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Note

Surface Structure of Some *Candida* Yeast Cells Grown on *n*-Alkanes

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Abstract

The ultrastructure of the cell surface of *Candida tropicalis* and of *C. albicans* growing on *n*-alkanes was investigated by use of field emission scanning electron microscopy. Appearance of curious protrusions of 100–200 nm in diameter was recognized on the surface of the yeast cells. The protrusions, consisting of smaller subunits of about 50 nm in diameter, were scattered at a regular distance on the cell surface. The sectioned view of the cells grown on *n*-alkanes obtained by transmission electron microscopy showed existence of slime-like outgrowths on the periphery of the cell wall. These outgrowths, which were observed as electron-dense layers, developed across the cell wall, making channels connected to the cell membrane. The slime-like outgrowth was supposed to correspond to the above-mentioned protrusion on the cell surface.

Introduction

There have been several papers dealing with the surface structure of yeast cells by use of scanning electron microscopy.[1,2] These results have revealed that all of the *Candida* yeast cells cultivated on carbohydrates show smooth surface. During the course of our studies concerning the relationship between the function and structure of the *n*-alkane-utilizing yeast cells,[3~7] many interesting features were observed on the physiological activities and the ultrastructure of the cells: Morphological change depending upon the chain length of *n*-alkane substrate, development of microbodies relevant to a marked increase of catalase activity, and so on.

This paper deals with an investigation on the fine structure of the cell surface of *Candida tropicalis* and of *C. albicans* growing on *n*-alkanes by use of field emission scanning electron microscopy, a new technique for the investigation of the cell surface. In connection with this experiment, the structure of the cell wall is examined by transmission electron microscopy.

Materials and Methods

Candida tropicalis pk 233[8] and *C. albicans* IFO 0583 were cultivated aerobically in a medium containing a *n*-alkane mixture (C_{10-13}) as described previously,[3] and harvested at the exponential growth phase (8 hr or 16 hr).

Specimens for field emission scanning electron microscopy were prepared as follows. Yeast cells were fixed with glutaraldehyde-OsO_4, dehydrated with acetone and amyl acetate, and then treated in carbon dioxide with acetone and amyl acetate, and then treated in carbon dioxide according to the critical point drying method of Anderson.[9] The specimens were viewed with a Hitachi HFS-1 field emission scanning electron microscope. The details of the procedure will be published elsewhere.

For the specimens of transmission electron microscopy, yeast cells were fixed with glutaraldehyde-$KMnO_4$ as reported previously.[4] Sectioned view was observed with a JEM 100B electron microscope.

