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Baker's Yeast

Some Biochemical Aspects and Their Influence in Biotransformations

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ABSTRACT

Baker's yeast is becoming an important reagent for organic synthesis. However, on many occasions, there are problems with the experimental reproducibility, which in general is the result of the different origins of baker's yeasts. In order to explain these differences, NAD (P)$^+$ reduction inside intact living cells from different strains was measured. The method can select cells with better reduction power in a short period of time.

Index Entries: Biotransformation; *Saccharomyces cerevisiae*; pH; NAD(P)H; temperature; enatiomeric excess.

INTRODUCTION

The use of baker's yeast (*Saccharomyces cerevisiae*) in organic synthesis involving reactions, such as condensation (*1*), oxidation (*2,3*), reduction (*4–6*), and cyclization (*7,8*), is increasing (*9*). In the majority of cases, a chiral carbon is produced, that is responsible for the interest in these microorganisms for synthesis, together with other advantages, such as their low cost, nontoxic nature, ease of manipulation, and availability. The reduction reactions are catalyzed by intracellular enzymes (dehydrogenases) in these living cells (*9,10*) aided by the reduced form of pyridine

nucleotides [NAD(P)H] (*11*). The former are highly stereospecific; in other words, they can produce an unique stereoisomer (*12,13*). Based on this rational, *S. cerevisiae* would be a "perfect" reagent if its enzymes had the same conformation. Unfortunately, this is not true. There are several dehydrogenases, with opposite conformations, that compete for the artificial substrate. In order to solve this problem, some researchers have added compounds that inhibit enzymes of one conformation (*12,14*). This method has proven to be efficient and improves the enantiomeric excess, but in some cases, results in low yields (*15*).

Another problem faced by organic chemists is to repeat experiments made elsewhere. To try to explain this problem, baker's yeasts from different origins were studied. The variation in the production of NAD(P)H in the interior of these living cells with external factors, such as temperature and pH, was measured. Another aspect studied was the effect of compounds known to inhibit some dehydrogenases and their effects on NAD(P)H production.

MATERIALS AND METHODS

Chemicals

Lithium acetoacetate was obtained from Sigma Chemical Co. (St. Louis, MO) and the other reagents used were of analytical grade. Water was double-distilled and deionized using a Barnstead D3750.

NAD(P)H Oxidation Reduction

Pyridine nucleotide oxidation-reduction changes were monitored using the wavelength pair 340 and 370 nm. All experiments were conducted using an SLM Aminco DW 2000 split and dual-wavelength spectrophotometer as previously described (*16*).

Biological Materials

The baker's yeast strains (*S. cerevisiae*) were obtained from Fermipan (Holland), Itaiquara (Brazil), Nishin Seifun Co. (Japan), and Fleischmman (Brazil). The stock suspensions were prepared by adding 1.0 g dry or 3.5 g raw baker's yeast to 10 mL water with stirring at room temperature. The cells were counted in a optical microscope (Carl Zeiss) using a Neubauer chamber.

Oxygen Uptake Measurements

Oxygen consumption was measured with a Clarck-type electrode (Yellow Springs Instruments Co.) in a 1.0-mL thermostatized glass chamber equipped with magnetic stirring.

