

Conidial surface ultrastructure of human-pathogenic and saprobic *Cladosporium* species

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Abstract

Freeze-fracturing of outer wall layers of *Cladosporium* conidia revealed two types of ultrastructure, coinciding with taxonomic characteristics. The outer conidial layers were essentially smooth in the human pathogenic species, *C. bantianum*, *C. carrionii*, and *C. trichoides*. In contrast, mosaic arrays of rodlets on conidia were observed with freeze-fracturing in the saprobic species, *C. cladosporioides*, *C. coralloides*, *C. herbarum*, *C. sphaerospermum*, and *C. variabile*. Conidia of *C. elatum* were an exception among the saprobic species as they had smooth surfaces. The present study supports the suggestion that the human pathogenic *Cladosporium* species should be transferred to another genus.

Introduction

The anamorph genus *Cladosporium* Link:Fr. has melanized and dry, catenulate chains of conidia which are generally branched. More than 500 species have been described, although most of them should probably be reduced to synonymy. The genus is generally presumed to be polyphyletic (Von Arx 1983). In particular, the human pathogenic species seem to be unrelated to the saprobic ones by differing in growth at 37° C and hydrolysis of gelatin (Kwon-Chung et al. 1983, 1989; De Vries 1952).

Freeze-fracturing has revealed fine details of layers which lay near the surface of the conidial wall. In genera such as *Aspergillus* Mich., *Penicillium* Fr. and *Neurospora* Shear & Dodge, where conidial surface ultrastructure has been compared among species of the same genus, fine structure proved similar (Hess et al. 1968; Hess & Stocks 1969; Hallett & Beever 1981). Thus, the polyphyletic nature of the genus *Cladosporium* can be deduced, if significant differences are found in conidial surface ultrastructure between species of *Cladosporium*.

Cladosporium cladosporioides (Fres.) de Vries has been reported to have conidial walls finely ornamented

with rodlets (Latgé et al. 1988). In our freeze-fracturing study with *C. carrionii* Trejos, however, we found that this species has essentially smooth conidia. This finding prompted a comparative study on selected *Cladosporium* species.

Materials and methods

Strains used were stock cultures held in the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University (IFM) and Centraalbureau voor Schimmelcultures (CBS); they are listed in Table 1. Cultures were grown on potato dextrose agar (Difco) at 25° C for 1–3 weeks, and fixed for a few days at 4° C with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7. Conidial masses were transferred to the specimen holder of a freeze-etching apparatus (JFD-7000 of Japan Electron Optic Laboratory), and rapidly frozen in sherbet-like nitrogen of solid-liquid mixture at the melting point of -210° C. Cells were fractured and etched at -100° C for 20–60 s. Replicas were cleaned in hypochlorous acid for 3 h to overnight and in 70% sulphuric acid for 1 h, and observed under elec-

Table 1. Conidial wall ultrastructure of selected *Cladosporium* species.

Saprobic species		
<i>C. cladosporioides</i> (Fres.) de Vries	CBS 170.54	I*
<i>C. coralloides</i> Yamamoto	IFM 41451	I
<i>C. herbarum</i> (Pers.:Fr.) Link	CBS 399.80	I
<i>C. sphaerospermum</i> Penzig	CBS 193.54	I
<i>C. variabile</i> (Cooke) de Vries	IFM 41458	I
<i>C. elatum</i> (Harz) Nannf.	CBS 146.33	II
Human pathogenic species		
<i>C. bantianum</i> (Sacc.) Borelli**	IFM 4807	II*
<i>C. carrionii</i> Trejos	IFM 4809	II
<i>C. carrionii</i>	IFM 4805	II
<i>C. trichoides</i> Emmons**	CBS 173.52	II

* Type I. Wall layers finely ornamented with rodlets. Type II. Wall layers essentially smooth.

** Type species *C. bantianum* and *C. trichoides* were recently synonymized (Masclaux et al. 1995).

tron microscope Hitachi H-700 H at 80 KV or JEOL 1200-EX at 70 KV.

Results

Three types of cells were recognized in the specimens examined: vegetative mycelium, aerial mycelium, and conidia. In cells of the vegetative mycelium either the plasma membrane or the cytoplasm were revealed, and the wall layers were rarely observed. In contrast, fracturing of the cells of the aerial mycelium almost invariably occurred along wall layers lying near the cell surface.

