Note

Effect of Methanol Concentration on Levels of Methanol Dissimilating Enzymes in *Candida* sp. N-16Takaaki FUJII, Tsutomu MURAKAMI,
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Received January 24, 1984

The growth of methanol-utilizing yeasts is extremely inhibited when they are incubated in a medium containing an excess amount of methanol as a sole source of carbon.¹⁻⁵⁾ However, there is little information on enzyme levels in these yeasts concerned with the growth-inhibition by methanol. This note deals with the changes of activities of methanol dissimilating enzymes in cells of *Candida* sp. N-16 incubated in the presence of a high concentration of methanol.

The yeast was grown on a salt medium containing methanol and/or glucose at various concentrations at 30°C as described previously.⁶⁾ After incubation, cells were harvested by centrifugation, washed twice with 50 mM potassium phosphate buffer, pH 7.2, resuspended in 10 ml of the same buffer to a concentration of about 1.0 g (wet wt.) per ml, and then sonicated for periods of 2 min in a 200 W Ohtake ultrasonic disintegrator at 20 kHz. The total exposure time was 6 min. The sonic extracts were centrifuged at 10,000 × *g* for 30 min at 4°C. The resultant supernatant was stored in ice and assayed for enzyme activities. Alcohol oxidase, formaldehyde dehydrogenase and formate dehydrogenase were assayed essentially according to the methods described in the previous paper.⁶⁾ One unit of an enzyme is defined as the amount of the

enzyme catalyzing the transformation of 1 μmol of substrate or the formation of 1 μmol of product per 1 min. Catalase was assayed by the spectrophotometric method of Lück.⁷⁾ Catalase activity is expressed as Δ*E*₂₄₀ per min. Protein concentration in cell-free extracts was determined by the method of Lowry *et al.*,⁸⁾ using bovine serum albumin as the standard.

The changes of the growth of *Candida* sp. N-16 and its enzyme activities were investigated after methanol was added at different concentrations to batch cultures incubated in the 1% methanol salt medium for 40 hr. The concentration of residual methanol was about 0.6% at that time. When 1% methanol was added to growing cultures, the growth of the yeast progressed normally although a little decrease in growth rate was observed for a time after the addition of methanol. In the case of the addition of 3% methanol, the growth rate decreased considerably, but cells kept on growing and the final cell concentration was higher than that of the 1% methanol-fed culture. However, when 5% methanol was added, the growth stopped immediately. Activities of methanol dissimilating enzymes were assayed in cells from these methanol-fed cultures. Table I shows specific activities in cell-free extracts prepared from cells after 6 hr incubation after the methanol-addition. With the addition of 3% methanol, the specific activity of alcohol oxidase fell to 20% of the control value (no addition). On the other hand, activities of catalase, formaldehyde dehydrogenase and formate dehydrogenase remained at constant levels. No decrease in catalase activity was observed even on the addition of 5% methanol. These results indicate that alcohol oxidase is more easily inactivated by a high concentration of methanol than the other three enzymes.

Sahm and Wagner⁹⁾ reported that *Candida boidinii* showed diauxic growth in a medium containing both methanol and glucose as carbon sources. Figure 1 shows the growth of *Candida* sp. N-16 on a glucose-methanol medium, with methanol present at various concentrations (1 to 5%). The yeast was able to grow in all cultures tested. However, when the concentration of methanol was increased to about 4%, stopped after the

TABLE I. EFFECT OF METHANOL-ADDITION ON ACTIVITIES OF METHANOL DISSIMILATING ENZYMES IN GROWING CELLS OF *Candida* sp. N-16

Methanol was added to cultures growing in the 1% methanol-salt medium at the mid-exponential growth phase. Cells were harvested at 6 hr after methanol-addition.

Final concentration of methanol added (%)	Enzyme activities (units/mg protein)			
	Alcohol oxidase	Catalase	Formaldehyde dehydrogenase	Formate dehydrogenase
No addition	0.750	9.92	0.954	0.229
1	0.674	10.3	0.987	0.229
3	0.179	11.1	1.29	0.287
5	0.189	11.6	0.579	0.197

TABLE II. CHANGE IN ACTIVITIES OF METHANOL DISSIMILATING ENZYMES
IN *Candida* sp. N-16 GROWN ON GLUCOSE AND METHANOL

Culture conditions are given in Fig. 1. Cells were harvested at the cultivation times indicated.

Growth substrate	Cultivation time (hr)	Enzyme activities (units/mg protein)			
		Alcohol oxidase	Catalase	Formaldehyde dehydrogenase	Formate dehydrogenase
0.5% Glucose	16	0.021	0.627	0.106	0
0.5% Glucose + 1% methanol	16	0.019	0.696	0.157	0
	23	0.018	0.602	0.110	0
	43	0.449	18.4	0.793	0.285
	77	0.405	7.90	1.08	0.337
0.5% Glucose + 4% methanol	23	0.014	1.09	0.249	0
	28	0.025	0.584	0.127	0
	70	0.028	7.76	0.487	0.112

FIG. 1. Growth of *Candida* sp. N-16 in Glucose Medium Containing Methanol at Various Concentrations.

