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ALCOHOL DEHYDROGENASE ACTIVITY IN THE YEAST *LIPOMYCES STARKEYI*

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SUMMARY

The oxidation of ethanol and the NAD-dependent alcohol dehydrogenase (alcohol:NAD^+ oxidoreductase, EC 1.1.1.1) activity were studied in the aerobic yeast *Lipomyces starkeyi*. Cells grown on glucose as a carbon source contain little ethanol dehydrogenase activity, but do contain an enzyme which oxidizes cinnamyl alcohol. After growth on ethanol, a second enzyme appears which reacts with both ethanol and cinnamyl alcohol. Both enzymes are located in the cytosol. The appearance of the ethanol dehydrogenase activity in the presence of ethanol is suppressed by the simultaneous presence of glucose but not of citrate.

INTRODUCTION

The study of alcohol oxidation in yeast and the metabolic role of the various isozymes of alcohol dehydrogenase (alcohol:NAD^+ oxidoreductase, EC 1.1.1.1) have been subjects of recent interest. The relation of the isozymes to ethanol oxidation is not entirely clear. Both intra- and extramitochondrial oxidation of ethanol is possible in Baker's yeast[1–5]. To provide additional information as to the site and mechanism of ethanol oxidation in yeasts, we have studied the NAD-dependent alcohol dehydrogenase activity in the yeast, *Lipomyces starkeyi*. This strictly aerobic organism produces no ethanol, but it will grow on ethanol as the sole carbon source[6].

MATERIALS AND METHODS

Yeast strain. The yeast used in these experiments was *Lipomyces starkeyi* (ATCC 12659). The yeast was maintained on glucose–yeast base agar[5].

Preparation of cultures. Working cultures were prepared as described previously for *Lipomyces lipoferus*[7]. The media used had the following compositions per l: (1) glucose 15 g, Difco yeast base 6.7 g; (2) ethanol (99%) 15 ml, Difco yeast base 6.7 g; (3) citric acid 9.4 g, tripotassium citrate 13 g, Difco yeast base 6.7 g. Media including both glucose and ethanol or citrate and ethanol were employed also. The cultures grew at 25–27° in an Eberbach water-shaking bath. For respirometry 24-h

TABLE I

O_2 UPTAKE OF *L. starkeyi* GROWN ON GLUCOSE OR ETHANOL

Yeast (0.8–1.2 mg (dry wt.)/vessel) suspended in 0.05 M potassium phosphate buffer, pH 6.2. Substrate concentration, 0.025 M in buffer.

Substrate	Q_{O_2} *of cells grown on*	
	Glucose	*Ethanol*
Blank	20	38
Glucose	97	105
Acetate	31	179
Ethanol	22	256
Cinnamyl alcohol	18	69
Allyl alcohol	21	29

cultures were used, while for alcohol dehydrogenase measurements, the cells were harvested after 48 h of growth.

General methods. Respirometry, cell fractionation, electrophoresis, and measurement of alcohol dehydrogenase activity were performed as described previously[5]. The specific activity of alcohol dehydrogenase is reported as nmoles NADH produced per min per mg protein.

RESULTS

O_2 consumption

In Table I is shown the effect of certain substrates on the O_2 consumption of glucose- and ethanol-grown cells. Growth on ethanol, as opposed to glucose as the carbon source, produced a striking difference in the effects of the substrates used. The O_2 uptake of the glucose-grown cells was stimulated by glucose and also slightly by acetate. The alcohols were without effect. After growth on ethanol, the Q_{O_2} for ethanol rose to 2.5 times that for glucose. Acetate now produced also a greater effect than glucose. Cinnamyl alcohol, but not allyl alcohol, stimulated the O_2 uptake of the ethanol-grown cells.

TABLE II

ACTIVITY PROFILE OF NAD-DEPENDENT ALCOHOL DEHYDROGENASE FROM *L. starkeyi* GROWN ON GLUCOSE OR ETHANOL

Activities reported relative to the activity with cinnamyl alcohol as 100%. Specific activity with cinnamyl alcohol, glucose-grown cultures, 28; ethanol-grown cultures, 83.