The ultrastructure of conidial wall layers revealed by freeze-fracturing were of two different types, depending on the species examined. Type I. Outer wall layers exhibited parallel arrays of rodlets 10 nm apart and of various lengths (Figs 1–3), although in some small areas there were no rodlets observable. Parallel arrays of rodlets were interwoven and were composed of mosaic patterns. Similar rodlet patterns were also observed on aerial mycelia. Type II. All the layers revealed were essentially smooth (Figs 4–6). Rodlets were not observed. Aerial mycelium also had similar characteristics.

Species exhibiting type I conidia were *C. herbarum* (Pers.:Fr.) Link (Fig. 1), *C. cladosporioides* (Fig. 2), *C. variabile* (Cooke) de Vries (Fig. 3), *C. sphaerosper-*

um Penzig (Fig. 4), and *C. coralloides* Yamamoto, as summarized in Table 1.

The human pathogenic species, *C. trichoides* Emmons (Fig. 5), *C. bantianum* (Sacc.) Borelli (Fig. 6), and *C. carrionii* (Figs 7, 8), had type II conidia (Table 1). Also *C. elatum* (Harz) Nannf. has essentially smooth conidia.

Discussion

Freeze-fracture techniques have been applied extensively to fungal asexual and sexual spores from a wide range of taxa. The following ultrastructural types have been revealed. Type 0. Freeze-fracturing scarcely occurs along with any layers in the wall, but simply cross-fractures the cell wall. The spore surface as revealed by deep etching is smooth or globular, and is generally presumed to be hydrophilic. Type 0 is observed in asexual and sexual spores (Hawker & Madelin 1976), but none of the *Cladosporium* species studied here showed this type. Type I. Freeze-fracturing occurs along with layers near the wall surface, which are finely ornamented with a mosaic pattern of rodlets (see Figs 1–3). Type I is rather common in basidiospores and conidia borne on aerial mycelia (Bronchat & Demoulin 1971; Hawker & Madelin 1976). It has also been reported in ascospores (Figueras et al. 1988) and in Zygomycetes (Brain & Young 1979). Type II. Freeze-fracture occurs along layers near wall surfaces, which are smooth, i.e., not ornamented with the rodlets. The type appears rather uncommon but has been reported for sporangiospores of *Rhizopus* Ehrenb. (Hess & Weber 1973) and conidia of *Ascosphaera* Olive & Spiltoir (Liu et al. 1991). Multilamellar surface layers have also been reported for *Albugo* (Pers.) Roussel:Gray and *Phycomyces* Kunze (Tewari et al. 1980).

The rodlets are well characterized for *Neurospora* and *Schizophyllum* Fr. (Beever & Dempsey 1978; Woesten et al. 1993). They are responsible for water repulsion and aerial dispersal. A single mutation, loss of a protein hydrophobin, causes the conidium to be smooth on surfaces and wettable, and non-dispersant in water and air.

Conidia are known to readily disarticulate in saprophytic *Cladosporium* species, but remain coherent in human pathogenic ones (Kwon-Chung et al. 1989). We found that all human pathogenic species, *C. bantianum*, *C. carrionii*, and *C. trichoides*, have essentially smooth conidial wall layers, and that most saprophyt-

