Mycoscience 35: 327–330, 1994

Ubiquinone system of the form-genus *Chrysosporium*

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Accepted for publication 20 August 1994

The ubiquinone (coenzyme Q: Q) system of 17 strains of the form-genus *Chrysosporium* was analyzed by high performance liquid chromatography (HPLC) and found to show a heterogeneous distribution of the major ubiquinone. Q-9, Q-10 or Q-10(H_2) was found to be the major ubiquinone in 3, 9 and 5 strains, respectively. It was further demonstrated that the teleomorphs of the species characterized by Q-9 and Q-10 could be classified into two separate families, Arthrodermataceae (Q-9) and Onygenaceae (Q-10), which were defined within the revised order Onygenales by Currah. Teleomorphs of *Chrysosporium* species having Q-10(H_2) have not been found. This paper also includes the ubiquinone system of dermatophytes which relate to the form-genus *Chrysosporium* morphologically.

Key Words——*Chrysosporium*; coenzyme Q; dermatophytes; Onygenales; ubiquinone system.

Species of the genus *Chrysosporium* Corda are prevalently isolated from soil and human or animal tissues, especially from keratinous tissues of the body such as skin, hair and nail. Case reports on opportunistic infection caused by these fungi have recently appeared (England and Hochholzer, 1993; Echavarria et al., 1993).

Oorschot (1980) made a revision of *Chrysosporium* and characterized 22 species of the genus. The teleomorphs associated with several *Crrysosporium* species are known, and they were classified into the families in Gymnoascales by von Arx (1979) as follows: Gymnoascaceae (*Gymnoascus, Apinisia, Arthroderma, Pectinotrichum, Renispora* and *Rollandina*), Onygenaceae (*Aphanoascus*) and Ascosphaeraceae (*Bettsia*). In 1987, von Arx reviewed the Gymnoascales together with Eurotiales and Onygenales, and combined the three orders into the single order Eurotiales.

Currah (1985) undertook a classification of species formerly placed in either the Gymnoascaceae or Onygenaceae on the basis of previously underutilized or unscrutinized characteristics such as ascospore sculpturing, mode of conidium dehiscence, morphology of ascoma peridia, and degradative capacity of keratin and cellulose. Through this study, four families were defined within the revised order Onygenales: Arthrodermataceae, Onygenaceae, Gymnoascaceae and Myxotricaceae. These studies show that the teleomorphs of the form-genus *Chrysosporium* are mostly classified into the two families, Arthrodermataceae and Onygenaceae.

The dermatophytes, *Trichophyton, Microsporum* and *Epidermophyton*, are related to *Chrysosporium* by the morphological and degradative characteristics mentioned above. *Nannizzia* and *Arthroderma*, which were placed in the family Arthrodermataceae newly defined by Currah, are known to be the teleomorphic genera associated with many species of *Microsporum* and *Trichophyton*, respectively. As seen in the taxonomic definition of *Arthroderma* as the teleomorphic genus for *Chrysosporium georgiae*, the teleomorphic genera of *Chrysosporium* species and the dermatophytes are also closely related.

The ubiquinone system has been recognized as a useful criterion in the classification of fungal taxa and the elucidation of their genealogy (Yamada and Kondo, 1973; Kuraishi et al., 1991; Fukushima et al., 1991a, b, 1993a, b). Herein, the system of the form-genus *Chrysosporium* is analyzed on the basis of chemotaxonomical studies. The ubiquinone system of the dermatophytes is also examined.

Materials and Methods

Fungi Species of the form-genus *Chrysosporium* and the dermatophytes used in this study are listed in Tables 1 and 2, respectively. Two strains of *Emmonsia* were examined as the allied genus of *Chrysosporium*.

Cultivation As shown in Tables 1 and 2, the fungi were cultured in YM broth (containing 1% glucose, 0.5% peptone, 0.3% yeast extract and 0.3% malt extract) on a rotary shaker or on YM agar slants at 25°C. Cultivation periods for each *Chrysosporium* species are also listed in Table 1. Cells of all fungi listed in Table 2 were collected after cultivation for 7 days.

