Short Communication

**HETEROGENEITY OF UBIQUINONE SYSTEMS IN
THE GENUS *SPOROTHRIX***

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The genus *Sporothrix* Hektoen and Perkins is a group of little-differentiated Hyphomycetes. The conidia are non-catenulate or catenulate without marked differentiation of conidia of the first and second order (1,2). WEIJMAN and DE HOOG (3) pointed out that this genus was heterogeneous, containing fungi of hemiascomycetous, ascomycetous and basidiomycetous relationships, based on carbohydrate patterns of whole cells. They divided the members of the genus into three sections, i.e., *Sporothrix* sect. *Sporothrix*, *Sporothrix* sect. *Farinosa* Weijman and de Hoog and *Sporothrix* sect. *Luteoalba* Weijman and de Hoog. SMITH and BATENBURG-VAN DER VEGTE (4) also pointed out the heterogeneity of the genus *Sporothrix* on the basis of the ultrastructure of septa. Their results supported the proposition of the WEIJMAN and DE HOOG (3).

The ubiquinone (coenzyme Q) system has been proposed as a useful criterion for classifying yeasts and yeast-like fungi at the generic level (5–8). KURAISHI et al. (9) reported ubiquinone systems of many species in higher fungi and stated that the ubiquinone system was very useful in the classification of fungal taxa and in the elucidation of their genealogy. Until now, however, no information about the ubiquinone systems in the genus *Sporothrix* is available. We report on these systems here.

Fourteen strains of *Sporothrix* and *Stephanoascus* species were used in this study (Table 1). All strains except one were cultivated in YM broth supplemented 2% of glucose with shaking for 4 to 6 days at 25 or 28°C. *Stephanoascus farinosus* JCM 2935 was cultivated for 6 days at 17°C. Cells harvested by centrifugation (6,000 rpm, 20 min) were washed twice with deionized water. Extraction and isolation of ubiquinones from cells were carried out according to the method described by YAMADA and KONDO (5), with a slight modification. Wet packed cells (5–10 g) were saponified with methanol-sodium hydroxide-pyrogallol (80 ml, 8 g,

Table 1. Ubiquinone systems of *Sporothrix* species and related species.

Section and species	Strain	Other designation	Molar ratio of ubiquinone isoprenologues (%) ^a				
			Q-8	Q-9	Q-9(H ₂)	Q-10	Q-10(H ₂)
<i>Sporothrix</i> sect. <i>Sporothrix</i>							
<i>Sporothrix curviconia</i>	JCM 2922 ^b	CBS 959.73	—	1.0	1.6	tr	97.4
<i>Sporothrix inflata</i>	JCM 2920 ^b	CBS 239.68	—	0.9	2.2	0.6	96.2
<i>Sporothrix schenckii</i>	JCM 2915	CBS 359.36	—	—	1.4	0.7	97.9
<i>Sporothrix</i> sect. <i>Farinosa</i>							
<i>Sporothrix ghanensis</i>	JCM 2925	CBS 769.73	—	—	tr	3.1	96.9
<i>Sporothrix insectorum</i>	JCM 2918 ^b	CBS 756.73	—	—	3.8	1.3	94.9
<i>Sporothrix ranii</i>	JCM 2917 ^b	CBS 119.81	—	—	1.5	0.4	98.1
<i>Sporothrix sclerotialis</i>	JCM 2913 ^b	CBS 312.77	—	0.6	3.2	tr	96.2
<i>Sporothrix catenata</i>	JCM 2921 ^b	CBS 215.79	3.8	95.0	—	1.2	—
<i>Sporothrix foliorum</i>	JCM 2916 ^b	CBS 326.37	6.7	92.4	—	0.9	—
<i>Sporothrix fungorum</i>	JCM 2924 ^b	CBS 259.70	8.2	91.3	—	0.5	—
<i>Sporothrix guttuliformis</i>	JCM 2919 ^b	CBS 437.76	9.1	90.2	—	0.7	—
<i>Sporothrix</i> sect. <i>Luteoalba</i>							
<i>Sporothrix cyanescens</i>	JCM 2914 ^b	CBS 357.73	—	15.0	—	85.0	—
<i>Sporothrix luteoalba</i>	JCM 2923 ^b	CBS 209.48	—	14.1	—	85.9	—
<i>Stephanosascus farinosus</i>	JCM 2935 ^b	CBS 140.71	7.0	91.7	—	1.3	—

^a tr, trace amount; —, not detected.^b Type culture.

