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MORPHOLOGICAL CHANGE IN *CANDIDA TROPICALIS* pK 233 CAUSED BY ETHANOL AND ITS PREVENTION BY MYO-INOSITOL

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SUMMARY

The cells of *Candida tropicalis* pK 233 grew in filamentous form when cultivated in a synthetic medium supplemented with ethanol. The ethanol-grown cells excreted significant amounts of polysaccharides into culture medium. Myo-inositol added simultaneously with ethanol prevented both the morphological change and the extracellular production of polysaccharides.

The cells of *Candida* yeasts, especially *C. albicans*, are known to grow in filamentous (or mycelial) form under several conditions [1-5]. The morphological change is of interest since the phenomenon would be closely related to the mechanism and regulation of cell growth and to the structure and function of cytoplasmic membrane and cell wall. Hirai *et al.* [6] reported that the cells of *C. tropicalis* pK 233, a hydrocarbon-utilizing yeast, underwent the morphological change when grown in a hydrocarbon medium supplemented with corn steep liquor.

This communication deals with the development of filamentous form in *C. tropicalis* pK 233 cells growing on glucose in a synthetic medium containing ethanol. Myo-inositol was found to abolish the effect of ethanol. Extracellular production of polysaccharides occurring concomitantly with the morphological change is also described.

MATERIALS AND METHODS

C. tropicalis pK 233 cells precultured in a malt extract medium were grown at 30° C with vigorous shaking in a synthetic medium (pH 6.0) containing (per 100 ml) : glucose 1.65 g, $(NH_4)_2SO_4$ 500 mg, KH_2PO_4 170 mg, Na_2HPO_4 448 mg, $MgSO_4 \cdot 7H_2O$ 12.5 mg, $MnSO_4 \cdot 4H_2O$ 0.25 mg, KCl 42.5 mg, $FeCl_3 \cdot 6H_2O$ 0.25 mg, casamino acids 500 mg and biotin 0.6 μg. Varying amounts of ethanol and myo-inositol were added when necessary. The addition of ethanol was done aseptically after autoclaving the other components of medium. Growth of cells was

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measured turbidimetrically at 430 nm and turbidity was converted to dry cells per ml of culture. The morphological change of cells was examined under a microscope. Cellular myo-inositol was determined by bioassay using *Saccharomyces carlsbergensis* 4228 (ATCC 9080). Cellular polysaccharides (glycogen, glucan and mannan) were fractionated according to the method of Trevelyan and Harrison [7,8] and assayed by an anthrone method. Extracellular polysaccharides were precipitated by adding ethanol to the culture at a final concentration of 70 % and assayed in the same way as above.

RESULTS

The cells of *C. tropicalis* pK 233 exhibited filamentous form when grown with ethanol in a synthetic medium containing glucose as carbon source (Table 1). With 1 % ethanol, only a small number of cells grew in filamentous form and the majority maintained yeast-like growth. Substantially all the cells were found to be filamentous in the presence of more than 1.5 % ethanol. Cell growth was also affected by ethanol as shown in the table. However, the morphological change would be independent of the inhibition of growth since all cells grew in yeast-like form in the presence of other alcohols such as methanol, n-propanol, iso-propanol and n-butanol, all of which also inhibited cell growth (data not shown).

Table 2 shows that myo-inositol added with ethanol prevented the morphological change caused by ethanol. Upon addition of a physiologically significant amount of myo-inositol (1.3 μg per ml), a considerable number of cells grew in yeast-like form even in the presence of enough ethanol (1.5 %) to cause the morphological change in all cells. The proportion of cells in yeast-like form increased with increasing myo-inositol concentration and 5.0 μg myo-inositol per ml permitted all cells to grow in yeast-like form. At a higher concentration of ethanol (2.5 %), 2.5 μg myo-inositol per ml was required for the occurrence of yeast-like cells. All cells grew in yeast-like form with 5.0 μg myo-inositol per ml. Both ethanol and myo-inositol had no effects on the morphology of cells when added after cell growth started. This indicates that dimorphism is governed by some events occurring before or immediately after the beginning of growth.

Table 1 Influence of ethanol on the growth and morphology of *C.tropicalis* pK 233

Ethanol concentration (%)	Growth (mg dry cells per ml)	Cell form
0	9.0	Yeast-like
0.5	8.4	Yeast-like
1.0	7.8	Filamentous (Yeast-like)*
1.5	5.2	Filamentous
2.0	5.1	Filamentous
2.5	4.9	Filamentous

Cells were grown for 60 hours. Other conditions were the same as described in Materials and Methods.
* Most cells grew in filamentous form but a small number of cells maintained yeast-like growth.

