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Short Communication

The ubiquinone system in Oomycetes

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Ubiquinone (Coenzyme Q) systems of 32 strains belonging to Oomycetes (Saprolegniales and Lagenidiales) were investigated. The fungi were isolated from various animals with fungal infections and both freshwater and marine environments. The major component in the fungi was Coenzyme Q-9 without exception.

Key Words——Coenzyme Q-9; Oomycetes; ubiquinone.

Ubiquinone systems were introduced as a new taxonomic criterion for the yeast by Yamada and Kondo (1973). Since then, the systems have proved to be a useful criterion at the generic and super-generic level for various fungi (Yamada et al., 1976; Kuraishi et al., 1985; Kuraishi et al., 1990; Kuraishi et al., 1991). Ubiquinone systems have not hitherto been investigated for the fungi of Saprolegniales and Lagenidiales (Oomycetes): these fungi have been identified by their mode of zoospore production and morphological characteristics (Dick, 1973; Sparrow, 1973). However, the fungi sometimes fail to produce zoospores because of repeated subculture, stocking for long periods and decrease in activity due to nutritional requirements. Sexual reproduction is frequently not observed in pathogenic fungi isolated from aquatic animals, especially fungi belonging to Lagenidiales. Recently, classifications on the basis of some biological characteristics have been reported for Saprolegniales (Wolf, 1937; Scott and Warren, 1964; Gleason et al., 1970; Yuasa and Hatai, 1994). Bahnweg and Bland (1980) reported physiological differences between *Lagenidium callinectes* Couch and *Haliphthoros milfordensis* Vishniac. Phospholipid and steroid requirements were useful for classification of *H. milfordensis* and *Phytophthora epistomium* Fell & Master (Bahnweg, 1980). This paper contains an investigation of ubiquinone systems of aquatic fungi belonging to Saprolegniales and Lagenidiales and a discussion of whether they could be useful for classification.

Tested fungi were isolated from various aquatic animals with fungal infections and from various aquatic environments. The sources are listed in Table 1. The fungi belonging to Saprolegniales and Lagenidiales were isolated from freshwater and marine water, respectively. Species of the genera *Saprolegnia* and *Achlya* were obtained from lesions on the surface of salmonid species and tropical fishes, respectively, and included saprophytic fungi from freshwater environments. All *Aphanomyces* species were isolated from various aquatic animals with fungal infections. Four isolates of *A. astaci* Schikora were isolated from European crayfish. Two strains of *A. piscicida* Hatai were causative agents of mycotic granulomatosis in ayu, *Plecoglossus altivelis* Temminck & Schlegel. Other *Aphanomyces* species were obtained from various fishes affected with epizootic ulcerative syndrome (EUS) in Asian countries. Eight species of Lagenidiales were also pathogenic toward marine invertebrates. *Lagenidium* species were obtained from crustaceans. *Atkinsiella parasitica* Nakamura & Hatai was isolated from the rotifer *Brachionus plicatilis* Müller, a first food of crustaceans and fishes used for seed production. *A. awabi* Kitancharoen et al. was isolated from the abalone *Haliotis sieboldii* Reeve.

First, 24 strains of Saprolegniales were cultured in 1-L Erlenmeyer flasks containing 300 ml of GY broth for 5 days at 20°C. GY broth consisted of 10 g of glucose and 2.5 g of yeast extract in 1 L of distilled water. The mycelia were washed in sterilized tap water three times to remove any nutrients, then dried with filter papers. Seven species of Lagenidiales other than *A. parasitica* were cultured in plastic Petri dishes (8.25 cm diam) containing about 30 ml of PYGS broth (peptone 1.25 g, yeast extract 1.25 g, glucose 3 g in seawater 1 L) for 5 days at 25°C. Small colonies were harvested by centrifugation at 3,500 rpm for 10 min and washed three times in sterilized artificial seawater (Aqua-Ocean®, Japan Pet Drugs Co., Tokyo). *A. parasitica* was cultured on PYGS agar plates at 25°C for one month because its growth was very slow in the broth, while the mycelia on the agar plates were easily stripped off with tweezers. The mycelia were also washed three times with sterilized artificial seawater and dried with filter papers. Thus, about 20 g of mycelia from each strain were obtained to estimate ubiquinone.

The analysis was carried out according to Yamada and Kondo (1973) and Hara et al. (1989). Ubiquinone was extracted from the mycelia with hexane after saponification. It was purified by thin layer chro-

Table 1. Isolates used in this study.