Cells were incubated in the medium containing glucose and methanol at the concentrations indicated. Culture conditions are given in the text.

○—○, 1.0% glucose; ●—●, 0.5% glucose; △—△, 0.5% glucose + 1.0% methanol; ▲—▲, 0.5% glucose + 4.0% methanol; □—□, 0.5% glucose + 5.0% methanol.

cell-concentration reached the value expected from the consumption of glucose. Diauxic growth was observed when 1% methanol was added. When the yeast was incubated in the presence of 3% methanol, its growth also showed a diauxic curve (data not shown). Specific activities of methanol dissimilating enzymes in cells from these cultures were examined. Table II shows the results obtained for cells grown on 0.5% glucose–1% methanol and 0.5% glucose–4% methanol. The enzyme

activities remained at low levels while the yeast utilized glucose. When glucose had completely disappeared from the medium, the activities of catalase, formaldehyde dehydrogenase and formate dehydrogenase began to increase regardless of the concentration of methanol. On the other hand, a rise in the activity of alcohol oxidase was not observed in cells incubated in the presence of 4% methanol. These results indicate that the high concentration of methanol (over about 4%) gave rise to inhibition of alcohol oxidase synthesis.

Alcohol oxidase and catalase are located in microbodies as described in previous papers.^{10–15)} The mode of induction (or derepression) of these enzymes is different from that of formaldehyde dehydrogenase and formate dehydrogenase, which are located in the cytosol. Furthermore, it is known that the mode of degradation of the former enzymes is different from that of the latter, when cells grown on methanol are transferred to a medium containing the other substrates. However, there are certain discrepancies in the published data relating to regulation of the synthesis or the degradation of these four enzymes.^{16–22)} Our results demonstrate that alcohol oxidase is the most sensitive to methanol-inhibitory effects among methanol dissimilating enzymes.

Acknowledgments. We thank the Fermentation Research Institute for providing us with *Candida* sp. N-16.

REFERENCES

- 1) H. Asthana, A. E. Humphrey and V. Moritz, *Biotechnol. Bioeng.*, **13**, 923 (1971).
- 2) P. Pilát and A. Prokop, *Biotechnol. Bioeng.*, **17**, 1717 (1975).
- 3) S. Goto, R. Okamoto, T. Kuwajima and A.

- Takamatsu, *J. Ferment. Technol.*, **54**, 213 (1976).
- 4) J. R. Swartz and C. L. Cooney, *Appl. Environ. Microbiol.*, **41**, 1206 (1981).
- 5) T. Urakami, *Hakkokogaku*, **59**, 1 (1981).
- 6) T. Fujii and K. Tonomura, *Agric. Biol. Chem.*, **36**, 2297 (1972).
- 7) Lück, "Methods of Enzymatic Analysis," ed. by H. U. Bergmeyer, Academic Press, New York, London, 1963, pp. 885~894.
- 8) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
- 9) H. Sahm und F. Wagner, *Arch. Mikrobiol.*, **90**, 263 (1973).
- 10) J. P. van Dijken, M. Veenhuis, C. A. Vermeulen and W. Harder, *Arch. Microbiol.*, **105**, 261 (1975).
- 11) S. Fukui, S. Kawamoto, S. Yasuhara and A. Tanaka, *Eur. J. Biochem.*, **59**, 561 (1975).
- 12) R. Roggenkamp, H. Sahm, W. Hinkelmann and F. Wagner, *Eur. J. Biochem.*, **59**, 231 (1975).
- 13) H. Sahm, R. Roggenkamp and F. Wanger, *J. Gen. Microbiol.*, **88**, 218 (1975).
- 14) J. Voříšek and O. Volfová, *FEBS Lett.*, **52**, 246 (1975).
- 15) M. Veenhuis, J. P. van Dijken and W. Harder, *Arch. Microbiol.*, **111**, 123 (1976).
- 16) R. Roggenkamp, H. Sahm and F. Wagner, *FEBS Lett.*, **41**, 283 (1974).
- 17) C. Bormann and H. Sahm, *Arch. Microbiol.*, **117**, 67 (1978).
- 18) M. Veenhuis, K. Zwart and W. Harder, *FEMS Microbiol. Lett.*, **3**, 21 (1978).
- 19) T. Egli, J. P. van Dijken, M. Veenhuis, W. Harder and A. Fiechter, *Arch. Microbiol.*, **124**, 115 (1980).
- 20) T. Egli, Käppeli and A. Fiechter, *Arch. Microbiol.*, **131**, 8 (1982).
- 21) T. Egli, *Arch. Microbiol.*, **131**, 95 (1982).
- 22) T. Egli, Th. Haltmeier and A. Fiechter, *Arch. Microbiol.*, **131**, 174 (1982).
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