Ultrastructure of *Candida* Yeasts Grown on *n*-Alkanes

Appearance of Microbodies and Its Relationship to High Catalase Activity* **

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Abstract. Catalase activities of the cells growing on *n*-alkanes of various strains of *Candida* yeasts were markedly higher than those of the cells growing on glucose, ethanol or acetate. In connection with this, electron-microscopical studies revealed abundant appearance of specific microbodies having homogeneous matrix surrounded by single unit membrane in the hydrocarbon-growing cells. Localization of catalase activity in the microbodies, in addition to the mitochondria, was confirmed by cytochemical treatment of the cells with 3,3'-diaminobenzidine reagent.

Key words: Yeast -- Electronmicroscopy -- Microbody -- Peroxisome -- Catalase -- *n*-Alkane -- Utilizing Yeast.

Although extensive studies have been performed concerning the metabolism of hydrocarbon-utilizing microorganisms or production of useful substances by fermentation of hydrocarbons, only a few reports have been published about the ultrastructure of the yeast cells growing on hydrocarbons and its possible relationship to metabolism of these unusual substrates (Ludvik *et al.*, 1968; Meissel *et al.*, 1973).

During the course of studies on the physiology of hydrocarbon-utilizing microorganisms, we found that microbodies having homogeneous matrix surrounded by single unit membrane appeared profusely in the...
cells of various strains of Candida yeasts growing on n-alkanes (Osumi and Fukui, 1972). Such microbodies in plant and mammalian cells have been identified as “peroxisome” and/or “glyoxysome” and catalase has been confirmed as the specific marker enzyme of these organelles (De Duve and Baudhuin, 1966; Tolbert, 1971). On the other hand, we have found that the catalase activities of Candida yeasts growing on n-alkanes are significantly higher than those growing on glucose, especially at their exponential growth phase (Teranishi et al., 1974 b).

This paper deals with the relationship between the appearance of microbodies and the high catalase activity of Candida yeasts growing on n-alkanes.

Materials and Methods

Microorganisms. As the n-alkane-utilizing yeasts, the following strains were used in this study: Candida albicans IFO 0583, C. guilliermondii IFO 0566, C. lipolytica NRRL Y-6795, C. tropicalis IFO 0589 and C. tropicalis pK 233. The following yeasts unable to use hydrocarbons were also used for comparison: Saccharomyces cerevisiae ATCC 7753, Schizosaccharomyces pombe NYCY 132 and Rhodotorula mucilaginosa (a strain identified by Dr. M. Soneda, Nagao Institute, Tokyo, Japan). Candida tropicalis pK 233 (Tanabe et al., 1966) was supplied by the National Fermentation Research Institute, Chiba, Japan. These yeasts were maintained on malt extract-agar slants.

Cultivation of Yeasts. For inocula, yeasts were precultured on malt extract-agar slants for 24 h at 30°C, then transferred into a medium composed of 3.0% of malt extract and cultivated on a rotary shaker (220 rpm) at 30°C for 22 h. After centrifugation, the cells sedimented were resuspended in 25 ml of sterilized water. One ml of the suspension was added to 100 ml of the medium containing different kinds of carbon sources (Table 1) placed in 500 ml shaking flasks and cultured on a rotary shaker (220 rpm) at 30°C. The hydrocarbon substrate used throughout the study was a mixture of n-decane 23.9, n-undecane 46.8, n-dodecane 26.3 and n-tridecane 3.0 by weight.

Electron-Microscopy. Cells were fixed with 2% of glutaraldehyde for 2 h and post-fixed with 1.5% potassium permanganate for 16–22 h. Treatment with glutaraldehyde and osmium tetraoxide is generally used for fixation. However, we found that elimination of cell wall was necessary before the post-fixation with osmium tetraoxide in order to observe clearly the ultrastructure of our materials. Hence, to study the whole structure including cell wall precisely, we employed the glutaraldehyde-KMnO4 treatment in this study. After the fixation, cells were stained with 1.5% uranyl acetate for 1.5 h and dehydrated by serial passage into alcohol or acetone, and the specimens were embedded in a mixture of butyl- and methyl-metacrylate (7:3). Ultrathin sections were made with Porter-Blum ultramicrotome MT-1 type equipped with a glass knife. Sections were picked up on collodion-carbon-coated grids, stained with uranyl acetate for 15 min followed by staining with lead citrate for 1.5 min, and then coated with carbon for supporting them. Electron-micrographs were taken using a JEM-100 B Electron-microscope at 80 to 100 KV, with initial magnification of 2000 to 20000 times.

To detect the localization of catalase activity in the cells, we used 3,3’-diaminobenzidine (DAB) (Novikoff and Goldfischer, 1969). The reaction mixture was as follows: 20 mg of DAB · 4 HCl, 9.8 ml of 0.05 M 2-амино-2-methyl-1,3-propanediol buffer, pH 10, and 1% of H2O2. After the DAB treatment for 30 min, the