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SHORT COMMUNICATION

MARINE FUNGI: THE OCCURRENCE OF ERGOSTEROL AND CHOLINE

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Abstract—Ergosterol and choline were identified in several marine Ascomycetes and Fungi Imperfecti.

INTRODUCTION

THE SECONDARY metabolism of marine fungi is virtually unknown. Filamentous Ascomycetes and Fungi Imperfecti comprise most of the described marine fungal species; however, published accounts of their physiology are few, and are concerned chiefly with saline tolerance,[1] nutritional requirements,[2] or cellulolytic activities having potential economic as well as biological significance.[3] Marine yeasts also have been collected extensively, but their biochemistry has been investigated only in relation to taxonomy.[4] Non-filamentous lower forms have received the most attention from a purely chemical standpoint, including work on the occurrence of cholesterol in *Labyrinthula*,[5] and several studies on the uptake[6] and utilization[7] of materials in *Thraustochytrium roseum*.

Before the role of marine fungi can be appraised critically, the overall metabolic capabilities of several species representing all major taxonomic categories must be understood fully. Thus, we commenced a systematic study to determine what materials would be produced by marine Ascomycetes and Fungi Imperfecti, when grown under controlled laboratory conditions. Ultimately, we intend to determine if these organisms can synthesize compounds of biomedical importance.

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[2] P. L. SGUROS and J. SIMMS, *J. Bacteriol.* **88**, 346 (1964).
[3] S. P. MEYERS, in *Symposium on Marine Microbiology* (edited by C. H. OPPENHEIMER), p. 315, Thomas, Springfield, Ill. (1963).
[4] J. W. FELL and N. VAN UDEN, in *Symposium on Marine Microbiology* (edited by C. H. OPPENHEIMER), p. 329, Thomas, Springfield, Ill. (1963).
[5] H. S. VISHNIAC, *Acta Biochim. Biophys.* **26**, 430 (1957).
[6] P. A. SIEGENTHALER, M. M. BELSKY, S. GOLDSTEIN and M. MENNA, *J. Bacteriol.* **93**, 1281 (1967).
[7] M. M. BELSKY and S. GOLDSTEIN, *Arch. Mikrobiol.* **49**, 375 (1964).

RESULTS AND DISCUSSION

Ergosterol was identified in six isolates, representing three species of Ascomycetes and two Fungi Imperfecti (Table 1). Choline was detected with certainty in one of the Ascomycetes and an imperfect fungus, and probably occurred in isolates of *Ceriosporopsis halima* of the Ascomycetes.

Despite having striking morphological adaptations to an aquatic environment, marine Ascomycetes and Fungi Imperfecti are like many terrestrial fungi in being able to produce readily detectable ergosterol and choline.[8] Other fundamental similarities in secondary metabolism, plus being adapted for life submerged in sea-water, may qualify certain marine fungi for industrial fermentations in which the oceans can provide an inexpensive source of water and nutrients.

TABLE 1. OCCURRENCE OF ERGOSTEROL AND CHOLINE IN MARINE FUNGI

Organism	Isolate	Ergosterol*	Choline†
Ascomycetes			
Corollospora trifurcata (Höhnk) Kohlm.	R-1	+	−
	R-3	−	−
	R-562	−	−
Corollospora maritima Wedermann	R-19	+	+
	R-563	+	−
Cerioporopsis halima Linder	R-546	−	±
	R-552	+	±
Ceriosporopsis calyptrata Kohlm.	R-612	−	−
Halosphaeria appendiculata Linder	R-588	−	−
Fungi Imperfecti			
Zalerion maritima (Linder) Anastasiou	R-6	+	−
Pyrenochaeta sp.	R-10	+	+
Culcitalna achraspora Meyers et Moore	R-57	−	−
Flagellospora sp.	F-74	−	−
Clavatospora stellatacula Kirk	F-83	−	−

* Silica gel G plates developed in benzene–EtOAc (4:1) and sprayed with $SbCl_3$ in $CHCl_3$.

