two chemotypes [7]. Representatives of both chemotypes were reported to be able to synthesize clavine alkaloids.

Our earlier studies of *P. commune* VKM F-3088 (formerly *P. palitans* [4, 13]) showed that this *Penicillium* fungus produces fumigaclavines A and B, analogous to those of *Aspergillus fumigatus* [3] and different from the isofumigaclavines A and B synthesized by *P. roquefortii* [9]. The isomeric alkaloids, which have the same UV and mass spectra, were distinguished based on differences in their chromatographic mobilities and melting points (Table 1). Strain F-3088 also produced festuclavine, pyroclavine, α-CPA, small amounts of agroclavine and chanoclavine 1, as well as cyclopenin and cyclopenol. The synthesis of clavine alkaloids took place only upon surface cultivation and was accompanied by the intense formation of conidia. When cultivated in the submerged mode, the fungus neither synthesized clavine alkaloids nor produced conidia [15].

Of the seven *P. commune* strains studied, three (VKM F-3233, F-3491, and F-3086) synthesized α-CPA. Two of these strains (F-3491 and the strain type F3233) also synthesized clavine alkaloids analogous to those produced by strain VKM F-3088 (Table 2). Unlike the other producing strains, strain VKM F-3491 synthesized clavine alkaloids not only during surface but also during submerged cultivation. The three other *P. commune* strains studied (VKM F-262, F-687, and F-689) were found to be unable to synthesize clavine alkaloids, but were able to synthesize tryptophan-containing diketopiperazine alkaloids. Strain VKM F-262 synthesized mainly roquefortine C [16], whose synthesis was especially active upon submerged cultivation. Strain VKM F-687 produced roquefortine and meleagrin. When grown on medium 3, strain VKM F-689 synthesized brevianamides A and B.

Thus, this research made it possible to reveal three new producers of fumigaclavines A and B (strains VKM F-447, F-3233, and F-3491). Since F-447 and F-3233 are the type strains of the species *P. clavigerum* and *P. commune*, respectively, it might be expected that the other strains of these species available in the collection are also able to produce these alkaloids. However, it turned out that they are not. This can be explained by the fact that the other strains tested either lost such an ability during storage or require very specific conditions for the synthesis of these alkaloids.

It should also be noted that it is necessary to make complete qualitative and quantitative analyses of the secondary metabolites of fungi from the genus *Penicillium* with reference to its taxonomic structure. This will allow the significance of a particular characteristic to a particular species to be evaluated.

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