DISCOVERY OF ASCOMYCETE CHARACTERISTICS IN SPOROTHRIX SCHENCKII

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Sporothrix schenckii *has been studied by light microscopy, and also by transmission and scanning electron microscopy. Characteristics of Ascomycetes have been observed at the level of the cell-wall and in the synaptic system of the hyphae. Also the perfect state has been discovered. The four spored asci are formed directly from the mycelium and there is no fructification.* Dolichoascus schenckii *is the name suggested for this perfect state which constitutes a new genus of the Endomycetaceae.*

INTRODUCTION

Sporothrix schenckii is a fungus that can be a parasite on man or animals as well as a saprophyte on dead plants. It can be found in different states: either in the saprophytic mycelial phase corresponding to its growth in nature and in culture, or in the yeast — like cells obtained in complex cultural conditions corresponding to the parasitic state. Until now considered as a Deuteromycete, *Sporothrix schenckii* owed its generic name and place in classification to the characteristics of its asexual reproduction which was then the only one known. Our experiments have revealed ascomycete characteristics and the existence of sexual reproduction.

MATERIAL AND TECHNIQUES

Our research was practised on a strain of *Sporothrix schenckii,* preserved in the mycotheque of the parasitology laboratory of the Medical school of the University of Paris. It had been isolated in 1942 in a South-African patient suffering from sporotrichosis. After experimental reproduction of the lesions in animals we studied the cytology of the fungus. In light microscopy, we went carefully over the classical cytologic and histologic examinations. In electron microscopy, the specimens were prepared acoording to methods previously described by Thibaut (8). They were observed first with the transmission electron microscope (Hitachi HU 11 B, Siemens Elmiskop 1, Jem 100 U and Jem 100 B Jeol) and secondly with the scanning electron microscope (JSM U 3 Jeol).

RESULTS

The cultures of mycelial form on Sabouraud's glucose agar are more irregular in the center than on the edges. Their colour is more or less ochre (fig. 1). Microscopic examination shows a very fine creeping mycelium, septate, intertangled and branching out. From it grow conidias or asexual spores, the only ones discovered so far; these are either clustered around and along filaments and inserted by means of sterigmates, or clustered at the extremities of recumbant filaments where they form a characteristic bouquet (fig. 2).

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The mycelial form grown in Mariats shaken infusion, in anaerobiosis at 37ºC, changes into yeast - like cells (fig. 3). In an animal such as a mouse, inoculated with sporotrichosic pus or a culture of *Sporothrix schenckii,* a parasitic and very polymorphic form is observed in the lesions, but the most characteristic feature is the cigar shaped body (fig. 4). Exceptionally, among such rodents as the hamster, asteroid bodies can be seen (fig. 5).

Study in transmission electron microscopy of the hyphae of *Sporothrix schenckii* first showed us the existence in the cell-walls and septa of particular structures common to Ascomycetes and red algae, with Woronin bodies around the septa; it secondly showed the presence of the same synaptic system between the cells as that of the Ascomycetes. In the lateral cell-walls (fig. 6), going from outside to inside, we found the following formations, named after Chadefaud's terminology (4): a tubular vagina enveloping the hypha; a sub-vagina; a film lining the inside of the sub-vagina; a locula encasing each of the articles contained in the sub-vagina. Between the successive articles of the hyphae the septum is formed by the extremities of the loculae of the two articles separated by the septum and interlocular system placed between the two loculae.

The septum of *Sporothrix schenckii* has a central hole, but when the lengthwise section does not pass the level of the pore, the septum appears to be continuous. At the pore level there is a complex synaptic system (fig. 7). The pore is covered on each side with a dark lenticular disc, thinner on its edges. Between the discs and perpendicularly to them, thin trabecules can be seen. They seem to pass through the discs and radiate in a short fan-shaped spray outside the discs and into the cytoplasm of each one of the adjoining articles. The plasmalemma surrounds the border of the pore and insinuates itself between this border and that of each of the two discs. On either side of the central synaptic system we observed Woronin bodies, generaly two or three in number on each side of the septum and around the pore (fig. 7 and 8).

Observation of the mycelial form in scanning electron microscopy, enabled us to discover a number of notable pairs of filaments standing above the vegetative mycelium (fig. 9 and 10). Each of the coupled hyphae has a bulging head taking up 25% of their height. These heads grow close to each other and seem to unite. Very likely these hyphae are sexual organs between which there is copulation such as can be observed among the Endomycetaceae.