Abbreviations: JCM, Japan Collection of Microorganisms (RIKEN), Wako, Saitama, Japan; CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

1 g, respectively) at 75°C for 1 hr. Ubiquinones were extracted with hexane and isolated by preparative thin layer chromatography using Merck Kieselgel 60 F₂₅₄ plates (0.5 mm, 20 × 20 cm) with benzene as the solvent system. Ubiquinone isoprenologues were identified by high performance liquid chromatography (HPLC) by comparing their retention times with those of standard ubiquinones from Q-6 to Q-10 and Q-10(H₂). Q-9(H₂) was identified based on the equivalent number of the isoprene units (ENIU) values described by TAMAOKA et al. (10). HPLC was performed on a LC-6A liquid chromatograph (Shimadzu) fitted with a Zorbax ODS column (4.6 mm × 25 cm). Ubiquinone isoprenologues were eluted with a mobile phase of methanol-propan-2-ol (2:1, v/v) at 2.0 ml/min, and monitored at 275 nm. Standard ubiquinone isoprenologues, except for Q-8 and Q-10(H₂), were purchased from Sigma Chemical Co. (U.S.A.). Q-8 and Q-10(H₂) were prepared from *Citeromyces matritensis* JCM 2333 and *Rhodotorula hasegawae* JCM 1545, respectively.

The results are summarized in Table 1. The *Sporothrix* species had Q-9, Q-10 and Q-10(H₂) as the major ubiquinones.

S. curviconia, *S. inflata* and *S. schenckii*, in *Sporothrix* sect. *Sporothrix*, had Q-

10(H₂) as the major component and Q-9, Q-9(H₂) and Q-10 as minor components. Q-10(H₂) constituted 96.2–97.9% of the quinones in each species, and Q-9, Q-9(H₂) and Q-10 constituted 2.2% or less. It has been suggested that these species are closely related to species in the ascomycete genus *Ophiostoma* (3). KURAISHI *et al.* (9) reported that *Ceratocystis piceae* IFO 8662 (= *Ophiostoma piceae*) had Q-10(H₂). The ubiquinone system may also support the relationship between them.

Species assigned to *Sporothrix* sect. *Farinosa* were divided into two groups based on the ubiquinone system. *S. catenata*, *S. foliorum*, *S. fungorum* and *S. guttuliformis* had Q-9 as the major component which occupied 90.2–95.0% of the total quinones. The minor components, Q-8 and Q-10, occupied 3.8–9.1% and 0.5–1.2%, respectively. *S. catenata*, the type species of *Sporothrix* sect. *Farinosa*, is regarded as the anamorph of *Stephanoascus ciferrii* (11). YAMADA and SMITH (8) reported that *Stephanoascus ciferrii* had Q-9. *Stephanoascus farinosus*, another species of the genus *Stephanoascus* (2), also had Q-9 as the major component. *S. fungorum*, *S. guttuliformis*, *Stephanoascus ciferrii* and *Stephanoascus farinosus* have septa with either one central micropore or several scattered micropores (4). Therefore, these species with Q-9 may have a close relation to the genus *Stephanoascus* or other hemiascomycetous fungi. *S. ghanensis*, *S. insectorum*, *S. ranii* and *S. sclerotialis* had Q-10(H₂) as the major ubiquinone. It occupied 94.9–98.1% of the total quinones, and Q-9(H₂) and Q-10, the minor ubiquinones, constituted 3.8 and 3.1% or less, respectively. Another minor component, Q-9, was detected only in *S. sclerotialis*. Species with Q-10(H₂) may be related to ascomycetous fungi because hydrogenated ubiquinones are also present in various taxa in the Ascomycotina (9). According to de Hoog (personal communication), these species are not endomycetous- but ascomycetous-related. Investigation of ultrastructures of septa of species with Q-10(H₂) is needed to further elucidate the phylogenetic relationship of this group of *Sporothrix*.

S. luteoalba and *S. cyanescens*, which were assigned to *Sporothrix* sect. *Luteoalba*, had Q-10 as the major component and Q-9 as the minor component. In *S. luteoalba*, Q-9 and Q-10 occupied 14.1 and 85.9%, respectively. In the latter, Q-9 and Q-10 constituted 15.0 and 85.0% of the systems, respectively. This section is characterized by the presence of xylose in the cell wall and septa with dolipore as markers of basidiomycete affinity, though *S. cyanescens* lacked xylose in the cell wall (3, 4). Since the ubiquinone systems of basidiomycetous fungi are either Q-9 or Q-10 (9), these species may have a relation to basidiomycetous fungi having Q-10. WEIJMAN and DE HOOG (3) stated that *S. luteoalba* resembled cultural states of some *Dacrymyces* species. KURAISHI *et al.* (9) reported that *Dacrymyces* sp. had Q-10.

As mentioned above, the genus *Sporothrix* is heterogeneous with respect to the ubiquinone system. In addition to the carbohydrate patterns, septal pore ultrastructure, and ubiquinone system, other molecular approaches may be required to clarify the classification of the genus *Sporothrix*.

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