Substrate	*Relative alcohol dehydrogenase activity in cells grown on*	
	Glucose	*Ethanol*
Cinnamyl alcohol	100	100
Methanol	<1	17
Ethanol	4	63
Propanol	8	160
n-Butanol	4	106
n-Amyl alcohol	12	150
Allyl alcohol	14	64

Alcohol dehydrogenase activity

In Table II are shown the activity profiles of NAD-dependent alcohol dehydrogenase produced by the various alcohols used as substrates. The glucose-grown cultures showed activity with cinnamyl alcohol as substrate, but there was little activity with the other alcohols. After growth on ethanol there was a 2–3-fold increase in total cinnamyl alcohol dehydrogenase activity. There was now considerable ethanol dehydrogenase activity and activity with the other aliphatic alcohols.

Electrophoresis

The variations in substrate specificity brought about by growth on ethanol were reflected in the electrophoretic patterns of alcohol dehydrogenase activity shown in Fig. 1. In the glucose-grown cells there was one band of activity with cinnamyl alcohol

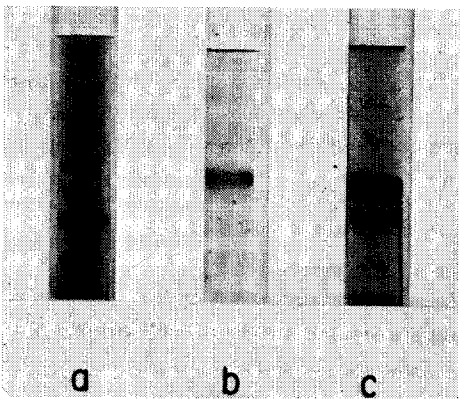

Fig. 1. Electrophoretic pattern of the NAD-dependent alcohol dehydrogenase activity. (a) Cells grown on glucose, gel developed with cinnamyl alcohol. (b) Cells grown on ethanol, gel developed with ethanol. (c) Cells grown on ethanol, gel developed with cinnamyl alcohol.

as substrate (Fig. 1a). On gels developed with ethanol, no activity was detected. In ethanol-grown cultures, an additional more slowly migrating band of activity was seen on gels developed with cinnamyl alcohol (Fig. 1c). This new band reacted also with ethanol (Fig. 1b).

Effect of carbon source

In Table III are shown the effects of various carbon sources on the alcohol dehydrogenase activity. While the growth on ethanol was less than on glucose, the specific activity of both the cinnamyl alcohol- and ethanol-dependent alcohol dehydrogenase activity was higher in the ethanol-grown cultures. After growth in glucose–ethanol medium the density of the culture and alcohol dehydrogenase activities were similar to those of the cultures grown on glucose alone. The least growth was achieved on citrate; the specific activity of cinnamyl alcohol dependent alcohol dehydrogenase was two thirds that of the glucose-grown cultures, and no ethanol-dependent activity was detected. If both citrate and ethanol were present in the medium, the growth of the cultures and the alcohol dehydrogenase activities were similar to those of the ethanol-grown cultures.

TABLE III

EFFECT OF GROWTH ON DIFFERENT CARBON SOURCES ON NAD-DEPENDENT ALCOHOL DEHYDROGENASE ACTIVITY

Preliminary cultures grown on glucose medium. Equal quantities of cells were transferred to the different media and allowed to grow 2 days.