Cell growth in filamentous form was accompanied by extracellular production of polysaccharides. As shown in Table 3, significant amounts of glycogen, glucan and mannan were detected in the filtrate of the culture growing in filamentous form with ethanol. The myo-inositol-supplemented cells, as well as the cells grown in the absence of ethanol, did not produce these polysaccharides extracellularly. Intracellular contents of the polysaccharides were not so much affected by the ethanol-induced morphological change.

Table 2 Effect of myo-inositol on the morphological change caused by ethanol

Ethanol (%)	Myo-inositol (μg per ml)	Cell form
0	-	Yeast-like
1.5	0	Filamentous
	1.3	Filamentous (Yeast-like)*[1]
	2.5	Yeast-like (Filamentous)*[2]
	5.0	Yeast-like
2.5	0	Filamentous
	1.3	Filamentous
	2.5	Filamentous (Yeast-like)*[1]
	5.0	Yeast-like

Culture conditions were the same as those in Table 1.
*[1] See the footnote of Table 1.
*[2] Most cells maintained yeast-like growth.

Table 3 Effect of ethanol and myo-inositol on intra- and extracellular polysaccharide contents

Additions to medium	Cell form	Polysaccharide contents*					
		Glycogen		Glucan		Mannan	
		Intra-cellular	Extra-cellular	Intra-cellular	Extra-cellular	Intra-cellular	Extra-cellular
None	Yeast-like	320	0	118	0	70	0
Myo-inositol	Yeast-like	335	0	140	0	95	0
Ethanol	Filamentous	445	20	230	47	40	55
Ethanol and myo-inositol	Yeast-like	555	0	143	0	68	0

* Intra- and extracellular contents are expressed as mg per g dry cells and mg per ml, respectively.
Culture conditions were the same as those in Table 1 except that cultivation was carried out for 48 hours.

DISCUSSION

As reported by Hirai *et al.* [6], the cells of *C. tropicalis* pK 233 exhibited filamentous form in a hydrocarbon medium supplemented with corn steep liquor and no morphological change occurred when glucose was used as carbon source in place of hydrocarbons. Furthermore, the present authors found that glucose repressed the hydrocarbon-induced dimorphism resulting in yeast-like growth of cells even in the presence of hydrocarbons (unpublished data).

This communication clearly demonstrated that ethanol caused the occurrence of filamentous growth in a defined medium containing glucose as carbon source. The mechanism of the effect of ethanol still remains to be elucidated. Action of ethanol as solvent would not be important in the event since no other alcohols caused the morphological change in this yeast. As mentioned above, something occurring before or immediately after the beginning of growth determines the form of cells. A sufficient amount of glucose for repressing the utilization of ethanol as carbon source would remain in culture medium at early growth phase. So ethanol itself, not its metabolites, would be responsible for morphological change.

The most interesting finding is the preventing effect of myo-inositol on the ethanol-induced morphological change. The mechanism by which myo-inositol exerts its effect is also unclear. It would be related to the

structure and function of cytoplasmic membrane in which myo-inositol plays important roles as phosphatidylinositol. Analysis of the membrane composition will serve to elucidate this problem. Extracellular production of polysaccharides by the ethanol-induced filamentous cells is also of interest. This indicates that pronounced change occurred in cell wall as well as in cytoplasmic membrane during the development of dimorphism caused by ethanol. The effect of myo-inositol to prevent the extracellular production of polysaccharides suggests that the structure and function of cytoplasmic membrane closely relate to the formation and structure of cell wall.

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REFERENCES

1. Gilardi, G. L. (1965) Bacteriol. Rev. 29, 406-424
2. Chattaway, F. W. and Holmes, M. R. (1968) J. Gen. Microbiol. 51, 367-376
3. Mardon, D., Balish, E. and Phillip, A. W. (1969) J. Bacteriol. 100, 701-707
4. Yamaguchi, H. (1974) J. Gen. Appl. Microbiol. 20, 87-99
5. Yamaguchi, H. (1974) J. Gen. Appl. Microbiol. 20, 217-228
6. Hirai, M., Shimizu, S., Teranishi, Y., Tanaka, A. and Fukui, S. (1972) Agric. Biol. Chem. 26, 2335-2343
7. Trevelyan, W. E. and Harrison, J. S. (1952) Biochem. J. 50, 298-303
8. Trevelyan, W. E. and Harrison, J. S. (1956) Biochem. J. 63, 23-33