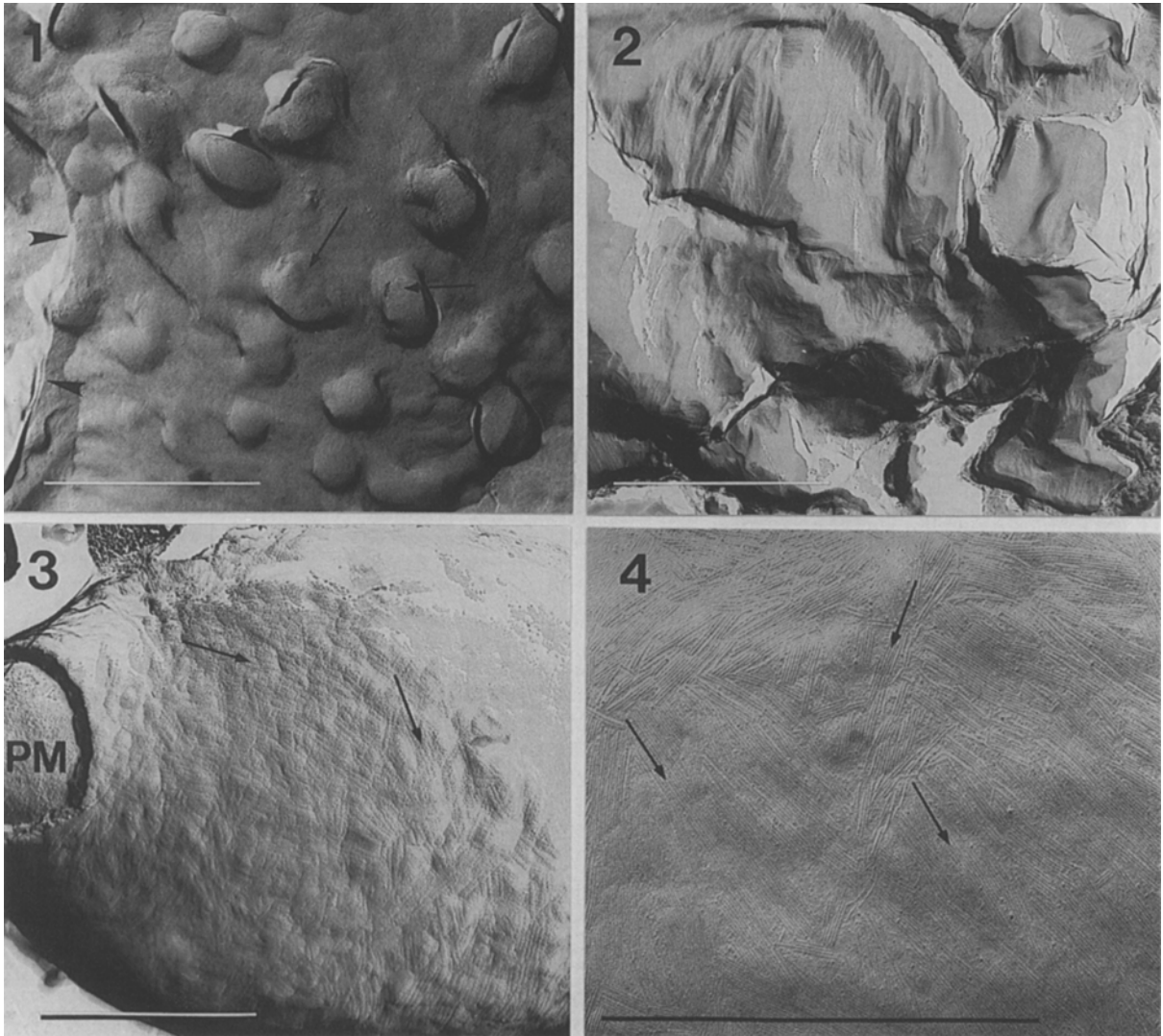


Fig. 1–4. Freeze-replicas of conidial wall layers of saprobic *Cladosporium* species. Note the existence of fine ornamentation by rodlets (arrows). Bars = 1 μ m. Fig. 1. *C. herbarum*. Note also that the surface layer was exposed by etching (large arrows). Fig. 2. *C. cladosporioides*. Fig. 3. *C. variabile*. The plasma membrane (PM) was exposed by fracturing. Fig. 4. *C. sphaerospermum*.

ic species of *Cladosporium* display rodlet-ornamented conidial wall layers. It may be supposed that saprobic *Cladosporium* conidia are dispersed more readily by air than pathogenic *Cladosporium* species.

Smooth walls of conidia are not necessarily correlated with pathogenicity, since in dermatophytes conidia with rodlets are common (Hasegawa 1975; Hawker & Madelin 1976; Hashimoto et al. 1976). Furthermore, in systemic pathogenic species, *Histoplasma capsulatum* Darling and *Blastomyces dermatitidis* Gilchrist & Stokes, which are phylogenetically related to dermatophytes (Bowman & Taylor 1993),

conidia and aerial mycelia are covered with rodlets (K. Takeo, K. Nishimura & M. Miyaji, unpublished). It should also be mentioned that freeze-fracturing is known to occur along with the hydrophobic interior of membrane bilayers (Branton 1971). Thus, smooth walls of pathogenic *Cladosporium* species should have hydrophobic layers near the wall surface, although hydrophobicity may be much lower than with conidia which have rodlets.

