*Chemistry of Natural Compounds, Vol. 45, No. 1, 2009*

# FATTY-ACID COMPOSITION OF CERTAIN SPECIES OF MARINE MYCELIAL FUNGI

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UDC 577.115:582.281

*The fatty-acid composition of seven strains of marine mycelial fungi was studied. GC and GC—MS showed that marine fungi of the genus* Penicillium *synthesized fatty-acid mixtures of saturated and unsaturated acids of similar compositions with different percent contents. Fatty-acid profiles of fungi associated with holothuria* Chiridota ochotensis *were characterized for the first time. Producers of branched 15:0 and dichloro- and cyclopropane-containing acids were observed.*

**Key words:** marine mycelial fungi, fatty acids, dichlorostearic acid, *Penicillium islandicum*, *P. puberulum*.

Marine mycelial fungi produce various types of biologically active compounds with unique chemical structures that are not found among metabolites of terrestrial fungi [1-4]. The biosynthesis of such compunds is a consequence of various marine adaptations characteristic of marine macro- and microorganisms. One factor for such adaptations may be the unusual composition of cellular and extracellular fatty acids of marine fungi that change the viscosity of membranes by distributing into the lipid bilayer of cell membranes or by incorporating into the fatty-acid part of membrane phospholipids. Fatty acids can change the membrane permeability for low-molecular-weight compounds that play an important role in interspecies relationships of organisms living in the marine environment. The fatty-acid composition of marine fungi is insufficiently studied. An unusually high percent of branched and unsaturated fatty acids in marine fungi has been reported several times [5-8]. It was demonstrated that marine fungi produce such important fatty acids as 16:0, 18:0, 18:1*n*9, and 18:2*n*6 in addition to 18:3*n*3 and 20:4*n*6 [9]. We studied the fatty-acid composition of marine fungi isolated during an expedition of the research vessel Akademik Oparin (Aniva Bay, Okhotsk Sea, 2004) and the composition of fractions of extracellular fatty acids (FA) from strains of facultative marine fungi *Penicillium puberulum* Bainier, *P. islandicum* Sopp, and *Verticillium tenerum* Nees and three strains of the fungal group *Anamorphic fungi* (*Agonomycetes*) (Table 1).

The results showed that the composition of fatty acids from two strains of *P. islandicum* (Nos. 6 and 7) (Table 2) had high contents of saturated and unsaturated fatty acids. Their fatty-acid compositions differed in percent content of these acids in mixtures and the presence of an appreciable amount (6.69%) of hydroxyacids in strain No. 6. This difference can be explained by the isolation of the fungi from different soil samples. Strain *P. puberulum* (No. 1) synthesized a large percent of saturated FA (89.34%). The FA composition of this species differed from that of *P. islandicum* by a lower content of unsaturated FA. The FA composition of *V. tenerum* (No. 4) and two strains of *Anamorphic fungi* (*Agonomycetes*) (Nos. 5 and 3) associated with holothuria lacked unsaturated FA and had high (99.9, 90.3, and 33.5%) contents of saturated acids. However, strain No. 3 synthesized an anomalously high percent of branched fatty acids that was apparently a signature of this strain of microorganism. A strain of *Anamorphic fungi* (*Agonomycetes*) (Table 2, No. 2) synthesized a dichloro-containing acid, 18:0 (13.83%). The retention time and mass spectrum of the dichloro-containing acid agreed with those for dichlorostearic acid given in the NIST98 database. Dichlorostearic acid was obtained previously from mycelium of the Yangiyul′ population of the pathogenic fungus *Verticillium dahliae* Kleb., which causes verticillic cotton wilt [10]. Chloro-containing fatty acids are as a rule minor components of various microorganisms [11, 12].

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 0009-3130/09/4501-0018 

TABLE 1. Sources of Isolated Fungal Strains

| Strain No. | Species | Source |
|---|---|---|
| 1 | *Penicillium puberulum* | Okhotsk Sea, Aniva Bay, 42 m depth, soil |
| 2 | *Anamorphic fungi* (*Agonomycetes*) | Okhotsk Sea shelf, Maisk Bay region, 57 m depth, soil |
| 3 | *Anamorphic fungi* (*Agonomycetes*) | Isolated from holothuria *Chiridota ochotensis*, Okhotsk Sea |
| 4 | *Verticillium tenerum* | Isolated from holothuria *Chiridota ochotensis*, Okhotsk Sea |
| 5 | *Anamorphic fungi* (*Agonomycetes*) | Isolated from holothuria *Chiridota ochotensis*, Okhotsk Sea |
| 6 | *Penicillium islandicum* | Okhotsk Sea, Aniva Bay, 42 m depth, soil |
| 7 | *Penicillium islandicum* | Okhotsk Sea, Aniva Bay, 42 m depth, soil |