Species	Strains	Host/Habitat
Saprolegniales		
Saprolegnia parasitica Coker	ATCC 42062	brown trout, *Salmo trutta* Linnaeus
S. parasitica	WS 10	lake water
S. parasitica	NJM 8629	coho salmon, *Oncorhynchus kisutch* Walbaum
S. diclina Humphrey	NJM 9218	pond water
S. ferax (Gruithuysen) Thuret	ATCC 36146	lake water
S. hypogyna (Pringsheim) de Bary	ATCC 52721	lake water
Achlya prolifera Nees von Esenbeck	NJM 8505	pond water
A. prolifera	NJM 9117	dwarf gourami, *Colisa lalia* Hamilron & Buchanan
A. flagellata Coker	NJM 9116	dwarf gourami, *C. lalia*
Aphanomyces astaci Schikora	FDL 401	European crayfish
A. astaci	FDL 420	European crayfish
A. astaci	FDL 423	European crayfish
A. astaci	FDL 445	European crayfish
A. piscicida Hatai	NJM 8997	ayu, *Plecoglossus altivelis* Temminck & Schlegel
A. piscicida	NJM 9030	ayu, *P. altivelis*
Aphanomyces sp.	NJM 9114	ayu, *P. altivelis*
Aphanomyces sp.	NJM 9201	dwarf gourami, *C. lalia*
Aphanomyces sp.	E-PHIL	snakehead, *Ophicephalus striatus*
Aphanomyces sp.	SIPA	snakehead, *Channa striata* Bloch
Aphanomyces sp.	G2PA	blue gourami, *Trichogaster trichopterus* Pallas
Aphanomyces sp.	24PS35	striped mullet, *Mugil cephalus* Linnaeus
Aphanomyces sp.	10PS38	*Cillago ciliata*
Aphanomyces sp.	34PS8	*Mugil* sp.
Aphanomyces sp.	84-1240	Atlantic menhaden, *Brevoortia tyrannus*
Lagenidiales		
Lagenidium giganteum Couch	ATCC 36492	*Culex* larvae
L. callinectes Couch	ATCC 24973	blue crab, *Callinectes sapidus* Rathbun
L. myophilum Hatai & Lawhavinit	ATCC 90216	northern shrimp, *Pandalus borealis* Krφyer
L. myophilum	NJM 9131	coonstripe shrimp, *Pandalus hypsinotus* Brandat
L. myophilum	NJM 9331	coonstripe shrimp, *P. hypsinotus*
Lagenidium sp.	NJM 9338	mangrove crab, *Scylla serrata* Forsskål
Atkinsiella parasitica Nakamura & Hatai	NJM 9231	rotifer, *Brachionus plicatilis* Müller
A. awabi Kitancharoen et al.	NJM 9354	abalone, *Haliotis sieboldii* Reeve

matography on silica gel plates (layer thickness 0.25 mm, size 20×20 cm, Merck), using benzene as the developing solvent.

Purified ubiquinone isoprenologues were identified by high performance liquid chromatography (HPLC), by comparison with standard ubiquinones from Q-6 to Q-10 (H_2). HPLC was done on a CCPD liquid chromatograph (Tosoh, Tokyo) fitted with a TSK gel ODS-80T_M column (5 Mic, 150×4.6 mm i.d., Tosoh, Tokyo). Ubiquinone isoprenologues were eluted with a mobile phase of methanol-isopropyl ether (3 : 1, v/v) at a speed of 1 ml/min, and monitored at 275 nm. Standard ubiquinone isoprenologues, except for Q-6 and Q-10, were prepared from the cells of *Candida methanolica* Oki & Kouno ATCC 26175 (Q-7), *Pichia pastoris* (Guilliermond) Phaff IAM 12267 (Q-8), *Rhodosporidium toruloides* Banno IFO 0559 (Q-9) and *Erythrobasidium hasegawae* (Yamada & Komagata) Hamamoto et al. IFO 1058 (Q-10 (H_2)) according to the method of Yamada and Kondo (1973). Q-6 and Q-10 were purchased from Sigma Chemical.

The results are summarized in Table 2. All fungi investigated, without exception, had one type of major ubiquinone, Coenzyme Q-9. *S. ferax* (Gruithuysen) Thuret ATCC 36146 and *S. hypogyna* (Pringsheim) de Bary ATCC 52721 had Q-6 and Q-8, respectively, as a minor component. Thus there was no difference in major ubiquinone types among the tested fungi, while two types of minor ubiquinone were present.

In conclusion, the fungi belonging to Saprolegniales and Lagenidiales could not be separated into any groups based on thier ubiquinone systems. However, ubiquinone systems in more fungi belonging to the class Oomycetes may be required for definite taxonomic decisions.

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Table 2. Coenzyme Q system in Saprolegniales and Lagenidiales.

