

Null and electrophoretic mobility mutants in the structural gene for L-lactate dehydrogenase of *Saccharomyces cerevisiae*

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A mutant lacking L-lactate dehydrogenase (EC 1.1.2.3) of *Saccharomyces cerevisiae* was isolated by its inability to grow on minimal medium with L-lactate as a carbon source. A simple activity gel assay for visualization of this enzyme and the two D-lactate dehydrogenases in this organism (EC 1.1.2.4 and 1.1.1.28) was developed. This enabled us to screen spontaneous and ethylmethanesulfonate-induced back mutants for electrophoretic mobility. Two mutants with a mobility faster than that of the wild type were isolated, and proved to be allelic to the L-lactate dehydrogenase negative mutant.

Activity staining Structural gene mutation

1. INTRODUCTION

The FAD-dependent lactate dehydrogenases of the yeast *Saccharomyces cerevisiae*, unlike the NAD-dependent enzymes of many other organisms, appear essentially to catalyze the conversion of lactate to pyruvate [1]. Since the intermediate which stores reducing power during anaerobic growth in this organism is ethanol rather than lactate, a freely reversible reaction resulting in the production of large amounts of lactate during anaerobic growth would be highly disadvantageous. Ethanol has the advantage that it has little effect on intracellular pH and therefore can be tolerated by the yeast cell (though not by the cells of higher organisms) at high concentrations.

The essential irreversibility of the lactate dehydrogenase (LDH) reaction in yeast is seen most clearly with the cytochrome *c*-associated L- and D-LDHs (EC 1.1.2.3 and 1.1.2.4, respectively), which are located between the inner and outer membranes of the mitochondrion. Lactate is converted to pyruvate, and the reduced FAD which results immediately passes its electrons to cytochrome *c*, whence they enter the electron

transport chain [2]. The primary direction of the D-LDH found in the cytoplasm (EC 1.1.1.28), also FAD-dependent but not associated with a cytochrome [3], is less clear.

Yet, the reaction appears not to be completely irreversible. Yeast can produce detectable quantities of L-lactate when grown on other carbon sources [4]. The dynamics of the reaction and its potential reversibility have been the subject of a series of studies [5]. It appears that the FAD-dependent component of the reaction may be reversible, although whether this occurs under physiological conditions is not clear.

Another interesting aspect of this set of enzymatic reactions is that their study may provide a greater understanding of yeast bioenergetics. For example, Pajot and Claisse [6] found that it was possible to grow yeast on lactate as a carbon source in the presence of very low levels of antimycin A. This was, they suggested, because the site of action of the inhibitor block still permitted operation of the electron transport chain subsequent to cytochrome *b*. Growth was observed at levels of antimycin A up to 10 ng/ml. Unanswered questions remain, however. Presumably, the tricarbox-

glycic acid cycle is unable to operate if the reducing equivalents generated cannot be drained off to oxygen; thus, the inhibited cell would have no source of dicarboxylates and tricarboxylates when grown on lactate. It may be that, while these inhibitor levels are sufficient to prevent growth on glycerol, they may not be sufficient to prevent growth on pyruvate. Pyruvate generated from lactate would then be a necessary adjunct for growth, and such growth would show that the electron transport chain was not completely blocked.

For these and other investigations, it will be useful to have both mutants lacking in activity of particular LDHs and simple methods to assay for their properties and activities. We present here the growth properties of an *ldhl* mutant in *S. cerevisiae*, lacking the cytochrome *c*-associated L-lactate dehydrogenase activity. We also provide a simple method for visualizing the activity of the native enzyme on a non-denaturing horizontal starch gel, and use this separation method to screen for a back mutant showing altered electrophoretic mobility. The segregation of this back mutant indicates that the *ldhl* mutation is in a structural gene for the enzyme.

2. MATERIALS AND METHODS

2.1. Yeast strains and mutant selection

Strain X2180-1A (*MATa SUC2 mal gal2 CUP*) was mutagenized with ethylmethanesulfonate (EMS) [7] and approx. 10000 colonies were scored for inability to grow on MV (yeast nitrogen base without amino acids, from Difco, 0.75%) with 1% L-lactate (Sigma) as a carbon source. Of 4 mutants found, only one clearly segregated 2:2 for the character in the next generation. Back mutants were isolated by plating a second generation segregant of the mutant on the same medium at 10^8 cells/plate and isolating 30 spontaneous revertants, which arose at a rate of 5×10^{-9} after mutagenesis with EMS.