TABLE 2. Fatty-Acid Composition of Studied Fractions of Marine Mycelial Fungal Strains, % of Total Fatty Acids

| Acid | Studied fungal strain | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Saturated | | | | | | | |
| 16:0 | 69.18 | 73.09 | 33.54 | 78.18 | 79.12 | 21.70 | 40.13 |
| 18:0 | 20.16 | 9.94 | Tr. | 21.81 | 11.27 | 1.34 | 2.84 |
| $\Sigma_{Sat.}$ | 89.34 | 83.03 | 33.54 | 99.99 | 90.39 | 23.04 | 42.97 |
| Unsaturated | | | | | | | |
| 18:1 | 10.65 | - | - | - | - | 12.34 | 56.73 |
| 18:2 | - | - | - | - | - | 57.90 | Tr. |
| $\Sigma_{Unsat.}$ | 10.65 | | | | | 70.24 | 56.73 |
| Branched | | | | | | | |
| 15:0 methyl-14 | - | - | 66.45 | - | - | - | - |
| Cyclopropane | | | | | | | |
| 9,10-cyclo 17:0 | - | 3.14 | - | - | - | - | - |
| Chloro-containing | | | | | | | |
| dichloro-18:0 | - | 13.83 | - | - | - | - | - |
| Hydroxyacids | | | | | | | |
| 9-hydroxy 9:0 | - | - | - | - | - | 6.69 | - |
| Total fatty acids, % of hexane fraction | 27.22 | 70.87 | 100 | 37.94 | 71.21 | 100 | 88.42 |

1 - *Penicillium puberulum*; 2, 3, 5 - *Anamorphic fungi* (*Agonomycetes*); 4 - *Verticillium tenerum*; 6, 7 - *P. islandicum*.

About 300 chlorinated fatty acids of natural origin are presently known. Sources of chloro-containing metabolites are marine microorganisms, algae, and marine invertebrates [12]. The presence of chlorinated and oxidized fatty acids in fatty-acid mixtures of fungi No. 2 and 6 may be indicative of highly active oxidative enzymatic systems in these fungi [13, 14].

## EXPERIMENTAL

**Cultivation of Fungi and Isolation of Fatty-Acid Mixtures.** Strains *Penicillium puberulum*, *P. islandicum*, *Verticillium tenerum*, and strains of the fungal group *Anamorphic fungi* (*Agonomycetes*) were cultivated on wort agar in marine water [15] for two weeks at room temperature. Cultures were extracted three times with ethylacetate. The extracts were evaporated to dryness. Total fractions of fatty-acids were obtained by chromatography of the ethylacetate extract over silica gel using hexane:ethylacetate (0→10%).

**Analysis of Mixtures of Acid Methyl Esters.** Methyl esters of fatty acids were analyzed in a GC (HP-5SM, 0.2 mm × 30 m column, Agilent 6850, Germany). Temperature program: injector 270°C; 100°C (1 min), 10°C/min to 270°C (20 min); detector 300°C (He carrier gas). GC—MS of the mixtures was performed using the same conditions in an HP GC System 6890 with an HP-5 ms column (30 m × 250 mm × 0.25 μm) with 5% phenylmethylsiloxane (MSD5973, Hewlett—Packard, USA) (He carrier gas). The products were identified by comparison of their mass spectra with those of known compounds using the NIST98 database. Table 2 lists the fatty-acid composition of the studied fungi.

**Mass Spectrum of Dichlorostearic Acid Methyl Ester.** (EI, 70 eV, *m*/*z*): 367.1 $[M]^+$, 330.9 $[M - HCl]^+$, 294.1 $[M - 2HCl]^+$, 263.2 $[M - 2HCl - OCH_3]^+$, 235.0 $[M - 2HCl - OCH_3 - CO]^+$, 221.0 $[M - 2HCl - OCH_3 - CO - CH_2]^+$, 207.1 $[M - 2HCl - OCH_3 - CO - C_2H_4]^+$, 137.1 $[M - 2HCl - OCH_3 - CO - C_7H_{14}]^+$.


## ACKNOWLEDGMENT

The work was supported by grants of the FEB RAS No. 06-III-B-05 and 06-III-A-06-184, grants of the RFBR No. 06-04-96001 and 06-04-48578, and the RAS Presidium Program Molecular and Cellular Biology.


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