莢膜の厚さの異なるCrytpococcus neoformans分芽胞子の微細構造について

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Ultrastructure of Thin and Thick Capsuled Cells in *Cryptococcus neoformans*

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A capsule is a slim layer around the microorganisms including fungi. Encapsulated yeasts were classified in genus *Cryptococcus*. *Cryptococcus neoformans* Sanfelice, 1984, opportunistic pathogen to humans and animals, was revealed to be the anamorph of basidiomycetous yeast, *Filobasidiella neoformans* Kwon-Chung, 1975 [12]. The capsule of this yeast has been analyzed for immunologic and physiological charactaristics in relation to pathogenicity [2, 6, 16]. The size of the capsule is known to depend on the culture condition [6]. The fine structure has also been studied on the yeast cells including their capsule regardless the size of the capsule [1, 7, 10, 11, 17, 18]. However, the fine structure of intracellular organelles might be different depending on the capsule size, since the capsule components were actively produced in thick capsuled cells.

In this paper, the fine morphology of the yeast cells with thin and thick capsules is described. Two strains of *C. neoformans* var. *neoformans* (VUT-77034 : *F. neoformans* var. *neoformans* maiting type α and VUT-77035 : *F. neoformans* var. *neoformans* maiting type a) were examined. They were donated by Dr. K.J.Kwon-Chung (National Institute of Health, USA) and preserved by subculturing on 1% malt extract agar slant in our laboratory. The organisms of these two strains were cultured on these media at 25°C at a 3-day interval, and then at the same temperature for 24 hr on 4 consecutive days. The capsule size was determined by the light microscopic examination of India-ink preparations. The size of the capsule was thin (about 0.5 μm) on 1% malt extract agar or 1% pepton agar with 15% and 20% glucose, middle (about 0.5–2 μm) with 5% and 10% glucose and thick (more than 2 μm) with 0% and 1% glucose (Fig. 1). The capsule size was increased in media with lower amount of glucose and thinner in media with higher amount of glucose as Dykstra *et al.* [6] reported. He suspected that suppresion of capsule size was attributed at least in part to the increased osmolarity of the medium.

For the electron microscopic examination, the yeast cells of the two strains incubated on 1% malt extract agar supplemented with 0%, 5% and 15% glucose respectively were harvested and fixed for 6 hr or 12 hr with 2.5% glutaraldehyde containing cacodyrated buffer (ph 7.4) and post fixed with 1% osmium tetroxide in the same

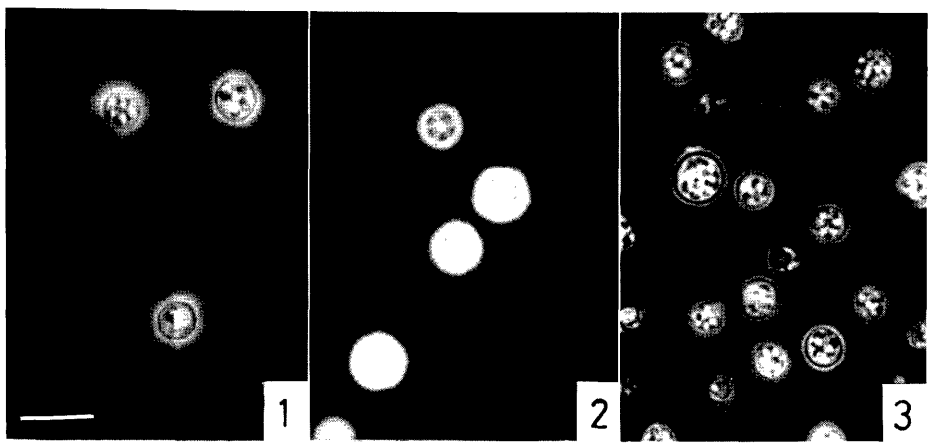

Fig. 1. *C. neoformans* (VUT-77035) cultured at 25°C for 24hr on 1% malt extract agar with (1) 0% glucose, (2) 5% gulcose, (3) 15% glucose. ×400, bar=10μm.