Conidia of *C. elatum* were an exception in the saprobic group. They essentially had smooth surfaces. This species, which possesses coherent conidial chains,

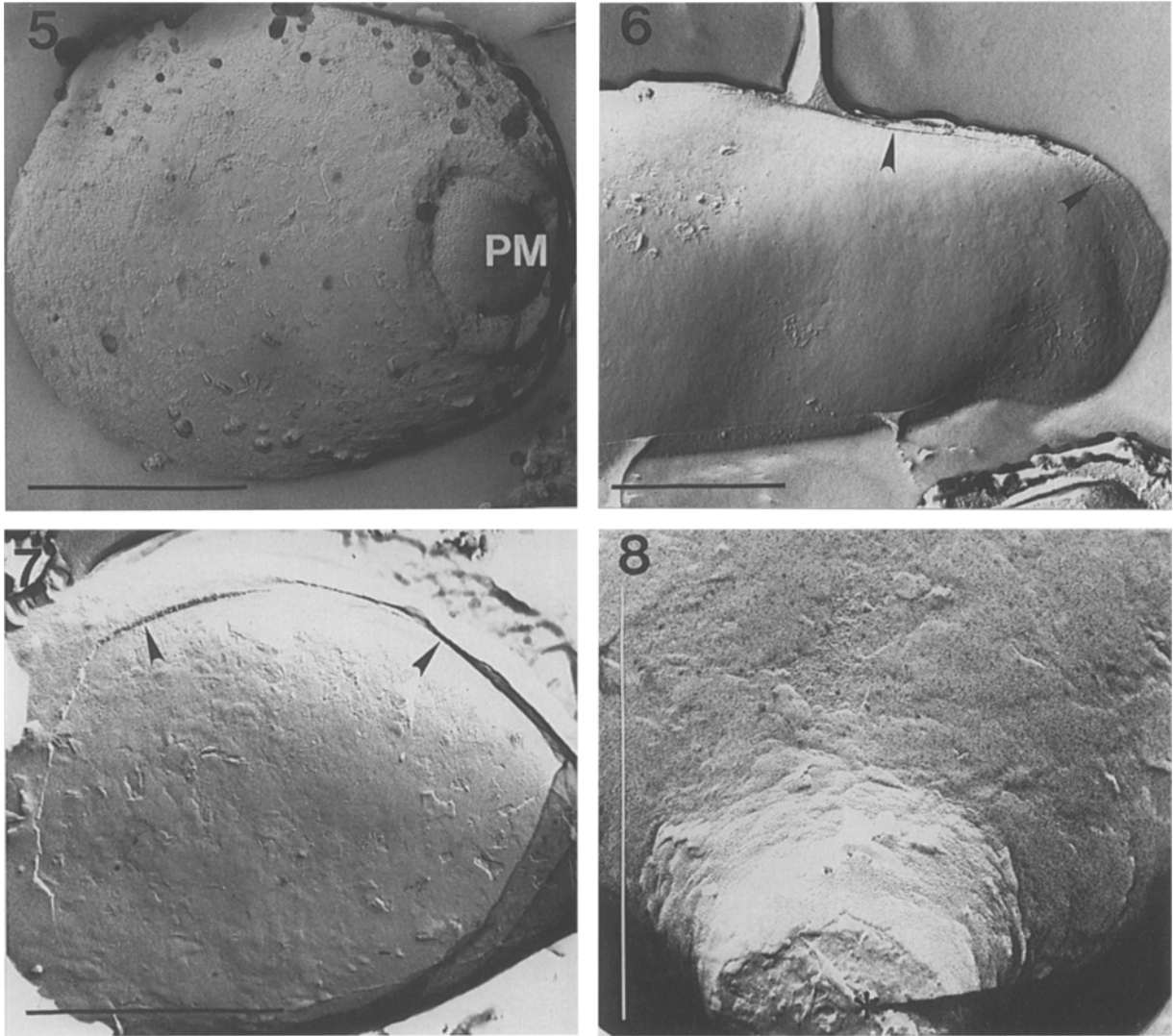


Fig. 5–8. Freeze-replicas of smooth conidial walls of human pathogenic *Cladosporium* species. Ridges between the fractured layer and the surface layer are shown (arrows). Bar = 1 μm . Fig. 5. *C. trichoides*. The plasma membrane (PM) was exposed by fracturing. Fig. 6. *C. bantianum*. Fig. 7. *C. carrionii*. Fig. 8. *C. carrionii*. The conidial stalk (*) is shown.

probably belongs to another group of saprobic *Cladosporium* species.

The results presented here coincide well with a recent work on partial rRNA sequencing, indicating that human pathogenic species are phylogenetically distantly related to *Cladosporium* species studied (Masclaux et al. 1995). The differences seem large enough to propose that the human pathogenic *Cladosporium* species should be transferred to another genus.

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