† Al_2O_3 G plates developed in MeOH–CCl_4–HOAc (28:12:1) and sprayed with Dragendorff's reagent.

This study also suggests that fungi may play a direct role in supplying vitamins to marine nematodes, with which they are often associated in sediments.[9] Nutritional requirements are probably similar in mycophagous marine and free-living soil nematodes,[10] and the latter grow well in culture media containing choline or lecithin.[11] A requirement for activated ergosterol or vitamin D_2 has not been established, although invertebrates may utilize this compound in

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[9] S. P. MEYERS, W. A. FEDER and K. M. TSUE, *Science* **141**, 520 (1963).

[10] R. D. WINSLOW, in *Nematology: Fundamentals and Recent Advances with Emphasis on Plant Parasitic and Soil Forms* (edited by J. N. SASSER and W. R. JENKINS), p. 341, University of North Carolina Press, Chapel Hill (1960).

[11] E. C. DOUGHERTY, in *Nematology: Fundamentals and Recent Advances with Emphasis on Plant Parasitic and Soil Forms* (edited by J. N. SASSER and W. R. JENKINS), p. 297, University of North Carolina Press, Chapel Hill (1960).

the synthesis of other sterols.[12] Because marine fungal–nematode associations are abundant, defining the chemical interactions of these organisms seems basic toward a full appreciation for the economy of the oceans.

EXPERIMENTAL

Mycelial cultures of marine fungi were maintained on slants of medium 5 M containing: g/l.; glucose, 10·0; yeast extract, 1·0; agar, 20·0; Rila artificial sea-water salts, 40·0. Transfers were made from these stock cultures to 125-ml Erlenmeyer flasks containing 30 ml of medium 6 M: g/l.; glucose, 10·0; ammonium succinate, 1·0; yeast extract, 1·0; Rila salts, 40·0, and the flasks were placed on gyrotory shakers 4–7 days at 25°. Growth was homogenized in a sterile semimicro Waring blendor, and 5 ml of homogenate used as standard inoculum for experimental flasks of 6 M. These were uniformly harvested after 7 days on a shaker. TLC of mycelia indicated a major steroidal component and one amine in isolate R-19 (Table 1), suspected to be ergosterol and choline, respectively.

To establish, unequivocally, the presence of those compounds, the level of fermentation was increased to 2800-ml Fernbach flasks containing 300 ml of 6 M, inoculated with the homogenized contents of one 125-ml flask culture. Mycelia separated from the medium by suction filtration were dried in a forced-air dryer at 50°, and a pooled sample of dried mycelium was reduced to No. 40 powder in a Wiley mill. An 85-g sample of powder was extracted to exhaustion in a Soxhlet with petroleum ether (30–60°) followed by methanol, and the extracts concentrated further in a flask evaporator *in vacuo*.

Thin-layer chromatography on silica gel G of the petroleum fraction developed with benzene–EtOAc (4:1) and sprayed with a saturated solution of $SbCl_3$ in CHCl revealed the presence of ergosterol. Column chromatography of the petroleum fraction over alumina and eluted with benzene–$CHCl_3$ (1:1) separated a homogeneous material. Recrystallized from methanol, the m.p. (160–162°) was undepressed when mixed with reference ergosterol. The u.v. spectra (273, 283 and 294 nm) and similar i.r. spectra further verified the isolated material as ergosterol.

Choline was isolated as the insoluble reineckate salt from the methanol fraction using the method of Hogg *et al.*[13] and subsequent identification was accomplished according to the TLC procedure of Sullivan and Brady[14] plus comparison of i.r. spectra. Thin-layer chromatography of additional isolates, cultivated in the same manner in 125-ml flasks, was used to establish the presence of the same metabolites.

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[13] R. L. Hogg, J. L. Beal and M. P. Cava, *Lloydia* **24**, 45 (1961).
[14] G. Sullivan and L. R. Brady, *Lloydia* **28**, 68 (1965).