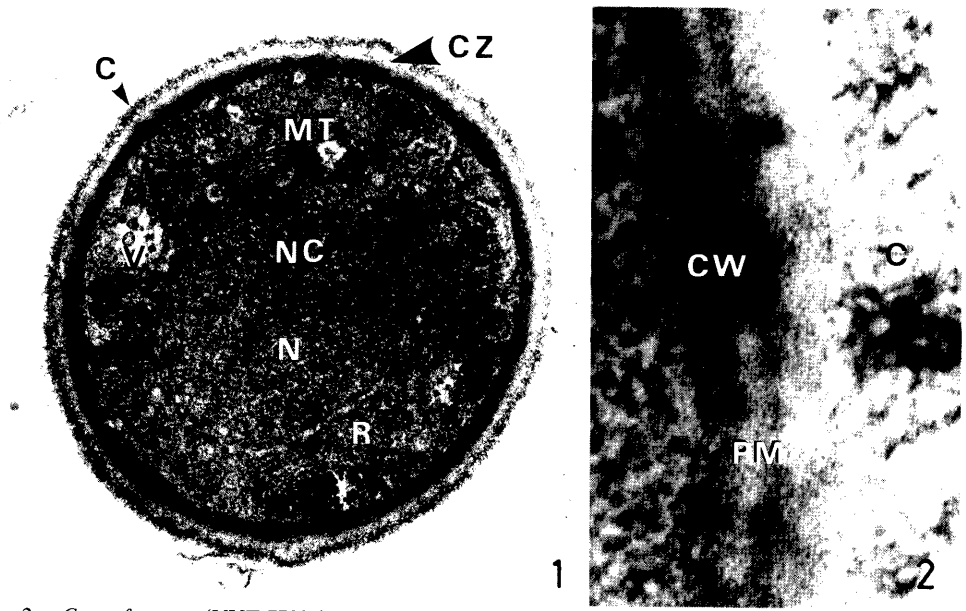

Fig. 2. *C. neoformans* (VUT-77035) cultured on 1% malt extract agar with 15% glucose. A 6-hr prefixed section. (1) ×24000 (2) ×120000, (C) capsule, (CW) cell wall, (CZ) clear zone, (MT) mitochondrion, (N) nucleus, (NC) nucleolus, (PM) plasma membrane, (R) ribosome, (V) vacuole.

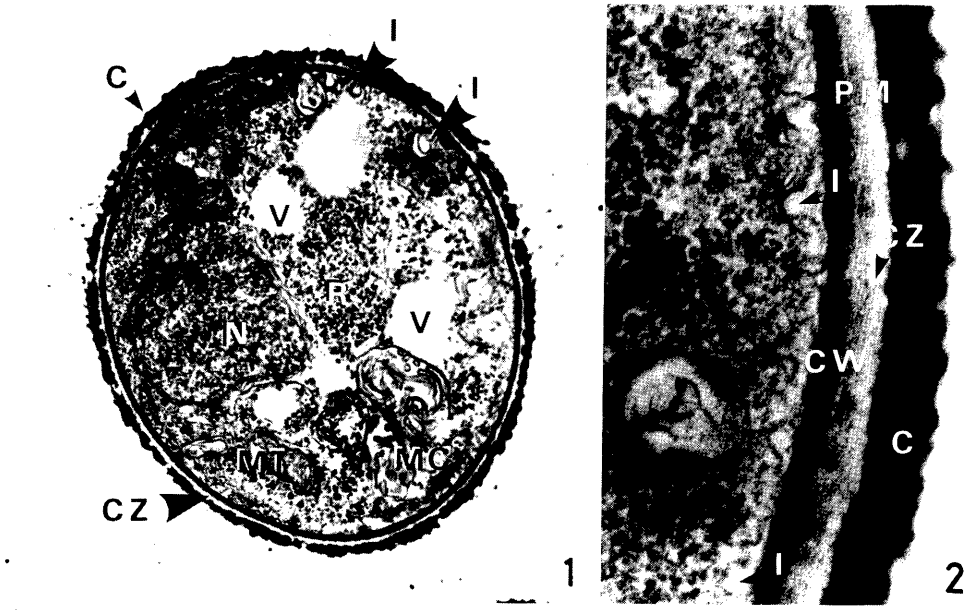

Fig. 3. *C. neformans* (VUT-77035) cultured on 1% malt extract agar without glucose. A 12-hr prefixed section. (1) ×15000 (2) ×80000, (C) capsule, (CW) cell wall, (CZ) clear zone, (I) invagination, (MC) membrane configuration, (MT) mitochondrion, (N) nucleus, (PM) plasma membrane, (R) ribosome, (V) vacuole.

buffer for 4 hr at 4°C. Each sample was dehydrated and embedded in Spurr low viscosity resin. Ultrathin sections were cut on Sovall ultramicrotome MT-5000 with glass knives and