Harvest of cells and extraction, purification and determination of ubiquinone The previously reported methods were adopted for this study (Fukushima et al., 1993b).

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Table 1. Principal ubiquinone systems in *Chrysosporium* examined.

Chrysosporium species examined	Teleomorphs	Q systems (%)	Families classified by Currah	Media (Days)
C. georgiae (Varsavsky & Ajello) van Oorschot CBS 272.66	*Arthroderma ciferrii*[1]	Q-9 (97)	Arthrodermataceae	YMA (7)
C. carmichaelii van Oorschot CBS 643.79		Q-9 (95)		YMA (7)
C. xerophilum Pitt IFO 30318	*Phanerochaete chrysosporium*[4]*	Q-9 (96)		YMB (7)
C. keratinophilum D. Frey ex Carmichael IFO 7584	*Aphanoascus keratinophilus* Punsola & Cano[1,2]	Q-10 (97)	Onygenaceae	YMB (7)
C. keratinophilum IFM 41432	*Aphanoascus keratinophilus*[1,2]	Q-10 (92)	Onygenaceae	YMB (7)
C. indicum (Randhawa & Sandhu) Garg CBS 117.63	*Aphanoascus terreus* (Randhawa & Sandhu) Apinis[1]	Q-10 (94)	Onygenaceae	YMB (7)
C. tropicum Carmichael IFO 7587	*Aphanoascus* species[1]	Q-10 (97)	Onygenaceae	YMB (5)
C. queenslandicum Apinis & Rees CBS 280.77	*Nannizziopsis vriesii* (Apinis) Currah[3]	Q-10 (96)	Onygenaceae	YMB (5)
C. queenslandicum CBS 662.78	*Nannizziopsis vriessi*[3]	Q-10 (89)	Onygenaceae	YMB (5)
C. pannicola (Corda) van Oorschot & Stalpers CBS 116.53		Q-10 (88)		YMA (7)
C. farinicola (Burnside) Skou CBS 688.71	*Bettsia alvei* (Betts) Skou[1]**	Q-10 (97)		YMA(15)
C. sulfureum (Fiedler) van Oorschot & Samson CBS 634.79	*Bettsia* species[1]**	Q-10 (95)		YMA (7)
C. inops Carmichael IFO 7583		Q-10(H_2) (100)		YMB (9)
C. merdarium (Link ex Greville) Carmichael IFO 31954		Q-10(H_2) (100)		YMB (7)
C. merdarium IFO 6812		Q-10(H_2) (100)		YMB (7)
C. merdarium (Link ex Greville) Carmichael var. *roseum* W. Gams & Domsch IFO 30317		Q-10(H_2) (100)		YMB (7)
C. pseudomerdarium van Oorschot CBS 631.79		Q-10(H_2) (100)		YMA (7)

[1] Oorschot (1980), [2] Cano and Guarro (1990), [3] Currah (1985), [4] Boekhout et al. (1989).
*Aphyllophorales, **Eurotiales.

Table 2. Principal ubiquinone systems in genera allied to *Chrysosporium* examined.