Examination by transmission electron microscopy of section cut from cultures of old yeast cells proved to be confirmation of the existence of sexual reproduction. Indeed we were able to discover (1) typical asci and ascospores (fig. 11 and 12). The mature ascus is oblong-shaped with thinning extremities. It is 5 μ long and 2,5 μ wide. It normally contains four ascospores, slightly more elongated, 3 μ in lenght and 1 μ in width. There is no fructification. According to the incidencce of the section, the appearance of the ascus may vary, as may the number of visible ascospores.

Since the discovery of asci and ascospores the species *Sporothrix schenckii* can no longer be considered as a Deuteromycete, but it must now be definitely connected to the class of the Ascomycetes. The well developed mycelium, the absence of fructification, the formation of the ascus through the copulation of two particular hyphae should lead us to classify it in the family of the Endomycetaceae. But in this family we did not find any speccies to which it could be related. On account of its four ascospores and its biology, it seems nearer the *Eremascus* genus. However it has neither globulous ascospores nor thick mycelium. Considering the very fine mycelium of *Sporothrix schenckii* (1 μ wide and often less), its characteristics vegetative multiplication system, and, most of all, the obviously elongated shape of its asci and ascospores, the species *schenckii* appears to us as a new type of fungus, whose name we have suggested to be: *Dolichoascus* Thibaut and Ansel 1970.

DISCUSSION

Chadefaud's experiments in optical microscopy detected that the cell-walls of ascomycete hyphae include two layers: the external one called the vagina of the hypha, and the internal one formed by the loculae of the cells. Again we discovered these elements within the ultrastructure

of the mycelial filaments of *Sporothrix schenckii*. The various layers of the fungal cell-wall described by Chadefaud (4) are of course much more evidently visible in electron microscopy. Schrantz (7) made the same observations in the cell walls of vegetative hyphae among the *Pustularia cupularis* and the *Galactinia plebeia* Ascomycetes.

The septal perforations were studied in electron microscopy by several botanists: Carroll (3) with *Saccobolus* and *Ascodesmis*, Schrantz (7) with *Galactinia plebleia* observed synaptic systems offering many relationships with *Sporothrix schenckii*. On the other hand these men showed that the Woronin bodies, also observed by us, are typical of the Ascomycetes only.

Could the perfect state of *Sporothrix schenckii* be interpreted differently? It could not be confused with that of endoconidia, such as Saez (6) described them in the *Geotrichum* genus, for these are formed in single files, one after another and are not grouped like ascospores. Indeed there are endospores in old cultures of *Sporothrix schenckii*, but they are very different from ascospores and cannot possible be confused (1). Nor could they be intra-hyphal hyphae like those Carbonnel and Rodriguez (3) observed in *Paracoccidioides brasiliensis*.

Our observations agree with those of Vuillemin (10) who, on the subject of the *Endomyces crateriformis* studied by Hudelo, Sartory and Montlaur (5), which is a pathogenic species discovered in a cutaneous lesions in man, found that the process of asexual reproduction of this parasites placed it — from the vegetative point of view — among the *Sporotrichum* (which, to-day, we should call *Sporothrix.*). In the reverse — we ourselves were led to consider the perfect atate of *Sporothrix schenckii* as an Endomycetaceae.

CONCLUSION.

Our research on *Sporothrix schenckii* has shown cytologic characteristics of Ascomycetes at the level of the cell wall, the septa and the synaptic system of the hyphae and, besides, a perfect state of sexual reproduction unknown until now. Therefore our experiments bring new taxonomic elements which change the category of this species completely, and it can new be classified with the Endomycetaceae.

RESUMO

Sporothrix schenckii *foi estudado em microscopia óptica e em microscopia eletrônica. Foram observados caracteres citológicos de Ascomicetos nas paredes das células e no aparelho sináptico dos hyphae. Foi descoberta a reprodução sexuada. Os ascos de quatro esporos são formados diretamente sobre o micélio e não há frutificação.* Dolichoascus schenckii *é o nome proposto para este estádio perfeito que constitui um novo gênero de Endomycetaceae.*

REFERENCES

1. ANSEL, M. & THIBAUT, M. — Une nouvelle Endomycetaceae: *Dolichoascus* nov. gen. Découverte de la reproduction sexuée par asques chez le *Sporotrichum schenckii* (Hetkoen et Parkins 1900). C.R. Acad. Sci., *270:* 2171-1173, 1970.

