Production of Yeast Cells from Methanol

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In recent years, many studies on yeast cell production from methanol have been reported.¹⁻⁴⁾ The present authors isolated newly several methanol-utilizing yeasts from natural sources and selected one strain from them based on growth rate and cell yield. This paper deals with isolation and identification of methanol-utilizing yeast, some cultural conditions and the components of the cell.

To isolate methanol-utilizing yeasts from natural sources, soil or natural materials were added in the large test tube containing the isolation medium and were shaken for four or seven days at 30°C. The isolation medium contained 10 g methanol, 3 g (NH₄)₂SO₄, 2 g KH₂PO₄, 0.4 g MgSO₄·7H₂O, 0.01 g FeSO₄·7H₂O, 0.01 g CaCl₂·2H₂O, 50 γ biotin, 500 γ thiamine-HCl, 1 g yeast extract and 0.3 g streptomycin in 1000 ml tap water (pH 5.5). The enrichment culture was repeated four times. Then, 0.1 ml of the shaken media was spread on agar plates containing the isolation medium to form yeast colonies.

For the test of cell production, 5 ml seed culture broth was transferred into 50 ml of the medium in a 500 ml shaking flask and incubated for 24 to 60 hr at 30°C on reciprocal shaker. Cell growth was determined by measuring optical density at 660 nm and weighing dried cells.

Residual methanol concentration was analyzed by gas-liquid chromatography under the following conditions: column, PEG 1000 3 mm×1 m of stainless

steel; temperature, isothermal at 100°C; carrier gas, helium; carrier gas flow rate, 40 ml/min; detector, TCD; injection, 5 μl of broth; and equipment, Shimadzu Gas Chromatography GC-3AH.

Thirty-two strains were isolated from about 1500 samples of natural materials, such as soil, sewage and rind of fruit. Eight strains showed good growth on methanol. The most excellent utilizer was selected from three strains having high growth rate and cell yield by batch culture using jar fermenter. Operation conditions of jar fermenter were as follows, working volume, 16 liters; cultural temperature, 30°C; aeration, 16 liters/min; agitation speed, 400 rpm; pH, 5.5; inoculum size, 500 ml of seed culture and cultivation time, 40 hr. pH was adjusted by adding NH₄OH.

Table I indicates that strain F433 is inferior to other strains, F432 and S773. Although F432 showed growth as well as S773 in this experiment, S773 was selected as the most excellent strain, considering the result of the experiment using 500 ml shaking flask. It was found that more than 20% of fed methanol escaped in vapor when initial methanol concentration was 1% (w/w). But when its concentration was 0.5%, lost methanol decreased considerably.

Maximum cell yield was obtained under the following conditions, cultivation temperature, 26.5°C; pH, 4~6; methanol concentration, 0.5% (w/w); corn steep liquor concentration, 2 g/liter; nitrogen sources, 3 g (NH₄)₂SO₄. Maximum cell yield and specific growth rate were 49.5% (w/w) and 0.2 hr⁻¹, respectively.

Strain S773 could not grow on methanol at the concentration more than 3% and its cell yield to fed methanol increased with decreasing of methanol concentration. This strain required natural nutrients for the growth. The sufficient amount of yeast extract, corn steep liquor and whisky soluble for the maximum growth of the strain were 1 g/liter, 2 g/liter and 2 g/liter, respectively.

The typical time course showing growth and methanol consumption under pH 5.5 is presented in Fig. 1. Exponential growth was observed through cultivation when initial concentration was 0.5%.

When yeast is used as feed or food supplement, it

TABLE I. SELECTION TEST OF YEAST FOR CELL PRODUCTION
BY BATCH CULTURE WITH JAR FERMENTER

Strain No.		S773	S773	F432	F433
Fed methanol	(g)	157	79	157	157
Recovered methanol	(g)	36	10	37	34
Consumed methanol	(g)	121	69	120	123
Cell dry weight	(g)	47	31	41	46
Cell yield	(wt %)	39	45	34	35
Specific growth rate	(hr ⁻¹)	0.14	0.2	0.15	0.11

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FIG. 1. Typical Growth Curve of Strain S773.

●—●, Cell concentration.

○—○, Residual methanol concentration.

is desired for yeast cell to have not only high cell yield and growth rate but also high protein content and low RNA content. Crude protein and RNA contents were determined by semimicro-Kjeldahl method and Schmidt-Thanhauser-Schneider method, respectively. The crude protein and RNA contents in strain S773 were 45.7% and 3.4%, respectively. The former was as much as other methanol yeasts and hydrocarbon yeasts, however, the latter was less than *Kloeckera* sp. reported by Ogata *et al.*¹⁾ and strain DL-1 reported by Levine and Cooney.⁴⁾

As strain S773 was comparatively excellent as single cell protein producer, taxonomical studies of the strain were carried out. From the results of morphological and physiological studies, it was identified as close resembling *Candida boidinii*, as shown in Table II. But it differed from *Candida boidinii* in the following points, 1) Succinic acid was utilized but *dl*-lactic acid was not. 2) Maximum temperature of the growth was 40°C.

TABLE II. TAXONOMICAL STUDIES OF STRAIN S773

Growth in malt extract broth: After two days at 25°C, cells were long ovoid to cylindrical (3.0~5.0) × (6.0~10.0) μ, isolated or in pairs.	
Growth on malt extract agar: After two days at 25°C, colonies were white colored, raised and smooth.	
Ascospore formation:	negative
Ballistospore formation:	negative
Pseudo-true mycelium formation:	positive
Fermentation:	
Glucose	positive
Galactose, Maltose, Lactose,	
Raffinose, Trehalose	negative
Assimilation of carbon compound:	
Glucose, Mannose, Fructose, Xylose, Mannitol,	
Dextrin, Glycerin, Ethanol, Succinic acid	positive
Galactose, Lactose, Maltose, Raffinose, Sucrose,	
Trehalose, Methyl glucoside, Mellibiose, Arabi-	
nose, Soluble starch, Inulin, Salicin, <i>dl</i> -Lactic	
acid, Citric acid	negative

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