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COENZYME Q SYSTEMS IN THE GENUS COROLLOSPORA AND ALLIED MARINE FUNGI

AKIRA NAKAGIRI

Summary

The ubiquinone systems of 13 species of the genus Corollospora and 3 species of allied marine fungi were determined by high performance liquid chromatography and found to contain ubiquinone Q-10, Q-10(H_2), and Q-10(H_4). Eleven species of Corollospora have Q-10(H_2) as the major ubiquinone, whereas two species of the genus have mainly Q-10(H_4). By considering not only the major ubiquinone but also the minor ones, ubiquinone systems were found to be useful for the taxonomy of the genus, because the ubiquinone data supported well the species classifcation proposed on the basis of morphology.

Keywords: ubiquinone, Corollospora, marine fungi.

The isoprene side chains of ubiquinone molecules have been investigated as a chemotaxonomic characteristic that has been considered useful for the taxonomy of bacteria (13), yeasts (12, 14, 15, 16, 17), and fungi (4, 9). The distribution of ubiquinone systems in certain fungal groups has been investigated and applied to the taxonomy of smut and rust fungi (10), Aspergillus and its teleomorphs (3), and Penicillium and related genera (2). Takachi et al. (11) examined the ubiquinone systems in marine fungi including ascomycetes, basidiomycetes, and deuteromycetes and found that the types of isoprene side chain of ubiquinones of marine

fungi corresponded with those of the terrestrial fungi that are classified into the same taxonomic group as the marine species.

In the genus Corollospora (Halosphaeriaceae, Sphaeriales, Pyrenomycetes, Ascomycotina), 14 species have so far been described from marine environments, especially from sandy beach (1, 5). Some of them are known to have anamorph states. Ascospore morphology is mainly used for classification at the species level, but some species whose ascospores are similar in shape may be difficult to identify. For precise identification of these species, it is necessary to compare other characteristics such as the peridial wall structure of the ascocarp (8), anamorphs, or chemotaxonomical characteristics. In this study, I investigated a distribution of ubiquinones in the genus Corollospora and allied fungi. The possibility of using the data as an aid to identification or classification of the species will be discussed.

Materials and Methods

Strains. The strains used here are listed in Table 1. The ubiquinone systems of 25 strains in 13 species of the genus Corollospora, and 2 strains in 1 anamorphic species and 4 strains in 2 species of allied fungi were investigated.

Cultivation. All strains were cultured on seawater starch agar medium (SWSA: 1% soluble starch, 0.1% soytone, 1.5% agar, in 20% salinity artificial seawater, pH 8.2) in tubes for two weeks. Agar medium containing mycelium was broken into small pieces with a sterilized stick. The agar pieces were inoculated in 5 ml of a liquid medium (SWS: as SWSA but without agar) in a tube. Four tubes for each strain were incubated on a shaker for one week at 28 C. The grown mycelium was used as a "seed" for the cultivation in 1-liter Erlenmeyer flasks containing 250 ml of SWS. Four flasks for each strain were incubated on a rotary shaker for one to three weeks at 28 C. The grown mycelial balls were washed in distilled water and freeze-dried for preservation before extracting ubiquinones.

Extraction and purification of ubiquinones. Dried mycelia were pow-

dered in a mortar. About 1 g of the mycelium powder was suspended in 240 ml of chloroforme/methanol (2:1, v/v). Ubiquinones were extracted for 8 hr under continuous stirring. After separating the mycelial powder by filtration, secondary extraction were done with fresh solvent for 8 hr. After the mycelial powder had been removed by filtration, the two extracts were combined and evaporated to dryness in a rotary evaporater. The dried extract was dissolved in ca. 1 ml of acetone and the solution was used for purifying ubiquinones under thin-layer chromatography (TLC). TLC was performed using 0.5 mm layers of Merck Kiesel-gel HF254 and benzene as a developing solvent. Vitamin-K_1 (SIGMA) and ubiqinone-10 (SIGMA) were used as standards. Ubiquinones were detected under ultraviolet light. The silica gel containing ubiquinones was scratched from the plates and the ubiquinones were eluted with acetone. The acetone was evaporated off, and the dried materials were dissolved in ca. 0.5 ml of ethanol. The ethanol solution was preserved at -20 C before analysis by high performance liquid chromatography (HPLC).

