The Wall Particle of the Genus *Cryptococcus*: Large Size and Characteristic Distribution

By K. TAKEO, I. UESAKA AND M. NISHIURA

*Division of Bacteriology, Chest Disease Research Institute,
and Leprosy Research Laboratory, Faculty of Medicine,
Kyoto University, Kyoto, Japan*

(Received 8 March 1974)

A feature of the genus *Cryptococcus* is the production of extracellular substance as a capsule. In this paper we describe a characteristic ultrastructural feature of the wall of the genus *Cryptococcus* and suggest its relation to the ability to produce capsular substances.

METHODS

All but two of the organisms used were kindly supplied by the Institute for Fermentation, Osaka, Japan. They were grown at 25 or 37 °C for 1 to 3 days on a potato-yeast agar supplemented with 10 to 20 % glycerol (Takeo, Uesaka, Uehira & Nishiura, 1973*a*). The growth was transferred directly, or after fixation with glutaraldehyde in the cases of *Cryptococcus neoformans* and *Candida albicans*, to the specimen holder of the apparatus and freeze-fractured or etched. Fixation and other methods were as described previously (Takeo *et al.* 1973*a*).

RESULTS AND DISCUSSION

All eight species and 11 strains of *Cryptococcus* examined showed two strata in the wall. This was not found in any of the 14 other organisms, of eight genera, studied. As shown in Fig. 1(*a*), the outer stratum abounded with particles which were sparse in the inner stratum. These particles were about 20 nm in diameter, i.e. larger than the plasmalemma particle, which was about 10 nm in size (Moor & Mühlethaler, 1963).

The other genera of yeasts observed showed a different arrangement of the wall. As shown in Fig. 1(*b*), particles in the wall were distributed randomly and most were of about the same size as plasmalemma particles. The results obtained are summarized in Table 1.

Most strains of *Cryptococcus* used produced a capsule when grown on the potato-yeast medium. Although taxonomical classification is based on inositol assimilation (Phaff & Fell, 1970), capsule formation is a distinct morphological characteristic which is shared by most strains of this genus (Phaff & Fell, 1970). *Cryptococcus unguiculatus* is known to be non-encapsulated, but it also shows the peripheral accumulation of particles in the wall. However, this organism excreted large amounts of mucous substance into the medium. Since the wall particles of *Cryptococcus* were concentrated in its outer part, we may suppose that they are related to the ability of this genus to produce either capsular or other extracellular material of a polymerized nature (Takeo, Uesaka, Uehira & Nishiura, 1973*b*). This speculation is supported by the fact that *Leucosporidium capsuligenum* also produces a capsule and also possesses the large wall particles. We have not been able to find a structural connexion between these large particles and the capsular microfibrils in specimens prepared by the freeze-etching technique (Takeo *et al.* 1973*a*), although many attempts

Fig. 1. (a) Freeze-fractured *Cryptococcus albidus* showing the wall (W) and external surface of the cell membrane with invaginations (I). Note the accumulation of large particles (arrow) in the outer part of the cell wall. These large particles are also found deep in the cell wall, which indicates that they are not artifacts comprising capsular substance aggregated on the outermost part of the wall. The plasmalemma particles (P) are smaller than the wall particles. The freeze-fracture technique does not reveal the capsule (Takeo *et al.* 1973a). (b) Freeze-fractured *Pichia membranaefaciens* showing the wall (W) and external surface of the cell membrane with invaginations (I). The particles seen in the wall (arrow) are about the same size as the plasmalemma particles (P). Freeze-fracturing was done without any chemical treatment.

Table 1. Size and distribution of particles in the walls of various yeast species

Wall particle larger than plasmalemma particle, and dense in outer part of wall	Wall particle approx. same size as plasmalemma particle, and distributed randomly
<i>Cryptococcus albidus</i>	<i>Torulopsis anomala</i>
<i>C. albidus</i> var. <i>ovalis</i>	<i>T. glabrata</i>
<i>C. laurentii</i>	<i>T. holmii</i>
<i>C. laurentii</i> var. <i>magnus</i>	<i>T. molishiana</i>
<i>C. luteolus</i> (2 strains)	<i>T. spherica</i>
<i>C. neoformans</i> (3 strains)	<i>Rhodotorula glutinis</i>
<i>C. terreus</i>	<i>R. pallida</i>
<i>C. uniguttulatus</i>	<i>R. rubra</i>
	<i>Candida albicans</i>
	<i>Trigonopsis variabilis</i>
	<i>Debaryomyces hansenii</i>
	<i>Hansenula anomala</i>
	<i>Pichia membranaefaciens</i>
	<i>Saccharomyces cerevisiae</i>

have been made to do so. This feature of the wall observed by the freeze-etching technique is a prominent characteristic of the genus *Cryptococcus*, unlike a number of non-capsulated yeasts studied.

The authors thank Professor S. Raffel, Stanford University, for his critical reading of the manuscript.

REFERENCES

- MOOR, H. & MÜHLETHALER, K. (1963). Fine structure in frozen-etched yeast cells. *Journal of Cell Biology* **17**, 609–628.
- PHAFF, H. J. & FELL, J. W. (1970). *Cryptococcus*. In *The Yeasts: A Taxonomic Study*, pp. 1088–1145. Edited by J. Lodder. Amsterdam: North-Holland Publishing.
- TAKEO, K., UESAKA, I., UEHIRA, K. & NISHIURA, M. (1973*a*). Fine structure of *Cryptococcus neoformans* grown *in vitro* as observed by freeze-etching. *Journal of Bacteriology* **113**, 1442–1448.
- TAKEO, K., UESAKA, I., UEHIRA, K. & NISHIURA, M. (1973*b*). Fine structure of *Cryptococcus neoformans* grown *in vivo* as observed by freeze-etching. *Journal of Bacteriology* **113**, 1449–1454.