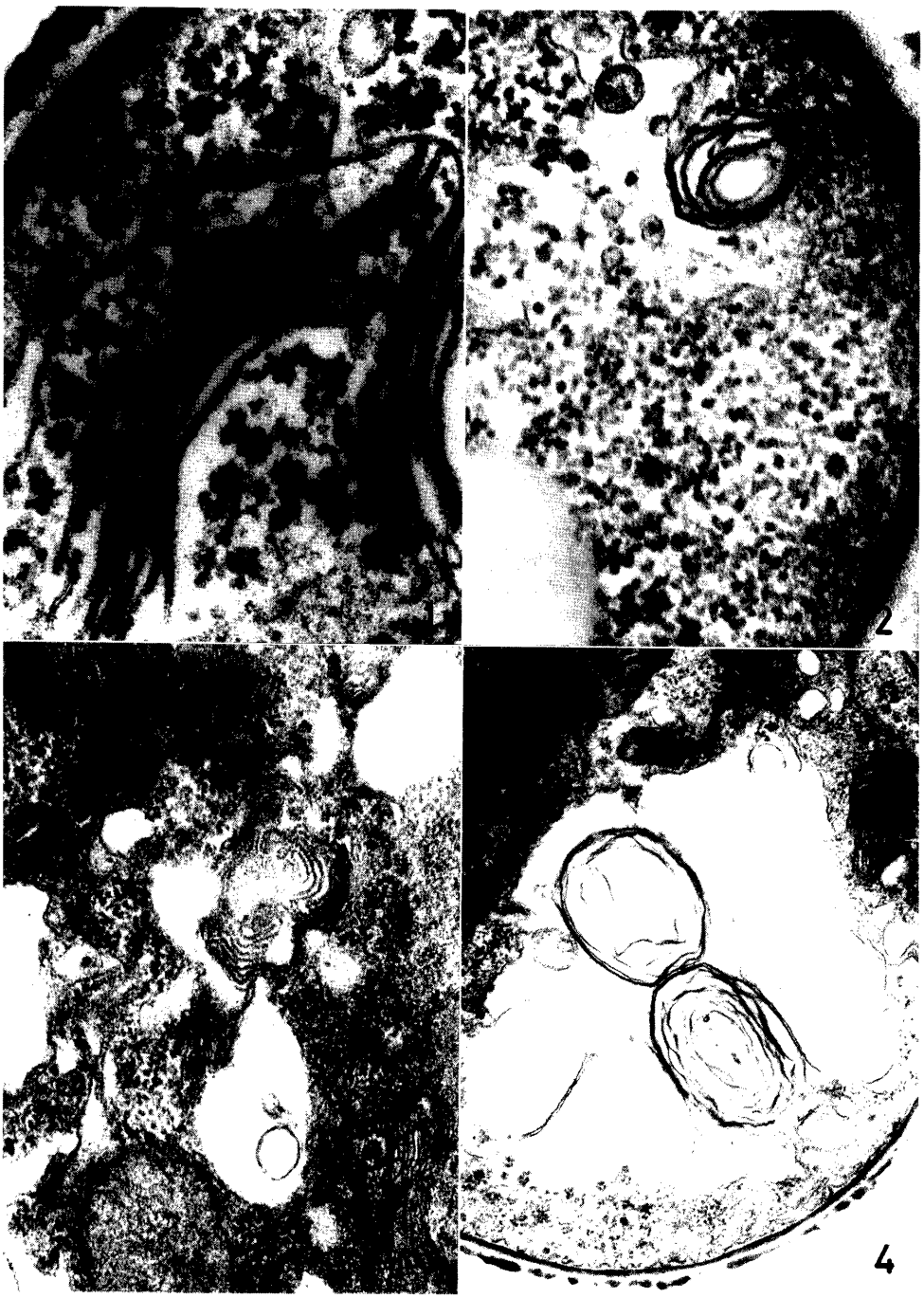

Fig. 4. Various shaped membrane configurations in the thick capsuled cells of *C. neoformans*. (1) ×60000 (2) ×60000 (3) ×37000 (4) ×20000.

stained with saturated uranylacetate and lead citrate. The sections were examined and photographed with a JOEL electron microscope 100S, employing of 80 kV.

