

FREE FATTY ACIDS FROM THE MARINE FUNGI *Cladosporium cladosporioides*, *Talaromyces wortmanii* AND *Wallemia sebi*

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Marine mycelial fungi are known to produce biologically active compounds with unusual chemical structures [1, 2]. These compounds are synthesized because of the need to adapt to specific marine habitats. One of the consequences of this adaptation is the unusual composition of cellular and extracellular fatty acids. It was shown that marine fungi produce fatty acids such as 16:0, 18:0, 18:1n9, 18:2n6, 18:3n3, and 20:4n6 [3] with an unusually high percentage of branched and unsaturated fatty acids [4]. However, the fatty-acid composition of marine fungi isolates are poorly studied.

In continuation of studies of secondary metabolites of marine fungi, hexane fractions and free-fatty-acid fractions from cultures of marine isolates of the strains *Cladosporium cladosporioides* (bottom, 26.7 m deep, Okhotsk Sea), *Talaromyces wortmanii* (sponge *Heniacidon assimilis*, Okhotsk Sea), and *Wallemia sebi* (bottom, 6 m deep, Sea of Japan, Troitsa Bay) were studied.

Fungus strains were cultivated in standard agar–wort medium prepared from seawater [5]: *C. cladosporioides* for 7 d; *T. wortmanii*, 14; and *W. sebi*, 30. This corresponded with the times for reaching a steady-state condition for these fungus species grown in culture. Cultures were extracted with EtOH. The extracts were evaporated to dryness. The resulting solids were dissolved in EtOH (10%) and extracted successively with hexane, EtOAc, and BuOH. The hexane fractions were evaporated at reduced pressure and analyzed by GC–MS by comparing mass spectrometric fragmentation of standard compounds using the NIST98 database. Table 1 presents the results.

Hexane fractions of the studied marine fungi contained fatty acids 16:0, 18:0, and 18:1n9 as the ethyl esters. *C. cladosporioides* also contained insignificant quantities of the isopropyl esters of hexadecanoic and phthalic acids. The isolate of *W. sebi* produced three hydrocarbons with C-17, C-25, and C-32 linear carbon chains and with C-29 branched symmetrically. All studied fractions of the marine fungi contained dibutyl- and di-(2-ethylhexyl)phthalates, the content of which in the hexane fraction of *C. cladosporioides* culture was unusually high, 48.8 and 43.9%, respectively. Phthalates isolated from marine fungi can inhibit cathepsin B, an enzyme for protein catabolism [6].

The EtOAc fraction of each culture was chromatographed over a column of silica gel with elution by a gradient of hexane:EtOAc (100:0→90:10) to isolate the free fatty acids. The resulting total acids were analyzed by GC–MS as the methyl esters (methylation by diazomethane in ether) and pyrrolidides [7]. The derivatives were identified by comparison of their mass spectra with those of standard compounds using the NIST98 database. Table 2 presents the results.

All studied strains produced oleic (18:1,n9) and linoleic (18:2,n9,12) acids. *C. cladosporioides* and *W. sebi* produced nonadecarboxylic acid in small quantities (0.6 and 1.5%). The studied strains of marine fungi are capable of producing both normal and branched fatty acids. The results showed that 16:0 and 18:0 fatty acids in the marine fungi could contain one or two double bonds.

The BuOH extracts of the studied marine fungi strains did not contain free fatty acids.

The total fatty-acid fraction of *T. wortmanii* reduced the fertilizing capability of sperm from the sea urchin *Strongylocentrotus intermedius* at a concentration of 5 µg/mL and blocked egg cell development at a concentration of 8 µg/mL [8].

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TABLE 1. Composition of Hexane Fractions of Facultative Marine Fungi *C. cladosporioides*, *T. wortmanii*, and *W. sebi*, %

Compound	Marine fungus strain		
	<i>C. cladosporioides</i>	<i>T. wortmanii</i>	<i>W. sebi</i>
Ethyl ester of 16:0	0.9		4.6
Ethyl ester of 18:1n9	0.4	4.5	6.6
Ethyl ester of 18:0	0.3	5.9	3.0
Isopropyl ester of 16:0	0.2		
Heptadecane			2.7
Pentacosane			1.9
Dotriacontane			2.0
9-Octyleicosane			3.0
Isopropylphthalate	0.2		
Dibutylphthalate	48.8	10.8	0.9
Di-(2-ethylhexyl)phthalate	43.9	11.6	21.2

TABLE 2. Composition of Fatty-Acid Fractions of Facultative Marine Fungi *C. cladosporioides*, *T. wortmanii*, and *W. sebi*, %

Fatty acid	Marine fungus strain		
	<i>C. cladosporioides</i>	<i>T. wortmanii</i>	<i>W. sebi</i>
9:0	0.6		1.5
10-Me-12:0	6.4		0.8
12-Me-13:0	1.6		0.8
14:0		4.7	
15:0		6.4	
16:1n7	2.3		
16:0	22.2	56.4	10.2
17:0			6.2
18:2n9,12	5.0	2.6	9.8
18:1n9	5.1	14.2	3.3
18:0	5.6	15.1	3.8

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