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NOTES

Ultrastructure of the Septal Complex in Hyphae of *Cryptococcus laurentii*

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Electron microscopy of hyphae produced by *Cryptococcus laurentii* revealed typical basidiomycetous dolipore septa between the cells. Parenthesomes were not observed.

The basidiomycetous nature of *Cryptococcus laurentii* is indicated by several different lines of evidence. One of the first of these stems from the finding of similarities between *C. laurentii* and the haplont of *Tremella mesenterica* (13). These similarities occur in their (i) morphologies, (ii) abilities to synthesize starch, (iii) carbohydrate assimilation patterns, and (iv) extracellular heteropolysaccharide structures. Subsequent studies on biochemical and ultrastructural aspects of *C. laurentii* support this view. The guanine plus cytosine content of *C. laurentii* falls in the range of those characteristic for yeasts belonging to the basidiomycetes rather than to the ascomycestes (12, 14). The capabilities of producing extracellular deoxyribonuclease (3) and urease (10) which are found in *C. laurentii* are with few exceptions, characteristic of basidiomycetous yeasts. The tryptophan pathway found in *C. laurentii* var. *flavescens* is more closely related to those found in the basidiomycetes than to those found in the ascomycetes (4). Electron micrographs of the cell wall of *Crytococcus* blastospores display the lamellar appearance that is common among the basidiomycetous yeasts (6). When imperfect yeasts are divided into two groups based on cell wall type, all those with lamellar cell walls give positive reactions with Diazonium Blue B, as do the basidiomycetous yeasts (15). Perhaps one of the strongest pieces of evidence is that mixtures of certain strains of *C. laurentii* produce hyphae, some of which are dikaryotic (7).

The dolipore septum characterizes all higher basidiomycetes and some basidiomycetous yeasts (1), including those of the genera *Filobasidiella* (9) and *Filobasidium* (11), both of which have anamorphs in the genus *Cryptococcus*. Because the micromorphology of the septal apparatus appears to be an important criterion in understanding phylogenetic relationships in fungi, we have examined the ultrastructure of hyphal septa in *C. laurentii*.

C. laurentii strain ATCC 26021, type α, was mixed with strain ATCC 26023, type **a**, or with strain NIH 230 on neutral V-8 juice agar. After 14 days of incubation at 25°C, pieces of agar block containing hyphae were transferred to fresh plates of V-8 juice agar. These secondary cultures produced dense mats of hyphae in 7 to 14 days at 25°C. Thin layers of hyphae were excised from the surface and were processed for electron microscopy as previously described (9). Nuclear staining was also performed on these cultures with a basic fuchsin method reported previously (8).

In Fig. 1a and b, the septal complex present in hyphae of crosses between strains of *C. laurentii* is shown. The dolipores produced in the hyphal septa are essentially identical in both pairs of cultures. The dolipore channel exhibits alternating bands of dark and light material and a dark central plug. Striated material is seen in each orifice (Fig. 1b, arrows). This morphology is very similar to that observed in the dolipore channels of *Filobasidiella neoformans* (9), *Filobasidiella arachnophila* (S. R. Khan, J. W. Kimbrough, and K. J. Kwon-Chung, manuscript submitted for publication), and the species of *Filobasidium* (11). Although *C. laurentii* and the haploid phase of *Tremella* spp. have many similarities (13), the septal pore apparatus in those *Tremella* species that have been examined includes a vesiculated parenthesome (2, 5). The lack of a parenthesome in *C. laurentii* suggests that it is more clearly related to *F. neoformans* (9) and *Filobasidium floriforme* (11), which also lack parenthesomes.