Results and Discussion

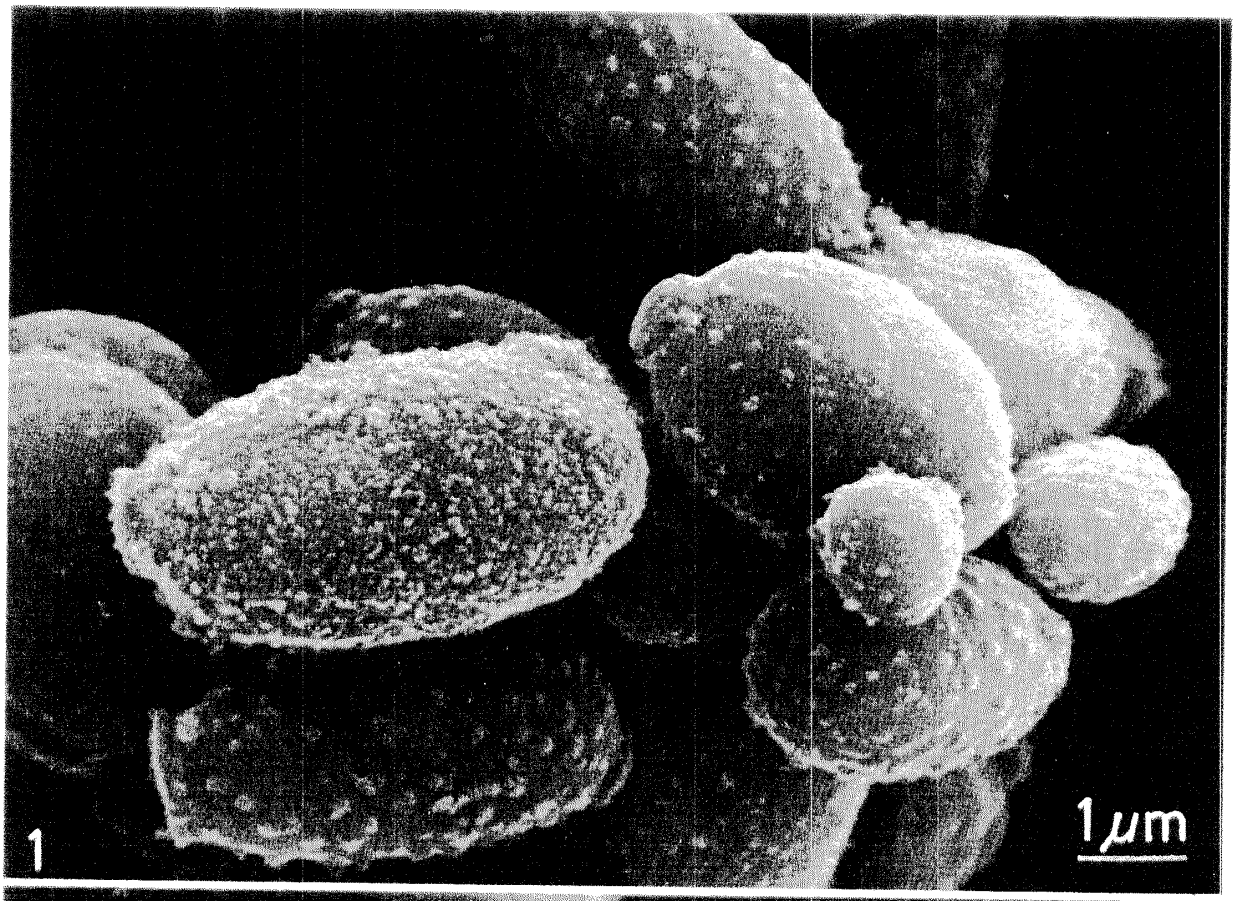
Figure 1 shows the surface of *Candida tropicalis* pK 233 cells grown on a *n*-alkane mixture (C_{10-13}) for 8 hours observed by field emission scanning electron microscopy. The most striking and interesting feature was appearance of curious protrusions on the surface. These protrusions, being about 100–200 nm in diameter, were uniformly scattered on the cell surface. A further enlarged picture (Fig. 2) revealed that the protrusions were composed of a group of subunits of about 50 nm in diameter. Such warty surface could be seen only on the cells growing exponentially in the *n*-alkane medium. When the yeast was cultivated on *n*-alkanes for a longer period, the cells showed smooth surface. Namely, disappearance of the protrusions occurred along with exhaustion of *n*-alkanes in the medium. When *n*-alkanes were added to the later phase culture and incubated for an appropriate period, the protrusions appeared again. The protrusions on the cell surface did not disappear even when the cells had been washed several times with 1% Tween 80 solution, 50% ethanol or *n*-hexane. This fact indicates that the protrusions were not small particles of *n*-alkanes attached to the cell surface.