Analysis of ubiquinones. HPLC was carried out on a Shimadzu Liquid Chromatograph LC-6AD with a ZORBAX (4.6 mm x 15 cm) column. Ubiquinones were detectecd by their absorbance at 275 nm by means of a Shimadzu Spectrophotometric Detector SPD-6A. Samples were eluted with methanol/isopropyl ether (4:1, v/v) at 1 ml/min at 30 C. The elution time and area of each peak of ubiquinone were calculated with Shimadzu Chromatopac C-R3A. Authentic samples of ubiquinones Q-6, 7, 8, 9, 10, 10 (H_2), 10 (H_4) were used as standards.

Results and Discussion

The distribution of ubiquinones in Corollospora and allied species is shown in Table 1, where all ubiquinones that constitute more than 1% of a total amount of ubiquinones are listed.

Ubiquinone Q-10 and its hydrogenated derivatives Q-10 (H_2) and Q-10 (H_4) were found in these fungi. This pattern corresponds to those of other pyrenomycetous terrestrial fungi (4, 9). Of 13 species of Corollospora, the following 11 species have Q-10 (H_2) as the major ubiquinone (74-100%): C. angusta, C. cinnamomea, C. colossa, C. filiformis, C. fusca, C.

Table 1. Distribution of ubiquinones in Corollospora and allied species.

Species	IFO No.	Ubiquinones*		
		Q-10	Q-10 (H_2)	Q-10 (H_4)
C. angusta Nakagiri & Tokura	32100		100	
	32101		100	
C. cinnamomea Koch	32125		96	4
	32126		97	3
C. colossa Nakagiri & Tokura	32103		100	
	32105		100	
C. filiformis Nakagiri	32106	12	86	2
C. fusca Nakagiri & Tokura	32107	2	98	
	32108	1	97	2
C. gracilis Nakagiri & Tokura	32110	1	99	
	32111		100	
C. intermedia I. Schmidt	32119	1	99	
	32120	1	99	
C. lacera (Linder) Kohlm.	32121	2	40	58
	32122	1	28	71
C. luteola Nakagiri & Tubaki	31315	4	95	1
	31316	5	95	
C. maritima Werdermann	32117	19	74	7
	32118	24	76	
C. pseudopulchella Nakagiri & Tokura	32112	1	95	4
	32113	2	80	18
C. pulchella Kohlm., Schmidt, & Nair	32123	1	7	92
	32124	1	6	93
C. quinqueseptata Nakagiri	32114	4	96	
	32116	1	96	3
Clavatospora bulbosa (Anastasiou) Nakagiri & Tubaki	(AN-847)		5	95
	(ATCC 14677)		3	97
Sigmoidea marina Haythorn & Jones	32159	1	97	2
	32160		100	
Varicosporina ramulosa Meyers & Kohlm.	32163		100	
	(AN-1017)		100	

* Numbers indicate percentages of total ubiquinones.

gracilis, C. intermedia, C. luteola, C. maritima, C. pseudopulchella, and C. quinqueseptata. Q-10(H_4) is the main ubiquinone in C. lacera (58-71%) and C. pulchella (92-93%). Q-10 is a minor ubiquinone in the genus, whereas relatively higher contents were observed in C. maritima (19-24%) and C. filiformis (12%).