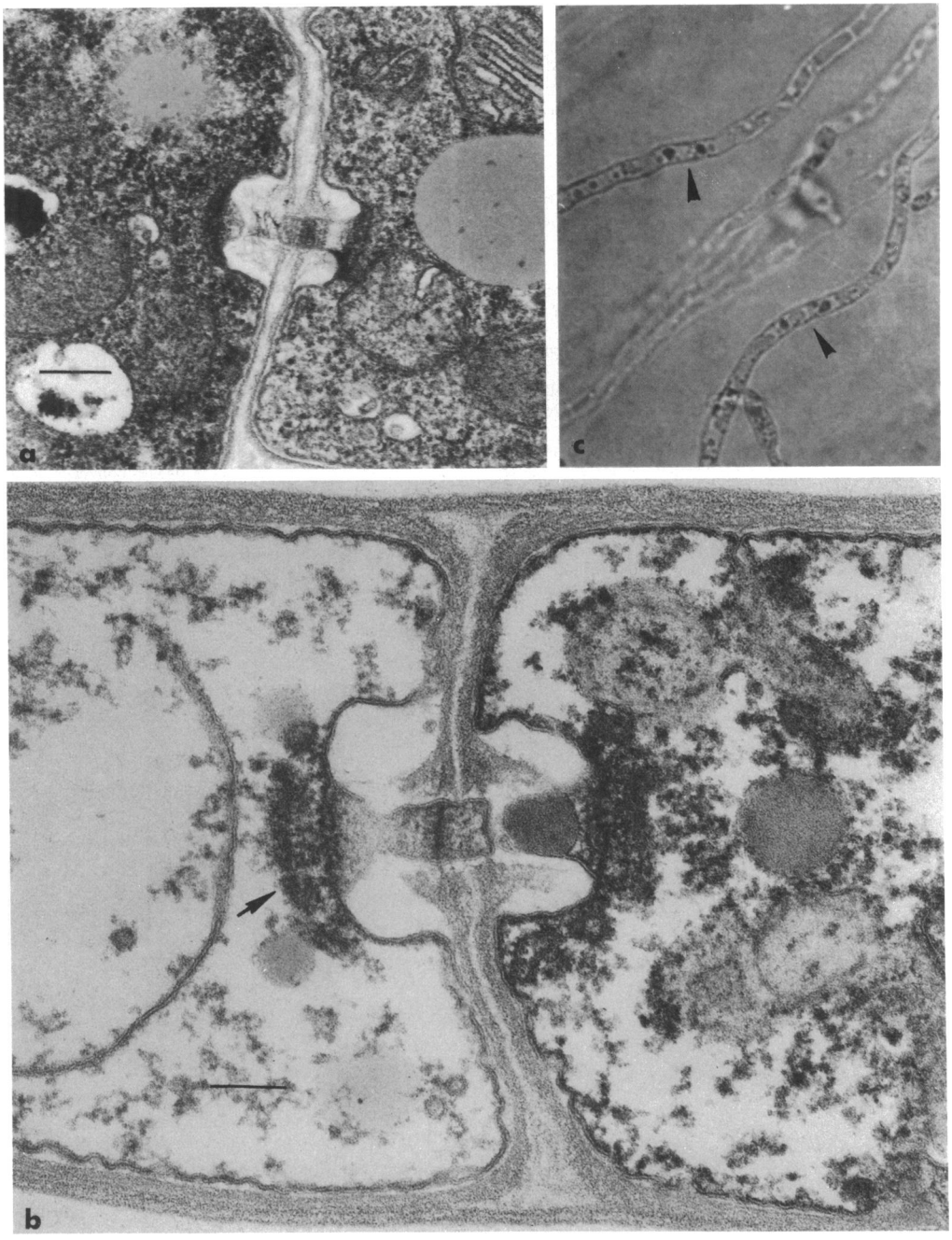

FIG. 1. *Hyphae of C. laurentii. (a) ATCC 26021 × ATCC 26023. Note the dolipore septum between the hyphal cells and the bands of dark and light material in the pore channel. Bar, 2.0 μm. (b) ATCC 26021 × NIH 230. Dolipore orifice is apparently occluded with striated material (arrow). Note lamellar cell wall. Bar, 1.0 μm. (c) ATCC 26021 × ATCC 26023. Nuclear staining reveals strands of dikaryotic hyphae (arrows) (magnification, ×1,200).*

Fig. 1b also illustrates the characteristic basidiomycete lamellar cell wall in the hyphae of *C. laurentii*.

Nuclear staining (Fig. 1c) confirms the observation of Kurtzman (7) that, although clamp connections are not produced, some dikaryotic hyphae are present.

The dolipore septa, lamellar cell walls, and

dikaryotic hyphae reported here confirm the affinity of *C. laurentii* to the basidiomycetes. The failure to find a pore cap associated with the dolipore suggests that the perfect form of *C. laurentii* may be related more closely to the fungi in the *Filobasidiaceae* rather than those belonging to the *Tremellaceae*.

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