2. CARBONNEL, L. M. & RODRIGUEZ, J. — Mycelial phases of *Paracoccidioides brasiliensis* and *Blastomyces dermatitidis:* an electron microscope study J. Bacterior., *96:* 533-543, 1968.

3. CARROLL, G. — The fine structure of the ascus septum in *Ascodesmis sphaerospora* and *Saccobolus kerverni.* Mycologia, *59*: 257-532, 1967.

4. CHADEFAUD, M. — Les végétaux non vasculaires. Tome I du Traité de Botanique systématique de M.CHADEFAUD et L. EMBERGER. Masson, Paris, 1960.

5. HUDELO, SARTORY et MONTLAUR. — cité par VUILLEMIN, p. 1930 in Les champignons parasites et les mycoses de l'homme. Lechevalier éd. 1930.

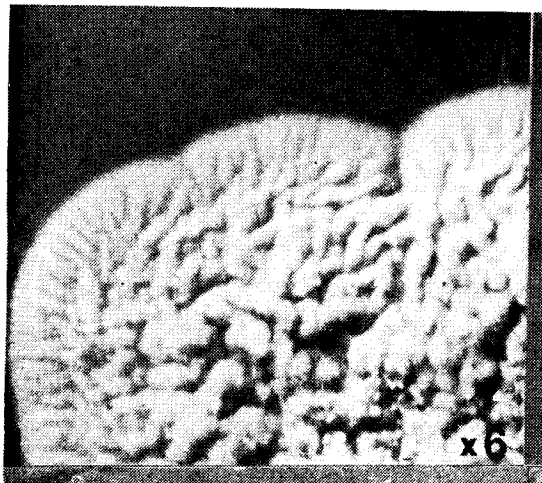
6. SAEZ, H. — Formation d'endospores chez *Geotrichum candidum*. Ann. Parasital. hum. comp. *44:* 197-204, 1969.

7. SCHRANTZ, J. P. — Etude cytologique en microscopie optique et electronique de quelques Ascomycètes. II. La paroi. Rev. Cytol. Biol. veg., *33:* 111-156, 1970.

8. THIBAUT, M. — Etude biologique, morphologique, cytologique et taxinomique du *Sporotrichum schenckii* (Hetkoen et Perkins) de Beurmann et Gougerot: *Dolichoascus schenckii* (H et P) Thibaut et Ansel, 1970. These de Doctorat d'Etat ès Sciences, Paris, *1970.*

9. THIBAUT, M. & ANSEL, M. — Observations microscopiques sur les phénomènes de senescence chez le *Sporothrix schenckii*. Ann. Parasitol. hum. comp. sous presse, 1972.

