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SHORT COMMUNICATIONS

Invaginations of the Cytoplasmic Membrane in Basidiomycetous Yeasts

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Electron microscopic cryofractography, which was used for the first time in this study to investigate the ultrastructural organization of basidiomycetous yeasts, showed a lack of plasma membrane invaginations, which are typical of ascomycetous yeasts, in *Cryptococcus humicola* and *Cryptococcus curvatus* cells and the occurrence of such invaginations in representatives of other genera of basidiomycetous yeasts, *Filobasidium capsuligenum* and *Phodotorula mucilaginosa.*

It is generally recognized that intramembrane particles (IMPs) and invaginations are typical ultrastructural elements of the cytoplasmic membrane (CPM) of yeasts. The ultrastructural studies of the cytoplasmic membrane of yeast, which were performed mainly with ascomycetous yeasts, allowed researchers to reveal and describe CPM invaginations, whose size and form depend on the yeast species, culture age, cultivation conditions, and other factors [1–3].

This work deals with the ultrastructural investigation of the CPM of basidiomycetous yeasts by electron microscopic cryofractography.

The following basidiomycetous yeast were studied: *Cryptococcus humicola* strain S [4], *Cryptococcus humicola* VKM Y-2238, *Cryptococcus humicola* VKM Y- 1613, *Cryptococcus curvatus* VKM Y-2230, *Filobasidium capsuligenum* VKM Y-1513, and *Rhodotorula mucilaginosa* VKPM Y-706. For comparison, two strains of ascomycetous yeasts, *Schwanniomyces occidentalis* VKM Y-395 and *Candida lipolytica* VKM Y-155, were also studied.

The yeasts were cultivated at 29–30°C either in a liquid medium with aeration (160 rpm) for 12–24 h or on wort agar (6° according to Balling) for 1–5 days. The liquid Yeast Nitrogen Base (Difco) medium was supplemented with glucose (5 g/l) and had pH 5.0. The growth phases of yeast cultures in the liquid medium were determined by measuring the culture turbidity with a KFK-2 photoelectrocolorimeter (1-cm cuvette, a wavelength of 540 nm, initial culture turbidity 0.1).

Cells were concentrated, frozen in propane (cooled to the temperature of liquid nitrogen), and fractured [5]. The fractured faces were subjected to vacuum etching for 3 min. Neither prefixation nor cryoprotectors were used.

The freeze-fractured cytoplasmic membrane of *S. occidentalis* cells exhibited the presence of IMPs and typical invaginations on its P face (Fig. 1), the length of these invaginations varying from 0.08 to 0.11 μm and their number being from 25 to 50 per 1 μm^2. The same structure of CPM was observed in *C. lipolytica* cells. These observations are consistent with literature data on the ultrastructural organization of yeast plasmalemma [1–3, 6].

The CPM of the logarithmic-phase *C. humicola* cells looked smooth and did not contain any invagina-

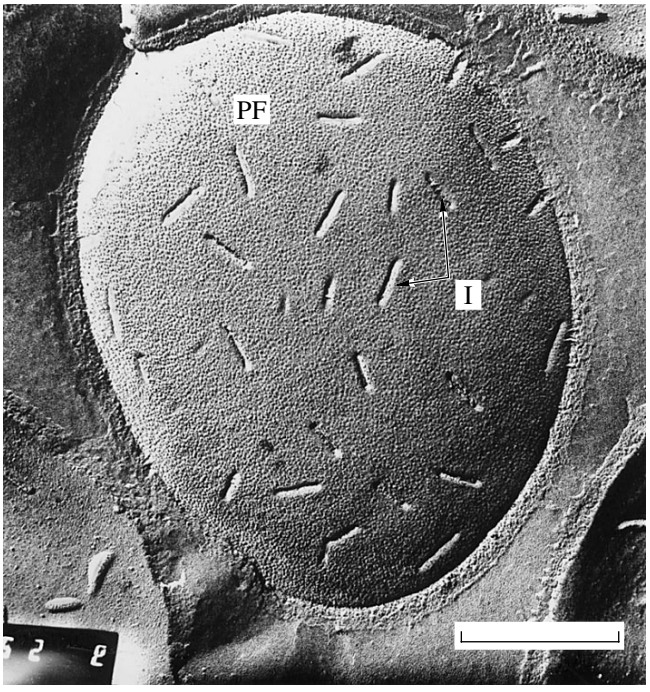

Fig. 1. The P face (PF) of the cytoplasmic membrane of the ascomycetous yeast *Schwanniomyces occidentalis*, as observed by the freeze-etching technique. I Here and in other figures: I, invagination; EF, E face. Bar = 0.5 μm.