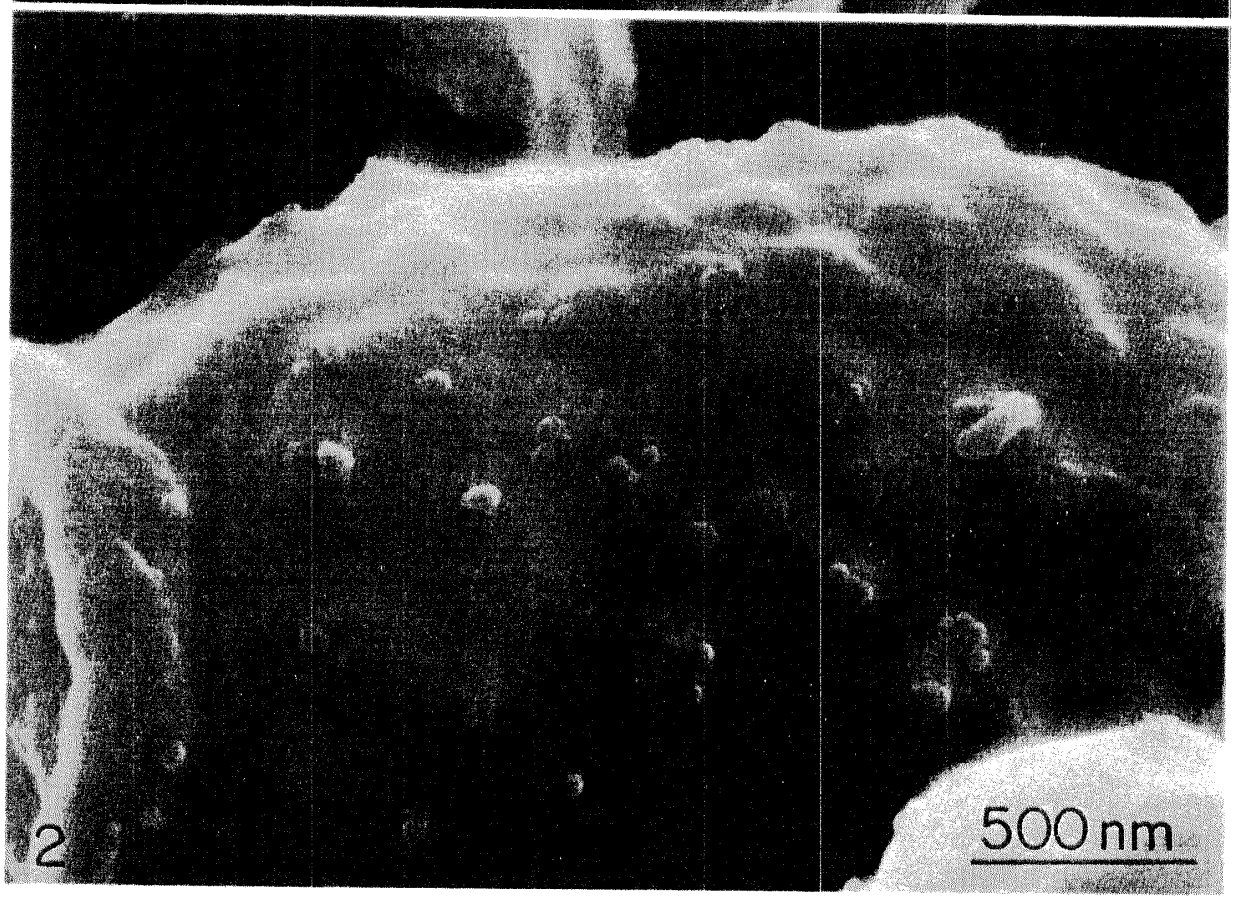
Similar protrusions were also found on the cell surface of *Candida albicans* IFO 0583 grown on *n*-alkanes, but not on the surface of neither *C. tropicalis* and *C. albicans* grown on glucose nor any other *n*-alkane-nonutilizable yeast cells, such as *Saccharomyces cerevisiae*, grown on glucose.

