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# CONTROL OF ETHANOL PRODUCTION BY YEAST: ROLE OF PYRUVATE DECARBOXYLASE AND ALCOHOL DEHYDROGENASE

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SUMMARY: The critical role of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) in determining the rate of ethanol production is confirmed using PDC constitutive mutants. By deriving strains with altered levels of these two enzymes, it has been found that a high level of both PDC and ADH is necessary for faster ethanol production.

## INTRODUCTION

We recently reported partial characterization of a fast and a slow ethanol producing strain of Saccharomyces cerevisiae with regard to some key enzymes (Sharma and Tauro, 1986). While invertase activity in both strains during batch fermentation was similar, the activities of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) were significantly different. Here we provide further evidence for the key role of pyruvate decarboxylase in determining the rate of ethanol production.

## MATERIALS AND METHODS

Details of the fast fermenting strain Saccharomyces cerevisiae 21, culture methods and the assay of pyruvate decarboxylase have been reported earlier (Sharma and Tauro, 1986). Enzyme activity is expressed in International Units ($\mu$ moles $min^{-1}$ $mg^{-1}$ protein). The haploid wild type strains of S. cerevisiae D-585-11C and D-587-48 and the pyruvate decarboxylase constitutive mutants R-144 $T_1B$ PDCsup and R-144 $T_1D$ PDCsup were from the laboratory of Prof. P.K. Maitra of TIFR, Bombay. Yeast strains were grown under stationary conditions without aeration or shaking, to reflect typical batch fermentations.
Spore progeny from strain 21 was derived by dissecting four-spored asci after treatment with glusulase (Sherman et al., 1974).

## RESULTS

Two approaches were used to verify the role of PDC in determining the rate of ethanol production by S.cerevisiae. The observation that strain 21 has a high PDC activity during later stages of fermentation

(Sharma and Tauro, 1986) led us to examine initial rates of ethanol production by recycled cells. In a second approach, a comparison of the initial rates of ethanol production by PDC-inducible versus PDC-constitutive strains of Saccharomyces was made.

In the first set of experiments, cells of strain 21 were collected by centrifugation at different times during a batch fermentation and immediately suspended in fresh warm fermentation medium and initial rate of ethanol production was determined (Table 1).

TABLE 1 Effect of PDC level on rate of ethanol production

| Times of cell harvest from first batch (h)* | Enzyme specific activity [/umol $min^{-1}$ mg $protein^{-1}$] PDC | ADH | Ethanol production (%v/v) in 2h |
|---|---|---|---|
| 24 | 0.303 | 24.84 | 0.55 |
| 36 | 0.490 | 27.92 | 0.85 |

*Biomass harvested after 24 or 36 h from fermentation medium containing sucrose 15%, peptone 0.5%, yeast extract 0.5%, pH 5.0 was used for the measurement of enzyme levels and for further fermentation on the same medium for initial rates measurement.

As shown in Table 1, the rate of ethanol production by cells collected at 36 h was about 55% faster than by cells collected at 24 h. Cells collected at 36 h had about 48% more PDC but only 12% more ADH as compared to cells collected at 24 h.

This observation was extended using genetically defined haploid yeasts. In wild type S.cerevisiae, PDC synthesis is inducible and its level remains extremely low when the yeast is cultured in a non-fermentable carbon source such as ethanol (Schmitt and Zimmermann, 1982). On the other hand, the constitutive strains have identical levels of PDC under either conditions. If cells with a higher PDC level produce ethanol faster, then a PDC constitutive strain previously cultured in ethanol medium should produce ethanol faster compared to the inducible strain pre-cultured in ethanol. Table 2 summarizes such data on the mutant strains.

As Table 2 shows, the level of PDC in constitutive mutants is similar irrespective of the prior growth conditions, while the enzyme is repressed in the wild type pre_cultured in ethanol medium. As expected, the constitutive mutant cultured in ethanol medium and with a

higher level of PDC produced ethanol faster than the wild type with a lower level of PDC.

In addition to high level of PDC, strain 21 has a high level of ADH. To verify whether the levels of ADH and PDC are related to ethanol production rate, strains with varying levels of these enzymes were derived from the parent strain 21 and the level of PDC, ADH and the ethanol production rates were determined when inoculated with identical cell mass into fermentation medium (Table 3).

TABLE 2 PDC, ADH levels and ethanol production rate by inducible and constitutive strains of S.cerevisiae*

| Strain:genotype | PDC specific activity# [/umol $min^{-1}$ (mg protein)$^{-1}$] in YEP-ethanol | in YEP-glucose | Ethanol production (%v/v) in 3 h after transfer# |
|---|---|---|---|
| D-585-11C wild-type haploid | 0.068 | 0.488 | 0.25 |
| D-587-48 wild-type haploid | 0.046 | 0.467 | 0.25 |
| R144 $T_1$ B PDCsup,constitutive | 0.523 | 0.501 | 0.47 |
| R144 $T_1$D PDCsup,constitutive | 0.506 | 0.491 | 0.51 |

*R144 was isolated from a PDC mutant pdc1-1;R144 $T_1$B and R144 $T_1$D are tetrad products from a cross R144 x WT (D-585-11C).

#Cells were grown in YEP+glucose or YEP+ethanol overnight at 30°C. PDC activity was measured and an equal biomass transferred to YEP+15% sucrose to monitor ethanol production.

TABLE 3: PDC, ADH levels and EtOH production in strain 21 and spore progeny

| Strain | Specific activity [/umol $min^{-1}$ (mg protein)$^{-1}$] | | Ethanol (%v/v) after 24h |
|---|---|---|---|
| | PDC | ADH | |
| 21 | 0.446 | 32.1 | 7.0 |
| 6C | 0.421 | 12.74 | 3.0 |
| 6D | 0.403 | 12.23 | 4.5 |
| 20 | 0.181 | 21.61 | 4.8 |

In Table 3, strain 21 has a higher level of both PDC and ADH and produces 7% ethanol in 24 h. Strains 6C and 6D have similar high levels of PDC but lower levels of ADH; they produce ethanol more slowly. In strain 20, which has been earlier reported as a slow fermenting strain (Sharma and Tauro, 1986), the level of PDC is very low while the level of ADH is higher than 6C and 6D; the rate of ethanol production is

slower than in 21. This suggests that for faster ethanol production the level of both PDC and ADH should be higher.

The use of PDC constitutive mutants and the derivatives from strain 21 with varying levels of ADH confirms that in S.cerevisiae both of these enzymes have a major role in determining the rate of ethanol production. The level of these enzymes is not constant during the fermentation, and the values represented here are maximum values. To determine the overall pattern of enzyme activity it will be necessary to determine the enzyme activity at different stages of fermentation.

## REFERENCES


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