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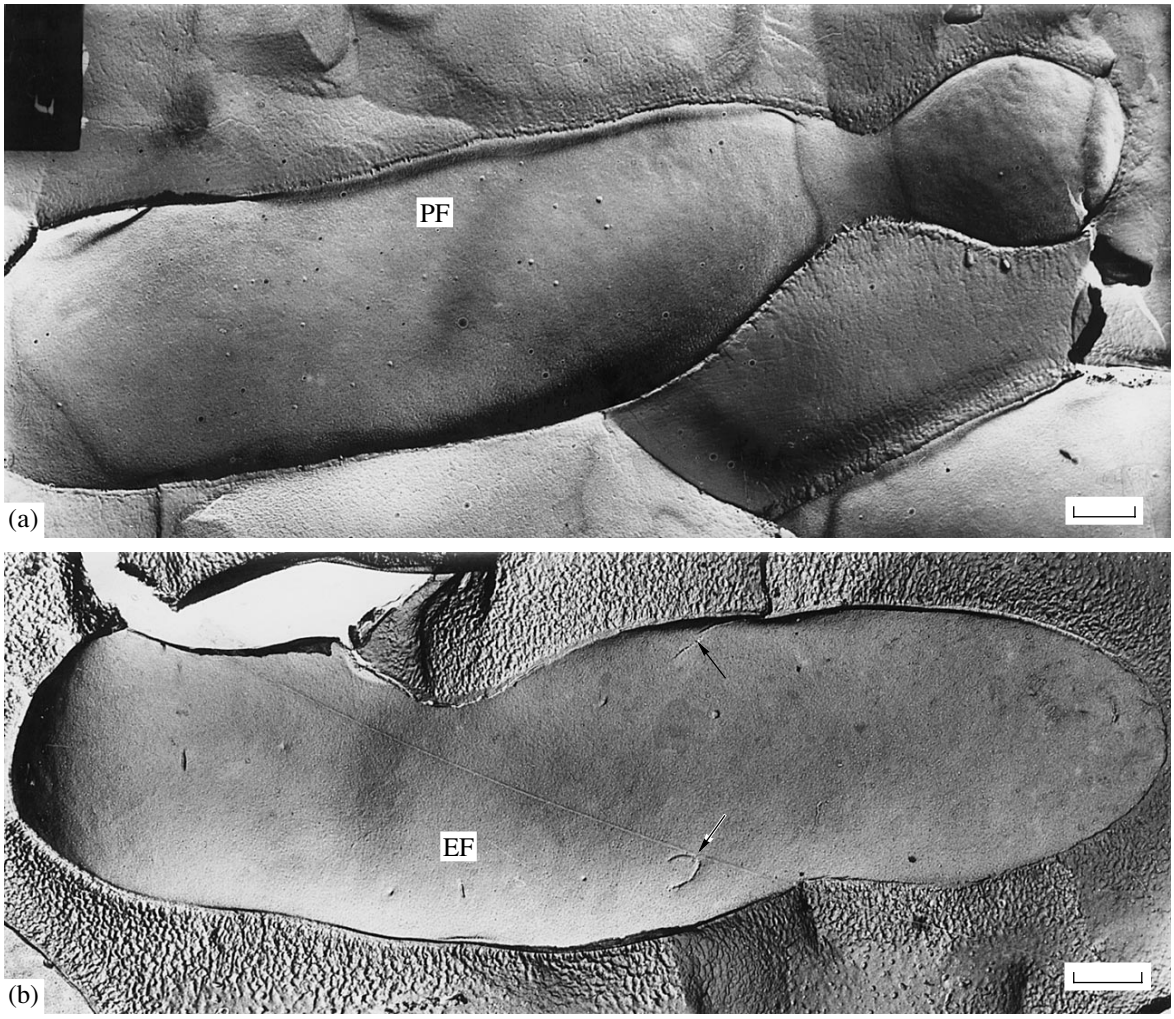

Fig. 2. The fracture faces of the CPM of the *C. humicola* strain S cells taken from (a) the logarithmic growth phase (P face) and (b) the stationary growth phase (E face). An invagination on the E face of the CPM is indicated by the arrow.

tions. The CPM of a bud and the isthmus between the mother cell and the bud had no invaginations either (Fig. 2a). Scarce invaginations (one per fracture area of 20–30 μm^2) could only be observed in the CPMs of stationary-phase yeast cells (Fig. 2b). However, these invaginations were curved in shape, narrower, and shorter (from 0.1–0.2 to 0.5–0.6 μm in length) in comparison with typical invaginations.

To elucidate whether typical invaginations are absent only in *C. humicola* strain S or in other strains of this species as well, we studied the *C. humicola* type strain VKM Y-2238 and *C. humicola* VKM Y-1613. The logarithmic-phase cells of both of these strains were found to lack CPM invaginations. As in the case of *C. humicola* strain S, atypical invaginations could be observed in the stationary-phase cells of these two strains, as well as in the allied *C. curvatus* species.

The ultrastructural study of *Rh. mucilaginosa* VKPM Y-706 and *F. capsuligenum* VKM Y-1513, other representatives of basidiomycetous yeasts, revealed typical CPM invaginations (Fig. 3). The occurrence of the CPM invaginations common for ascomycetous yeasts was also reported for the pathogenic basidiomycetous yeast *Cryptococcus neoformans* [2].