2.2. Electrophoresis and activity gel staining

Crude extracts of tetrads segregating for the mutant and the revertants were prepared from cells grown with aeration on complete medium to plateau. Cells were broken with an equal weight of 0.3 mm glass beads in an equal volume of cold 0.1 M K PO₄ buffer, pH 7.0, by two 1 min periods

of agitation in a vortex mixer. Horizontal starch gel separation of the crude extract proteins was carried out for 2.5 h [8]. L-LDH activity was visualized by staining the gel overnight at 25°C in the dark in the following stain buffer: 100 mM K PO₄, pH 7.0, to 100 ml of which was added 25 mg nitroblue tetrazolium, 4 mg phenazine ethosulfate and 100 mg of the sodium salts of L, D or DL-lactate (all chemicals from Sigma).

3. RESULTS

3.1. Electrophoretic patterns

The activity gel stain showed L-LDH activity only in the presence of lactate as a substrate. Three first-generation tetrads of the cross with wild type of the mutant unable to grow on lactate showed 2:2 segregation of the presence and absence of the heavily staining band near the origin. Of 30 EMS-induced revertants examined, all had regained the activity band on electrophoresis, but only two showed altered electrophoretic mobility. Both revertants showed identical mobility, 121% of the wild type, and may represent a single mutational event. A tetrad of one of these mutants segregating with wild type is shown in fig. 1.

Two faint, more rapidly migrating bands were present, although not consistently, in crude cellular extracts. These bands were not seen when L-lactate was used as a substrate, and the L-LDH band was not seen when D-lactate was used as a substrate. These two bands therefore represent the two D-LDHs.

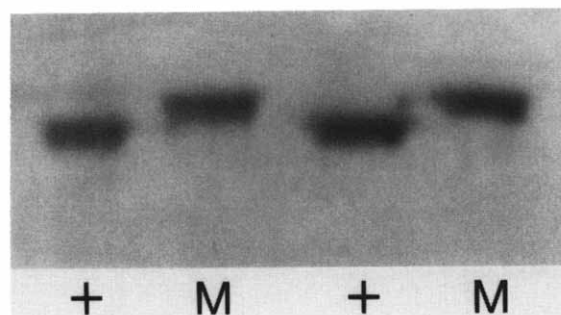


Fig.1. Activity pattern visualized after electrophoresis of a tetrad segregating 2:2 for a wild type and a mobility mutant L-LDH. (+) Wild type, (M) mutant segregant.

The origin is at the bottom of the picture.

3.2. Growth studies

The *ldhl* strain showed no detectable phenotypic effect except for its inability to grow on L-lactate. Growth on dextrose, ethanol, pyruvate and glycerol was indistinguishable from that of wild type.

4. DISCUSSION

The visualization of L- and D-LDH activity on a gel is accomplished because reduced FAD associated with the enzyme readily passes electrons to nitroblue tetrazolium and phenazine ethosulfate as a terminal acceptor. The reaction is simple and appears to be specific. No bands appeared which could not be accounted for by the LDH activity.

The isolation of a revertant of *ldhl* with altered electrophoretic mobility constitutes a direct demonstration that the *ldhl* mutation is in the structural gene. It remains to be seen whether the lesion is in the FAD-binding subunit or in the subunit which interacts with cytochrome *c*.

It is to be expected that the *ldhl* mutant would have no effect on growth on any other substrate, since the electrons provided by oxidation of lactate to pyruvate enter far down the electron transport chain and no other catabolic pathway ought to be disturbed. It should be possible to use the *ldhl* mutant to determine how much of the energy derived from lactate comes from this step and how much comes from the metabolism of pyruvate resulting from the L-LDH reaction. Further, it would appear from the lack of effect of the mutant on growth on other substrates that any production of

lactate by this enzyme is not essential to growth.

The availability of mutants lacking the L-LDH may prove useful in fermentations in which it is desirable to keep the production of lactic acid to a minimum.

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