Classification of these species based on morphology presents the following problems. The two distinct species C. maritima and C. gracilis have been often confused because their ascospores are similar in shape and size, though they are distinguishable by ascospore diameter, colony colour, presence or absence of chlamydospores, etc. (5). The data of ubiquinones showed a clear distinction between the two species (Table 2, A). The three species C. colossa, C. lacera, and C. quinqueseptata are distinguished by ascospore size and septation, peridial wall structure of ascocarps, etc. (5), but they have been misidentified by many authors. The distinction of these three species was suported by the ubiquinone data, which were distinctive for C. lacera against C. colossa or C. quinqueseptata (Table 2, B). Another example is that of C. pulchella and C. pseudopulchella, which are very similar in ascospore morphology, but distinguishable by the surface structure of the ascocarp wall and anamor-

Table 2. Comparison of ubiquinones in morphologically similar species of Corollospora.

(A)

Ubiquinones	C. maritima	C. gracilis
Q-10	19-24*	0-1
Q-10(H_2)	74-76	99-100
Q-10(H_4)	0-7	

(B)

Ubiquinones	C. colossa	C. lacera	C. quinqueseptata
Q-10		1-2	1-4
Q-10(H_2)	100	28-40	96
Q-10(H_4)		58-71	0-3

(C)

Ubiquinones	C. pulchella	C. pseudopulchella
Q-10	1	1-2
Q-10(H_2)	6-7	96
Q-10(H_4)	92-93	0-3

* Numbers indicate percentages of total ubiquinones.

phic state (5). The ubiquinone systems of these species are completely different, as C. pulchella has Q-10 (H_4) as the major ubiquinone, while C. pseudopulchella mainly possesses Q-10 (H_2) (Table. 2, C).

From the viewpoint of teleomorph-anamorph relationships, the ubiquinone systems of teleomorphs, anamorphs, and related species are summarized in Table 3, A-C. Corollospora pulchella is known to have an anamorphic state in Clavatospora bulbosa (7). The strains of C. pulchella, IFO 32123, 32124, are known to have both morphs in the life cycle (holomorphic), since they were isolated as an ascospore and produced conidia of Clav. bulbosa in culture. On the other hand, two strains of Clav. bulbosa, AN-847 and ATCC 14677, were isolated in the anamorphic state, and it has not been confirmed whether they have lost the ability of sexual reproduction (anamorphic). The ubiquinone systems of these four strains are similar, but a slight difference is seen between the holomorphic strains and anamorphic (?) strains (Table. 3, A). However, it is uncertain whether this difference corresponds to the type of life cycle of the strains. Two strains of C. luteola, IFO 31315, 31316, are holomorphic and have Sigmoidea luteola as their anamorph. Sigmoidea marina is a distinct species, but similar to S. luteola in conidium morphology (6).

Table 3. Comparison of ubiquinones in holomorphic, anamorphic, and allied species of Corollospora.

(A) Ubiquinones	C. pulchella - Clav. bulbosa	Clav. bulbosa
Q-10	1*	
Q-10 (H_2)	6-7	3-5
Q-10 (H_4)	92-93	95-97

(B) Ubiquinones	C. luteola - S. luteola	S. marina
Q-10	4-5	0-1
Q-10 (H_2)	95	97-100
Q-10 (H_4)	0-1	0-2

(C) Ubiquinones	C. intermedia - V. prolifera	V. ramulosa
Q-10	1	
Q-10 (H_2)	99	100
Q-10 (H_4)		

* Numbers indicate percentages of total ubiquinones.

No teleomorph is known so far. The same situation was observed in the relation between C. intermedia-Varicosporina prolifera and an allied species, V. ramulosa. The ubiquinone data show that there is a slight difference between holomorphic strains and allied species in the former case (Table. 3, B), and that similar patterns are found in both species in the latter case (Table. 3C).

From these observations, the ubiquinone system was found to be a useful character supporting morphology-based classification at species level in the genus Corollospora. In this study, I observed the minor as well as the major ubiquinones and found that they are more informative for species classification within a genus than the major ubiquinone alone, which has been treated as a sole character in many previous studies. Further research into the relationships between teleomorph-anamorph fungi and their allied species may reveal that the ubiquinone data are informative in investigating their phylogenetical relationship.

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