10. VUILLEMIN, P. — Les champignons parasites et les mycoses de l'homme. Lechevalier, Paris, 1931.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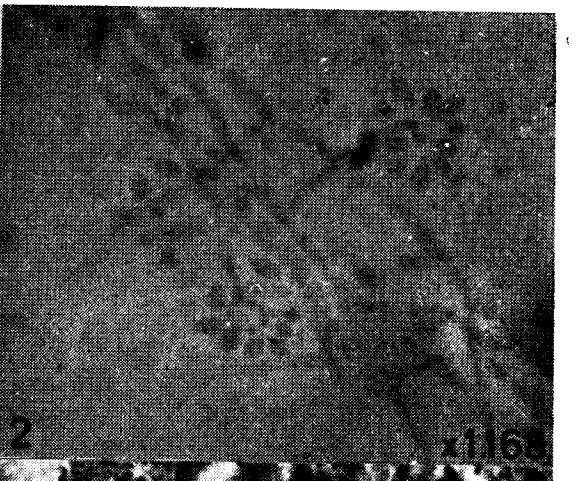
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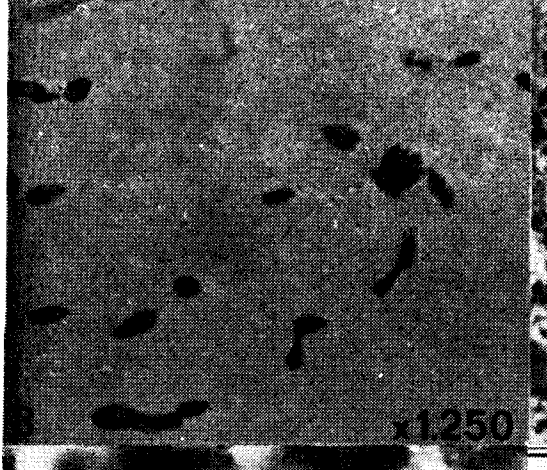
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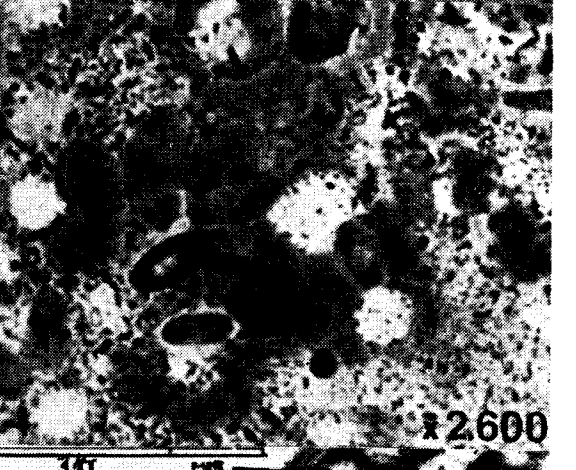
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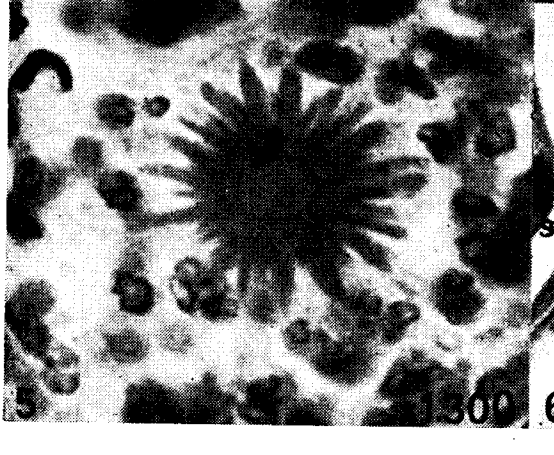
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Fig. 1 — Microscopic view of *Sporothrix schenckii* on Sabouraud's glucose agar.

Fig. 2 — Microscopic view of the mycelial phase. Bouquets of conidia. Coloration: methyl blue.

Fig. 3 — Microscopic view of the yeart — like cells. Coloration: methyl green.

Fig. 4 — Parasitic phase *in vivo*: cigar — shaped bodies in pus (mouse) Coloration: carbolic thionine.

Fig. 5 — Asteroid body in tissues (Hamster). Coloration: Hematoxylin and eosine.

Fig. 6 — Various constructive layers of the cell wall and septum on a hypha of *Sporothrix schenckii* (longitudinal section).
Fixation: glutaraldehyde and osmium tetroxyde.
Contrastation: uranyl acetate and lead citrate.
Abbreviations: P: Plasmalemma; I.S.: interlocular strip; I.R.: interlocular ring; L: locula; V: vagina; S.V.: sub vagina; F: internal film lining sub vagina.