Carbon source	$A_{540\ nm}$ *of culture*	*Specific activity with*	
		Ethanol	*Cinnamyl alcohol*
Glucose	3.6	2	28
Ethanol	1.5	47	83
Glucose and ethanol	4.2	1	32
Citrate	1.0	<0.5	19
Citrate and ethanol	1.8	35	56

Since glucose seemed to repress the appearance of the ethanol-dependent alcohol dehydrogenase, the importance of glucose and ethanol in the regulation of this enzymic activity was examined by washing and transfering cells grown on glucose to ethanol medium or to 0.05 M potassium phosphate buffer, pH 6.2. After incubation in the phosphate buffer for 22 h there was no increase in ethanol-dependent alcohol dehydrogenase activity. In ethanol medium, the cinnamyl alcohol-dependent specific activity increased from 25 to 58 and the ethanol-dependent activity from less than 1 to 26. During this period there was some growth of the cells as measured by a 15% increase in the absorbance of the culture.

Localization

The alcohol dehydrogenase was localized within the cell by measuring the activity in the mitochondrial and cytosol fractions prepared from protoplasts of ethanol-grown cells. All the ethanol-dependent activity was found in the cytosol. The mitochondrial fraction contained less than 1% of cinnamyl alcohol-dependent activity.

DISCUSSION

The use of a strictly aerobic organism simplifies the study of ethanol oxidation in the yeasts. *L. starkeyi* is a non-fermenting yeast, and after growth on glucose there is little or no ethanol dehydrogenase activity present. Ethanol does not affect the O_2 consumption of the intact cells. On the other hand, baker's yeast, an organism capable of fermentation, contains a mitochondrial and an extramitochondrial ethanol dehydrogenase after growth on glucose. These cultures of baker's yeast produce ethanol, and ethanol has a small effect on O_2 consumption[5].

Ethanol-grown cells of *L. starkeyi* show striking similarities to ethanol-grown baker's yeast. Ethanol stimulates the O_2 uptake of both species of yeast more than does glucose[5]. Q_{O_2} of *L. starkeyi* for ethanol reported here is in the same range as those of the more active species of Saccharomyces as reported by BARNETT AND KORNBERG[8]. As was found with baker's yeast[2,4,5], growth of *L. starkeyi* on ethanol is accompanied by the appearance of an alcohol dehydrogenase virtually absent during growth on

glucose. This enzyme in baker's yeast has been designated ADH II[9,5]. Like ADH II, the enzyme of *L. starkeyi* shows a strong affinity for the higher alcohols[4]. For the aliphatic alcohols tested, the specific activity tends to increase with increasing chain length (Table II). Also like ADH II the ethanol dehydrogenase activity of *L. starkeyi* is located in the cytosol[5]. Thus ethanol oxidation must be a function of this cell fraction. This differs from baker's yeast where both intra- and extramitochondrial oxidation is possible[1,3,5]. The resulting NADH would either be used in synthetic processes or oxidized by the mitochondria, either directly or by the shuttle of some substrate.

From the results presented here, it appears that both the absence of glucose and the presence of ethanol are necessary for the production of ethanol dehydrogenase by *L. starkeyi*. Unlike glucose, the presence of citrate does not suppress the appearance of this enzyme. Similarly, ADH-II does not appear during growth of baker's yeast on glucose, but does appear either on transfer to ethanol or during secondary growth on the alcohol produced during the fermentative phase of growth[2,4,5].

However, there are some noteworthy differences between *L. starkeyi* and baker's yeast. The total ethanol dehydrogenase activity of ethanol-grown *L. starkeyi* is only 3% of that seen in glucose-grown baker's yeast and 1% of that found in ethanol-grown baker's yeast. However the Q_{O_2} for ethanol is nearly twice as great in *L. starkeyi* as in baker's yeast[5]. Clearly the oxidation of ethanol by baker's yeast is not limited by the total alcohol dehydrogenase activity. Another difference is the relatively small effect on O_2 consumption of cinnamyl alcohol and allyl alcohol. In baker's yeast both these alcohols were reported to have a greater effect than ethanol[3].

It is interesting also that the glucose-grown cultures of *L. starkeyi* contain an alcohol dehydrogenase which reacts with cinnamyl alcohol but shows little or no reactivity with the aliphatic alcohols tested. As far as we are aware, no similar enzyme has been reported in Saccharomyces. The significance of this enzyme in the total metabolism of the yeast is not known.

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