Although invaginations of the yeast plasmalemma were first described as far back as the 1960s [1], their physiological role is still the subject of discussion [6, 7]. Takeo [7] reported that the CPM invaginations of the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* lack IMPs. The increased number of invaginations in the CPM of these yeasts were interpreted by the author as being related to a decrease in the metabolic activity of the membrane. A similar inference was made by Ratner *et al.* [6], who comparatively studied the ultrastructure of the CPM of lipolytically active and quiescent *S. lipolytica* cells.

The occurrence of CPM invaginations was also accounted for by the specific lipid composition of membranes, the presence of cytoskeleton structures and

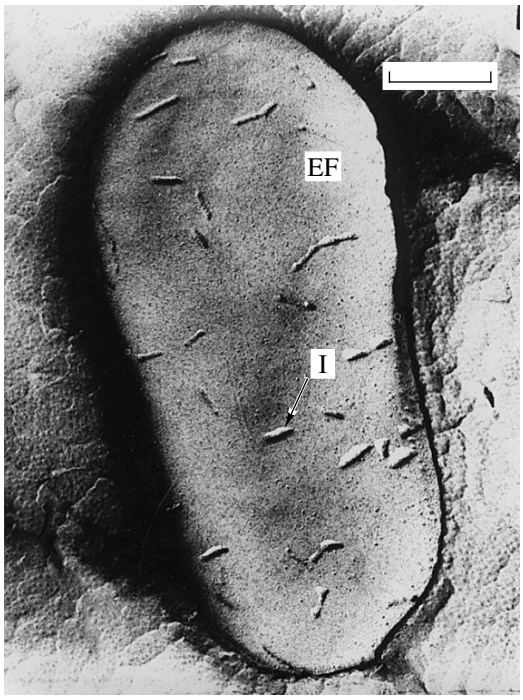

Fig. 3. The E face of the freeze-fractured membrane of *Rh. mucilaginosa*.

protein complexes [7], and sexual agglutination processes [8].

It should be noted that, although typical invaginations were absent from the CPM of the studied representatives of the genus *Cryptococcus* grown on standard media, the possibility cannot be excluded that invaginations of one or another type may appear in response to drastic changes in the cultivation conditions, which should induce some metabolic and ultrastructural alterations in the CPM.

To conclude, the lack of typical CPM invaginations in *C. humicola* and *C. curvatus* can be treated as a unique feature of the ultrastructural organization of the CPM of these yeast species. The presence or absence of CPM invaginations can be used as a discriminating marker of basidiomycetous yeasts and as an indicator of the functional activity of their plasmalemma.

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REFERENCES

1. Moor, H. and Mhlethaler, K., Fine Structure in Frozen–Etched Yeasts Cells, *J. Cell Biol.*, 1963, vol. 17, pp. 609–628.
2. Takeo, K., Uesaka, I., Uehira, K., and Nishiura, M., Fine Structure of *Cryptococcus neoformans* Grown *In Vitro* as Observed by Freeze–Etching, *J. Bacteriol.*, 1973, vol. 113, pp. 1442–1448.
3. Garrison, R.G., Vegetative Ultrastructure, *Yeast Cell Envelopes: Biochemistry, Biophysics, and Ultrastructure*, Arnold, W.N., Ed., Florida: CRC, 1981, vol. 2, pp. 139–160.
4. Dmitriev, V.V., Bibikova, I.I., Ofitserov, E.N., and Naumova, R.P., Yeasts Utilizing the Laurox-9 Surfactant, *Mikrobiologiya*, 1988, vol. 57, pp. 174–180.
5. Fikhte, B.A., Zaichkin, A.I., and Ratner, E.N., *Novye metody fizicheskogo preparirovaniya biologicheskikh ob"ektov dlya elektronno mikroskopicheskikh issledovanii: Zamorazhivanie-skalyvanie i zamorazhivanie-travlenie* (New Methods of Preparing Specimens for Electron Microscopic Studies: Freeze-Fracturing and Freeze-etching Techniques), Pushchino, 1973.
6. Ratner, E.N., Dmitriev, V.V., Zvyagintseva, I.S., and Fikhte, B.A., The Ultrastructure of Invaginations and the Density of Intramembrane Particles in the Plasmalemma of Lipolytically Active and Resting Cells of *Saccharomycopsis lipolytica*, *Mikrobiologiya*, 1986, vol. 55, pp. 174–180.
7. Takeo, K., Lack of Invaginations of the Plasma Membrane during Budding and Cell Division of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, *FEMS Microbiol. Lett.*, 1984, vol. 22, pp. 97–100.
8. Nakagawa, Y., Tanaka, K., and Yanagishima, X., Occurrence of Plasma Membrane Invagination Associated with Sexual Agglutination Ability in the Yeast *Saccharomyces cerevisiae*, *Mol. Gen. Genet.*, 1983, vol. 189, pp. 211–214.