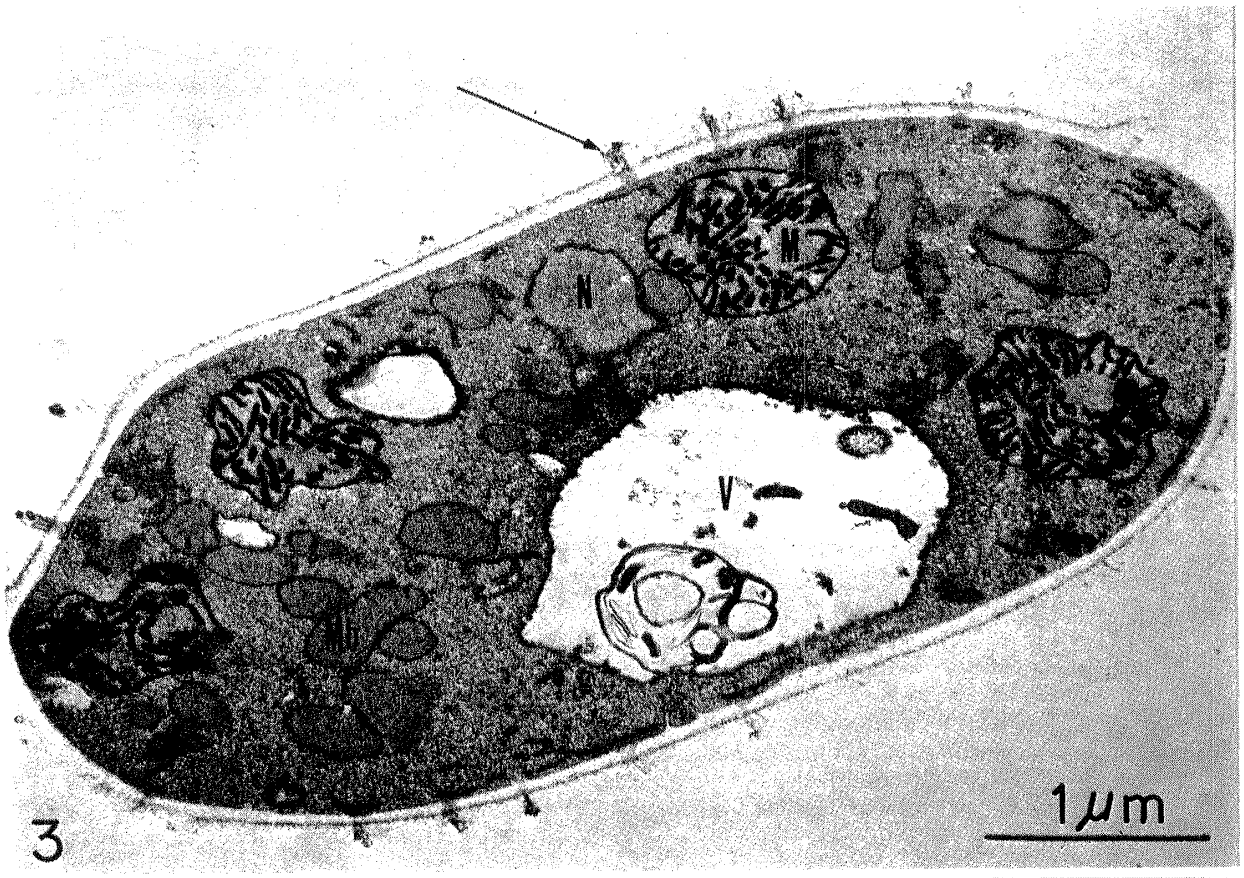
In the thin sections of the *C. tropicalis* cells viewed with the transmission electron microscope, outgrowths of slime-like substance, about 150 nm long and 200 nm wide, were recognized on the cell wall of the cells of exponential growth phase on *n*-alkanes (Fig. 3). Each of the outgrowths, which showed electron-dense layers, passed through the cell wall and connected to the cell membrane, forming a sort of a channel (Fig. 4). Beneath each channel, endoplasmic reticulum was recognized close to the cell membrane. This structure could not be observed in any yeast cells grown on glucose. In the case of the late phase culture of *C. tropicalis* growing on *n*-alkanes, the periphery of the cell wall looked homogeneous in electron density and the outgrowths were not recognized in accord with the disappearance of the protrusions. It seems likely that the slime-like outgrowth corresponds to the protrusion observed in field emission scanning electron microscpoy.