Species	Strains	Coenzyme Q system
Saprolegniales		
Saprolegnia parasitica	ATCC 42062, WS 10, NJM 8629	Q-9
S. diclina	NJM 9218	Q-9
S. ferax	ATCC 36146	Q-9, (Q-6)*
S. hypogyna	ATCC 52721	Q-9, (Q-8)*
Achlya prolifera	NJM 8505, NJM 9117	Q-9
A. flagellata	NJM 9116	Q-9
Aphanomyces astaci	FDL 401, FDL 420, FDL 423, FDL 445	Q-9
A. piscicida	NJM 8997, NJM 9030	Q-9
Aphanomyces spp.	NJM 9114, NJM 9201, E-PHIL, SIPA, G2PA, 24PS35, 10PS38, 34PS8, 84-1240	Q-9
Lagenidiales		
Lagenidium giganteum	ATCC 36492	Q-9
L. callinectes	ATCC 24973	Q-9
L. myophilum	ATCC 90216, NJM 9131, NJM 9331	Q-9
Lagenidium sp.	NJM 9338	Q-9
Atkinsiella parasitica	NJM 9231	Q-9
A. awabi	NJM 9354	Q-9

* The Coenzyme Q homologue enclosed in parentheses indicates a minor component.

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Literature cited

Barnweg, G. 1980. Phospholipid and steroid requirements of *Haliphthoros milfordensis*, a parasite of marine crustaceans, and *Phytophthora epistomium*, a facultative parasite of marine fungi. Bot. Marina **23**: 209–218.

Barnweg, G. and Bland, Ch. E. 1980. Comparative physiology and nutrition of *Lagenidium callinectes* and *Haliphthoros milfordensis*, fungal parasites of marine crustaceans. Bot. Marina **23**: 689–698.

Dick, M. W. 1973. Saprolegniales. In: "The fungi, vol. 4B," (ed. by Ainsworth, G. C., Sparrow, F. K. and Sussman, A. S.), pp. 113–144. Academic Press, New York.

Gleason, F. H., Rudolph, C. R. and Price, J. S. 1970. Growth of certain aquatic Oomycetes on amino acids I. *Saprolegnia, Achlya, Leptolegnia*, and *Dictyuchus*. Physiol. Plantarum **23**: 513–516.

Hara, N., Tubota, Y., Saito, K. and Suto, T. 1989. Biochemical characteristics and ubiquinone of 44 strains of *Trichosporon beigelii* and related organisms. J. Gen. Appl. Microbiol. **35**: 1–10.

Kuraishi, H., Aoki, M., Itoh, M., Katayama, Y., Sugiyama, J. and Pitt, J. I. 1991. Distribution of ubiquinones in *Penicillium* and related genera. Mycol. Res. **95**: 705–711.

Kuraishi, H., Itoh, M., Tsuzaki, N., Katayama, Y., Yokoyama, T. and Sugiyama, J. 1990. The ubiquinone system as a taxonomic aid in *Aspergillus* and its teleomorphs. In: "Modern concepts in *Penicillium* and *Aspergillus* classification," (ed. by Samson, R. A. and Pitt, J. I.), pp. 407–421. Plenum Press, New York.

Kuraishi, H., Katayama-Fujimura, Y., Sugiyama, J. and Yokoyama, T. 1985. Ubiquinone systems in fungi I. Distribution of ubiquinones in the major families of ascomycetes, basidiomycetes, and deuteromycetes, and their taxonomic implications. Trans. Mycol. Soc. Japan **26**: 383–395.

Scott, W. W. and Warren, C. O., Jr. 1964. Studies of the host range and chemical control of fungi associated with diseased tropical fish. Virginia Agr. Exp. Sta. Tech. Bull. **171**: 1–24.

Sparrow, F. K. 1973. Lagenidiales. In: "The fungi, vol. 4B," (ed. by Ainsworth, G. C., Sparrow, F. K. and Sussman, A. S.), pp. 159–163. Academic Press, New York.

Wolf, F. T. 1937. A nutritional study of *Achlya bisexualis* and *Saprolegnia ferax*. Am. J. Bot. **24**: 119–123.

Yamada, Y. and Kondo, K. 1973. Coenzyme Q system in the classification of the yeast genera *Rhodotorula* and *Cryptococcus*, and the yeast-like genera *Sporobolomyces* and *Rhodosporidium*. J. Gen. Appl. Microbiol. **19**: 55–77.

Yamada, Y., Nojiri, M., Matsuyama, M. and Kondo, K. 1976. Coenzyme Q system in the classification of the ascosporogenous yeast genera *Debaryomyces, Saccharomyces, Kluyveromyces*, and *Endomycopsis*. J. Gen. Appl. Microbiol. **22**: 325–337.

Yuasa, K. and Hatai, K. 1994. An attempt of classification by biological characteristics of some pathogenic water moulds from freshwater fishes. Nippon Kingakukai Kaiho **35**: 104–110 (in Japanese).