Fungi examined	Families classified by Currah	Q system (%)	Anamorphs or teleomorphs	Media
Arthroderma tuberculatum Kuehn IFO 8165	Arthrodermataceae	Q-9 (99)	*Chrysosporium* anamorph	YMA
Arthroderma tuberculatum CBS 473.77	Arthrodermataceae	Q-9 (97)	*Chrysosporium* anamorph	YMA
Microsporum canis Bodin IFM 45831	Arthrodermataceae	Q-9 (98)	*Nannizzia otae* Hasegawa & Usui	YMB
Microsporum gypseum (Bodin) Guiard & Grigorakis IFM 45631	Arthrodermataceae	Q-9 (98)	*Nannizzia incurvata* Stockdale	YMB
Trichophyton mentagrophytes (Robin) Blanchard IFM 45110	Arthrodermataceae	Q-9 (97)	*Arthroderma vanbreuseghemii* Takashio	YMB
Trichophyton rubrum (Castellani) Sabouraud IFM 45586		Q-9 (99)		YMB
Epidermophyton floccosum (Harz) Langeron & Milochevitch IFM 41111		Q-9 (86)		YMB
Aphanoascus fulvescens (Cooke) Apinis IFO 31723 (as *Anixiopsis fulvescens* (Cooke) de Vries var. *fulvescens*)	Onygenaceae	Q-10 (96)	*Chrysosporium* anamorph	YMB
Aphanoascus fulvescens (Cooke) Apinis IFO 31762 (as *Anixiopsis fulvescens* var. *stercoraria* (Hansen) de Vries)	Onygenaceae	Q-10 (94)	*Chrysosporium* anamorph	YMB
Renispora flavissima Sigler et al. CBS 709.79	Onygenaceae	Q-10 (55*)	*Chrysosporium* anamorph	YMA
Emmonsia parva (Emmons & Ashburn) Cif. & Montem. var. *parva* CBS 475.77		Q-10(H_2) (87)		YMA
Emmonsia parva (Emmons & Ashburn) Cif. & Montem. var. *crescens* (Emmons & Jellison) van Oorschot IFM 41471		Q-10(H_2) (98)		YMA

*Accompanied with Q-10(H_2) (45%).

Results and discussion

The known teleomorphs associated with the genus *Chrysosporium* are mostly assigned to two families, Arthrodermataceae and Onygenaceae in Onygenales, defined by Currah (1985).

Major ubiquinones and molar ratios of seventeen strains of *Chrysosporium* and the dermatophytes examined are summarized in Tables 1 and 2, respectively. Three ubiquinones, Q-9, Q-10 and Q-10(H_2), were found to be the major ubiquinones of the form-genus *Chrysosporium*, in 3, 9 and 5 strains, as shown in Table 1. Of the three *Chrysosporium* species having Q-9 as the major ubiquinone, the teleomorph of *C. georgiae* has been discovered and characterized to be *Arthroderma ciferrii* Varsavsky & Ajello, which belongs to Arthrodermataceae. *Arthroderma* has been defined as the teleomorphic genus for the dermatophytes as shown in Table 2. Two strains of *A. tuberculatum* and *A. vanbreuseghemii* Takashio, the teleomorph of *Trichophyton mentagrophytes*, also had Q-9 as the major ubiquinone. *Nannizzia* (*Arthroderma*), the teleomorphic genus assosicated with *Microsporum canis* and *M. gypseum*, also had Q-9 as the major ubiquinone. The major ubiquinone of *Ctenomyces serratus* Eidam was reported to be Q-9 by Kuraishi et al. (1985). These results showed the homogeneous distribution of the major ubiquinone (Q-9) in all of three teleomorphic genera placed in the family Arthrodermataceae. *Epidermophyton floccosum*, whose teleomorph has not been discovered, had Q-9 as the major ubiquinone.

C. xerophilum was reduced to the synonym of *Sporotrichum pruinosum* Gilman & Abbott (teleomorph: *Phanerochaete chrysosporium* Burds. & Eslyn) in the order Aphyllophorales by Boekhout et al. (1989). The species was found to possess the Q-9 ubiquinone system. This evidence further substantiated the conception of Kuraishi et al. (1985) that homobasidiomycetes are characterized by the Q-9 system.