The electron microscopy showed the nucleus sometimes with nucleolus, endoplasmic reticula,

mitochondria, ribosomes, lipid granules and vacuoles. The thin and thick yeast capsules were appeared to be intertwining microfibrils in 6-hr prefixed sections (Fig. 2), and to be tighten microfibrils in 12-hr prefixed sections (Fig. 3). For the prefixation time, thin and thick capsuled cells required 6 hr and 12 hr, respectively, to get clear photomicrographs. The cell wall was 30 to 80 nm in width and consisted of two layers. The outer layre was electron transparent and inner one was electron dense. The transparent area so called "clear zone" or "white rim", was distinct between capsule and cell wall in thick capsuled cells, however, it was sometimes lacking in thin capsuled ones. The plasma membrane located directly beneath cell wall appeared to be smooth in thin capsuled cells, and it was winding and had many invaginations in thick capsuled cells. The cytoplasma of both cells had mitochondria, smooth and rough endoplasmic reticula, small and large vaculoes and storage granules. The mitochondria were oval and long oval with curving in shape. However, an annurated type of mitochondria found by Edwards *et al.* [7], and golgi apparatus including dictyosome were not observed. These intracellular organelles were more abundant in the thick capsuled than in thin capsuled cells which appeared almost the same in electron microscopy as previously reported [1, 7, 10, 11, 17, 18]. Further more, thick capsuled cells had various intracellular membrane configurations such as multitublar body, concentrated lamellae, whirlpool, myelin-figures (Figs. 3 and 4). They were found to be either free in the cytoplasma or associated with plasma membrane, and were also within vacuoles.

These membrane structures were similar to those reffered to as "lomasome" [14, 15], "myelin figures" [8, 14], "mesosome" [9, 13, 15], or "plasmalemmasome" [8, 14], as frequently reported for other fungi. Chang and Tanaka [4] suggested that the formation of these structures might be initiated by the aggregation and convolution of endoplasmic reticulum in the cytoplasma, and that these structures might play different roles in physiological functions according to their developmental stages and their localities. The relation between these structures and intracytoplasmic membrane systems still remained unclear.

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REFERENCES

1. Al-Dooly, Y. 1971. *Sabouraudia* 9: 113–118.
2. Bulmer, G.S. and Sana, M.D. 1968. *J. Bacteriol.* 95: 5–8.
3. Calbonell, L.M. and Pollack, L. 1962. *J. Bacteriol.* 83: 1356–1357.
4. Chang, S.T. and Tanaka, K. 1971. *Cytologia* 36: 689–851.
5. Cole, G.T. and Aldrich, H.C. 1971. *J. Cell Biol.* 51: 873–874.
6. Dykstra, M.A., Friedman. L. and Murphy, J.W. 1977. *Infect. Immun.* 16: 129–135.
7. Edwards, M.R., Gordon, M.A., Lapa, E.W. and Ghiorse, W.C. 1967. *J. Bacteriol.* 93: 766–777.
8. Garrison, R.G. 1983. *In*: Fungi pathogenic for humans and animals Part A, (Howard, D.H. ed.) Marcel Dekker, N.Y.
9. Hashimoto, T. and Yoshida, N. 1966. *In*: Electron microscopy Vol. II (Uyeda, R. ed.) Maruzen, Tokyo.
10. Hiruma, M. and Kagawa, S. 1985. *Mycopathol.* 89: 5–12.
11. Iyo, S. 1965. *Jpn. J. Dermatol.* B 40–51.
12. Kwon-Chung, K.J. 1976. *Mycologia* 68: 942–946.
13. Lewia, S. and Calbonell, L.M. 1970. *J. Gen. Microbiol.* 62: 43–47.
14. Marchant, R. and Moore, R. 1973. *Protoplasma* 76: 235–247.
15. Moore-Landecker, E. 1971. *Cytologia* 36: 563–574.
16. Shinoda, T., Ikeda, R., Nishikawa, A. and Fukazawa, Y. 1980. *Jpn. J. Med. Mycol.* 21: 230–238.
17. Takeo, K., Uesaka, I., Uehira, K. and Nishimura, M. 1972. *J. Bacteriol.* 113: 1449–1454.
18. Tsukahara, T. 1963. *Jpn. J. Microbiol.* 7: 53–60.

要　約

莢膜の厚さの異なる *Cryptococcus neoformans* 分芽胞子の微細構造について：恩田千景，長谷川篤彦，友田勇（東京大学農学部家畜内科学教室）——*Cryptococcus neoformans* の分芽胞子について，莢膜の厚さの違いによる微細構造の差異を観察した。莢膜の厚い胞子では薄い胞子にくらべて透明層が明瞭で，形質膜は屈曲に富み，ミトコンドリアや小胞体が豊富であった。莢膜の厚い胞子には，多形性の細胞質内膜構造がしばしば観察され，いわゆる plasmalemmasome あるいは lomasome と考えられた。