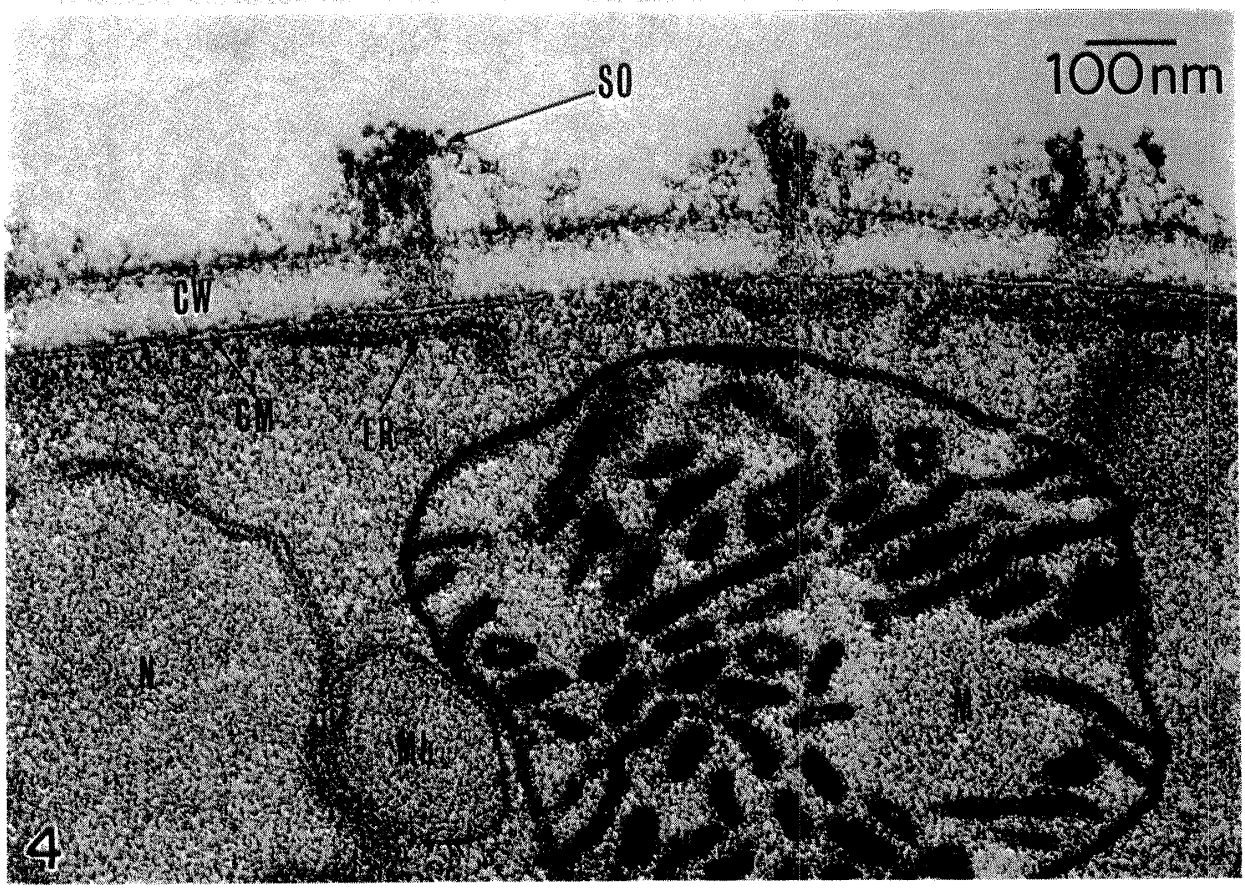
The electron microscopical studies presented here would provide new and interesting information concerning the structure of cell surface as welll as cell wall of *C. tropicalis* and of *C. albicans* growing on *n*-alkanes. By means of fluorescence microscopy, Meissel *et al.* [10] observed the penetration of polycyclic aromatic hydrocarbons having strong fluorescence into yeast cells through cell wall. Based on this observation, they assumed that this phenomenon would be the case for *n*-alkane. It is of great interest that analogous slime-like outgrowths were recognized on the section of the cell wall of *C. tropicalis* grown on *n*-alkanes (See, Fig. 4).

There might be an intimate relationship between the uptake of *n*-alkanes and appearance of the protrusions or slime-like outgrowths on *Candida* yeast cells.









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Legends to Figures

Fig. 1. Surface of *Candida tropicalis* pK 233 grown on *n*-alkanes for 8 hr.
Fig. 2. Higher view of protrusions of *Candida tropicalis* pK 233 grown on *n*-alkanes for 8 hr.
Fig. 3. Section of *Candida tropicalis* pK 233 cell grown on *n*-alkanes for 16 hr. Key to abbreviations: CM, cell membrane; CW, cell wall; ER, endoplasmic reticulum; M, mitochondrion; Mb, microbody; N, nucleus; SO, slime-like outgrowth; and V, vacuole.
Fig. 4. Section of *Candida tropicalis* pK 233 cell grown on *n*-alkanes for 16 hr.