Short Communication

ISOLATION AND PROPERTIES OF LARGE CELL STRAINS FROM A METHANOL-UTILIZING YEAST, *CANDIDA* SP. N-16 BY COLCHICINE TREATMENT

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It is well known that some of practical yeasts are high ploidy. The increment of ploidy leads to a larger cell than that of a wild strain. The growth of methanol-utilizing yeasts is accompanied by the development of microbodies in cells grown on methanol, and by mitochondria in cells grown on ethanol. These organelles concern the initial oxidative metabolism of alcohols (6). It is interesting to investigate the changes of enzyme activities and the profiles of organelles in high-ploidy cells. In a previous study (3), we described the isolation of some stable intraspecific hybrids from a methanol-utilizing yeast, *Candida* sp. N-16, through protoplast fusion. The DNA contents and cell volumes of the fusants obtained were much larger than that of the wild strain. The levels of alcohol-dissimilating enzymes in the fusant cells grown on methanol were higher than those of the wild strain. Colchicine is often used as an agricultural chemical to produce plants which have a high ploidy (4,5). It seems that a colchicine treatment of cells is an easy method for breeding a microorganism possessing a high ploidy. However, there are very few reports on effective isolation of high-ploidy cells from industrial yeasts by colchicine treatment. We obtained large cell strains having high growth rate, high ploidy and a huge vacuole by a simple method that involved isolation of large colonies from small colonies developed after wild cells treated with colchicine were spread on an agar plate and incubated. This note deals with the isolation of large cell strains from *Candida* sp. N-16 by colchicine treatment and some properties of these strains.

The wild-type strain of *Candida* sp. N-16 was originally obtained from National Institute of Bioscience and Human-Technology, Tsukuba, Japan. The yeast was grown on a basal salt medium containing 1% (w/v) methanol at 30°C as

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described previously (1). Large cell strains (Col-1-7) were derived from the wild-type strain by colchicine treatment as follows. Colchicine was added to the 18 h culture of the wild strain to give a final concentration of 0.05%. The culture was incubated for further 18 h. After treatment, cells were spread over 1% methanol agar plates and incubated for about 2 weeks at 30°C. Random observation of colonies developed on the agar plates showed that large colonies were always composed of larger cells than that of small colonies. So large colonies were selected for isolates. These isolated colonies were spread over the agar plates again, and the same colony selection was repeated once more. Consequently, 7 isolates consisting of large cells were obtained from about 2,000 initial large colonies. No large cell strains were obtained from the wild strain by incubation in the absence of colchicine. The growth of the wild and isolated strains was estimated by measuring the optical density at 610 nm. The DNA contents of cells were determined by measuring the absorbance at 260 nm, using herring sperm DNA as a standard.

Fig. 1. Micrographs of large cell strains obtained by colchicine treatment and wild strain of *Candida* sp. N-16 grown on the solid medium containing methanol.

Cells were incubated for 2 weeks on the solid medium containing 1% (w/v) methanol as a carbon source at 30°C. Bars in photographs indicate 30 μ m of length. A, wild strain; B, large cell strain Col-5; C, Col-6; D, Col-7.

DNA of the cells was extracted according to the method described previously (3). Protoplasts were prepared as described previously (3). The alcohol oxidase (EC 1.1.3.13) activity was assayed according to the method described in the previous paper (1).

Cells of the isolated strains (Col-5–7) became larger and more spherical shape than those of the wild strain under various growth conditions. Particularly, the strong tendency appeared when these strains were incubated on the agar plates. The vacuoles in these cells developed remarkably (Fig. 1). Similar results were also obtained with other strains (Col-1–4, data not shown). After 48 h incubation, cell sizes and DNA contents of these strains were examined (Table 1). The cell sizes and DNA contents were compared by measuring the protoplast sizes. The volumes of the protoplasts of the large cell strains and their DNA contents were about 2.9–3.9 and 1.4–2.1 times larger than those of the wild strain, respectively. It was confirmed that the growth rates of these strains were higher in a liquid culture than that of the wild strain in the 1% methanol liquid medium. Furthermore, levels of methanol-dissimilating enzymes in these strains were investigated. It was found that there were differences concerning the level of the alcohol oxidase activity between the isolated large cell strains and the wild strain (Table 2).

There are very few reports on effective isolation of high-ploidy cells from industrial microorganisms by colchicine treatment. Toyama and Toyama (7) reported the isolation of polyploidy strains from *Trichoderma reesei* by colchicine treatment and the enhancement of cellulase-production by the isolates. They described that the colchicine treatment would be applicable to the strain-improvement of fungi. However, it has not been reported that a unique strain remarkably developing a vacuole is obtained from yeasts by colchicine. This note

Table 1. Protoplast size and DNA content of large cell strains isolated from *Candida* sp. N-16 treated with colchicine.

Strain	Protoplast ^a size			Ratio of DNA content
	Diameter (μm)	Volume (μm^3)	SD ^b	
Wild strain	2.71 (1.5–4.1)	10.4 (1.77–36.1)	0.52	100 (42.8 $\mu\text{g}/10^9$ cells)
Isolate (Large cell strain)				
Col-1	4.14 (2.0–6.1)	37.2 (4.19–119)	1.04	141
Col-2	3.97 (2.0–5.6)	32.8 (4.19–92.0)	0.76	147
Col-3	4.27 (2.6–6.1)	40.8 (9.20–119)	0.76	193
Col-4	4.22 (3.1–5.6)	39.4 (15.6–92.0)	1.04	144
Col-5	4.04 (2.6–5.6)	34.5 (9.20–92.0)	1.04	156
Col-6	3.88 (3.1–5.1)	30.6 (15.6–69.5)	1.04	208
Col-7	4.03 (3.1–5.6)	34.3 (15.6–91.2)	1.04	166

^a Protoplasts were prepared from cells incubated for 48 h in the salt medium containing 1% (w/v) methanol at 30°C.

^b Standard deviation.

Table 2. Growth and alcohol oxidase activity of large cell strains isolated from *Candida* sp. N-16 treated with colchicine.

Strain	Growth ^a rate (h ⁻¹)	Final cell concentration (OD 610nm)	Alcohol oxidase activity ^b (units/mg protein)
Wild strain	0.066	12.0	1.21
Isolate (Large cell strain)			
Col-1	0.096	13.9	1.67
Col-2	0.073	13.8	0.73
Col-3	0.087	16.0	1.30
Col-4	0.087	15.2	1.35
Col-5	0.093	15.9	1.12
Col-6	0.093	13.1	2.03
Col-7	0.082	14.9	1.75

^a Cells were incubated in the medium containing 1% (w/v) methanol at 30°C.

^b The activity of alcohol oxidase was measured with cell-free extracts prepared from cells incubated for 42 h.

shows that the large cell strains possessing the high growth rate and a huge vacuole were easily obtained by the isolation of large colonies developed from cells of a methanol-utilizing yeast, *Candida* sp. N-16 treated with colchicine. It seems that the large colonies are due to the higher growth rate of the colchicine-treated cells. But causal relation between alcohol oxidase levels in the isolates and their growth rates has not been confirmed. An unusual microbody divided by a huge vacuole was sometimes observed in cells of the isolates (data not shown). Further investigations are being attempted to define the development of microbodies and huge vacuoles in cells of the isolated strains, and as to whether the colchicine treatment is a useful procedure for the strain-improvement of yeasts or not.

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