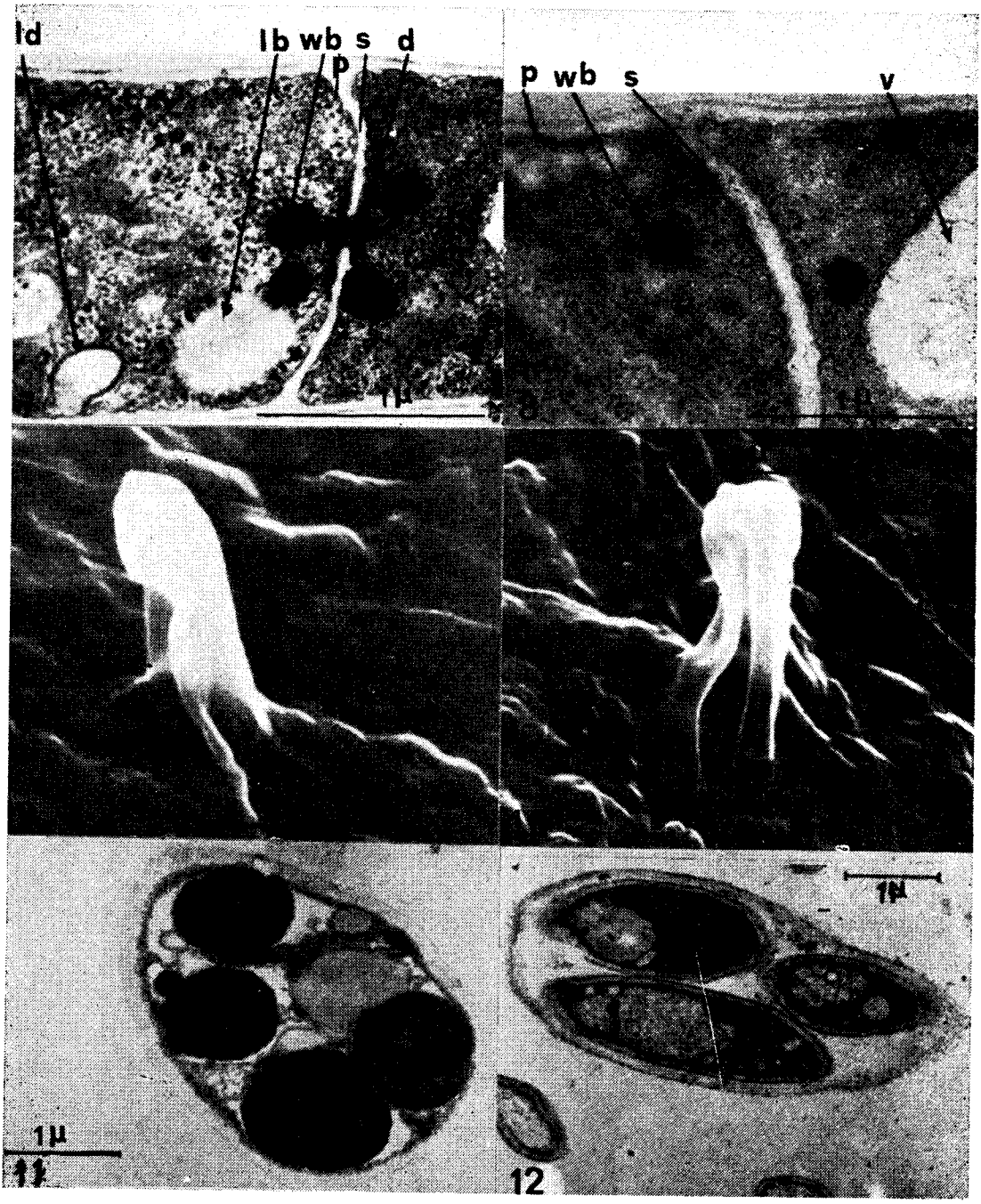
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Fig. 7 — Longitudinal section of a mycelial filament showing — on the level of septal pore, — the synaptic system composed of two discs crossed by trabecules. Four Woronin bodies can be seen near by.
Fixation: glutaraldehyde and osmium tetroxyde.
Contrastation: uranyl acetate and lead citrate.
Abreviations: L: lomasome; L.B.: lipid body; W.B.: Woronin body; P.: plasmalemma; S.: septum; D.: disc.

Fig. 8 — Longitudinal section in a hypha on the level of septum with no pore. Note two Woronin podies.
Fixation: glutaraldehyde and osmium tetroxide.
Contrastation: uranyl acetate and lead citrato.
Abbreviations: W.B.: Woronin bodies; P.: plasmalemma; S.: septum; V.: vacuole.

Fig. 9 — Sexual filaments of *Sporothrix schenckii*.
Fixation: formalin. Scanning electron microscopy.

Fig. 10 — *Sporothrix schenckii*, pair of sexual filaments.
Fixation: formalin. Scanning electron microscopy.

Fig. 12 — Section of an immature ascus.
Fixation: glutaraldehyde and osmium tetroxide.
Contrastation: uranyl acetate and lead citrate.

Fig. 12 — Mature ascus with three well differentiated ascospores.
Fixation: glutaraldehyde and osmium tetroxyde.
Contrastation: uranyl acetate and lead citrate.