Currah (1985) listed eighteen teleomorphic genera as members of the family Onygenaceae. Of these genera, *Aphanoascus, Nannizziopsis* and *Renispora* are associated with *Chrysosporium* anamorphs as shown in Tables 1 and 2, and the first genus name now tends to be used in place of *Anixiopsis* at present. All these *Chrysosporium* were subjected to ubiquinone analysis to demonstrate the homogeneous distribution of major ubiquinone, Q-10 (Table 1). The two varieties of *A. fulvescens* and *R. flavissima* had Q-10 as the major ubiquinone (Table 2). From these results, it was concluded that the three genera classified in the family Onygenaceae were homogeneous in the distribution of Q-10 as the major ubiquinone. The genus *Apinisia* is also listed in Onygenaceae by Currah, of which *A. graminicola* La Touche is the only known species (Arx, 1987). The major ubiquinone of the strain IFO 9161 was reported to be Q-9 by Kuraishi et al. (1985). As the anamorph of *A. graminicola* was cited to be *Chrysosporium*-like (Arx, 1987), the association between this fungus and genus *Chrysosporium* seems not to be established yet. For this reason, the above conclusion was mentioned without referring the ubiquinone datum of *A. graminicola* in this paper.

C. pannicola, C. farinicola and *C. sulfureum* were found to be *Chrysosporium* species having Q-10 as the major ubiquinone. The teleomorph of *C. pannicola* has not been found. The genus *Bettsia* is known to be the teleomorphic genus associated with the latter two *Chrysosporium* species. But it is placed in another order, Eurotiales.

The major ubiquinones of all fungi examined, except for *R. flavissima*, constituted more than 86% of the ubiquinone molecules found in a particular species. *R. flavissima* had a different ubiquinone profile, consisting of two ubiquinone molecular species present in similar molar ratios: Q-10 (55%) and Q-10(H_2) (45%). Similar profiles can be seen especially in fungi having hydrogenated ubiquinone molecules (Kuraishi et al., 1991). The significance of this ubiquinone system in fungal taxonomy remains to be examined.

C. inops, C. pseudomerdarium and three strains of *C. merdarium* had Q-10(H_2) as the major ubiquinone. The major ubiquinone of two varieties of *Emmonsia parva*, formerly placed in synonymy with *Chrysosporium*, was also found to be Q-10(H_2) (Table 2). The teleomorph of *C. merdarium* was reported to be *Gymnoascus uncinatus* Eidam by Oorschot (1980). Currah (1985) transferred *G. uncinatus* to *Uncinocarpus uncinatus* (Eidam) Currah in recognition of its keratinolytic capacities and some morphological characters, and furthermore reported the unrelatedness between *U. uncinatus* and *C. merdarium* by observing that none of twenty-seven strains of *C. merdarium* developed a teleomoph and none was keratinolytic. No teleomorph associated with the other two *Chrysosporium* species and *Emmonsia* has been found. This makes taxonomical discussion of *Chrysosporium* and related species having Q-10(H_2) as the major ubiquinone difficult.

As stated above, this study revealed the following new evidences: the form-genus *Chrysosporium* has a heterogeneous distribution of the major ubiquinones, Q-9, Q-10 and Q-10(H_2); the teleomorphs of the species characterized by Q-9 and Q-10 were classified into separate families, Arthrodermataceae and Onygenaceae, defined within the revised order Onygenales by Currah.

In addition, a series of studies on the ubiquinone system of fungi, including this report, allows the following discussion. As shown in Tables 1 and 2, the ubiquinone system was investigated for several strains in six species including *C. keratinophilum* and *C. merdarium*, and it was demonstrated to be homogeneously distributed in each species. Most fungi examined to date have shown a homogeneous ubiquinone distribution in different strains, although a few exceptions are known, such as *Ambrosiella hartigii* Batra, *Geosmithia cylindrospora* (G. Smith) Pitt, *Paecilomyces variotii* Bainier, *Penicillium duclauxii* Delacroix and *Talaromyces byssochlamydoides* Stolk & Samson (Kuraishi et al., 1991; Fukushima et al., 1991a, b, 1993a). These data suggest that one way in which the ubiquinone system is significant in the taxonomy of fungi is that a species can be defined as having a single ubiquinone system.

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