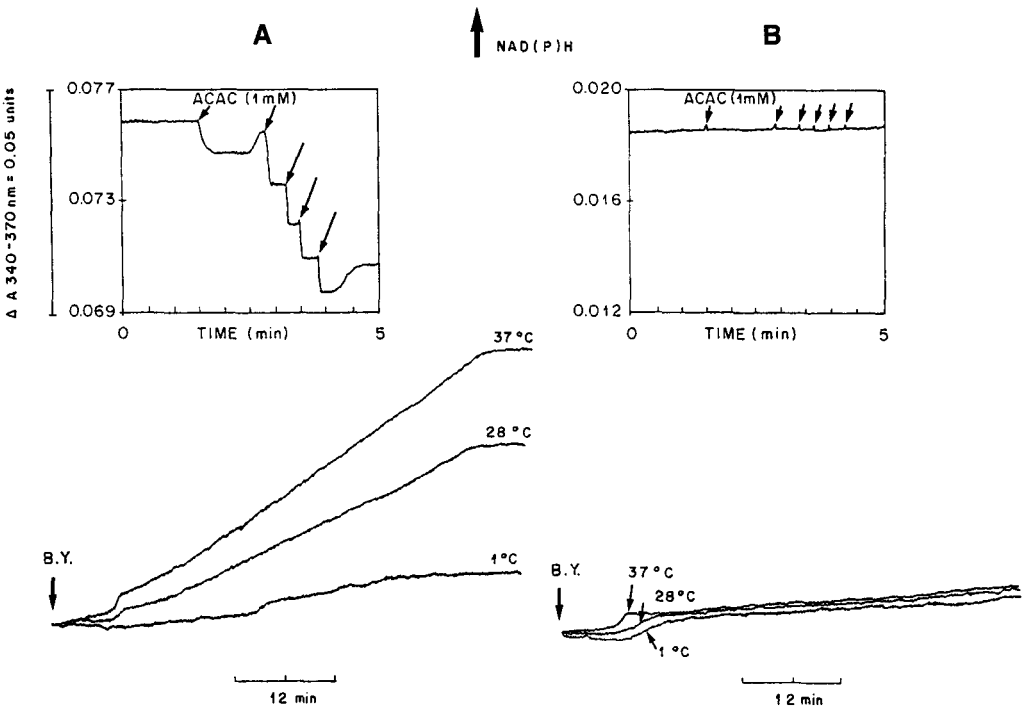


Fig. 1. Influence of temperature on the reduction of pyridine nucleotide by baker's yeast (*S. cerevisiae*). Baker's yeast (B.Y., 2.3 mg/mL) was added to double-distilled and deionized water, and pH of the medium was 6.0. (A) Commercial strains from Holland and Japan; (B) commercial strains from Brazil. Inset: Oxidation of NAD(P)H by multiple additions of lithium acetoacetate 1 m*M* (ACAC).

RESULTS AND DISCUSSION

The reduction of oxidized pyridine nucleotide [$NAD(P)^+$] by baker's yeast is presented in Fig. 1A,B. The former presents the Dutch and Japanese strains and the latter the Brazilian strains.

In order to assure that all experiments contained the same number of cells (correction of the stoichiometry), cells were counted using a Neubauer chamber.

Since glucose is not present in the reaction medium, the cells degraded their internal glycogen, which can supply glucose for 10–12 h (*17*). Commercial baker's yeast strains (*S. cerevisiae*) when added to water initiate the reduction of the oxidized forms of $NAD(P)^+$. This reduction continues until a steady state is reached (Fig. 1A). To verify the NAD(P)H oxidation, lithium acetoacetate was added to the medium (inset of Fig. 1). The absorbance falls because NAD(P)H is being oxidized and the acetoacetate transformed into β-hydroxybutyrate through internal *S. cerevisiae* dehydrogenases. It is worthwhile mentioning that acetoacetate (and others substrates) can

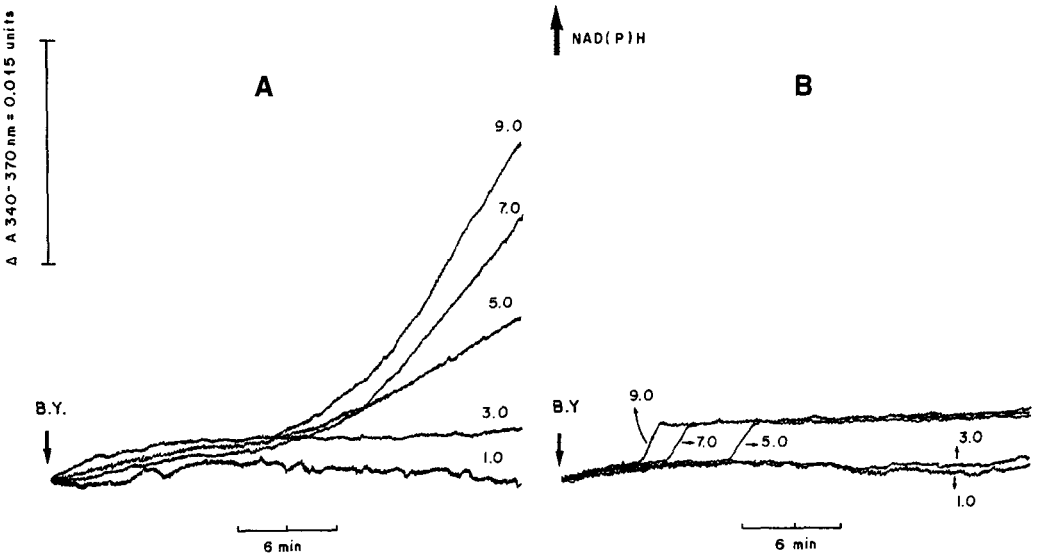


Fig. 2. Effect of pH on the reduction of pyridine nucleotide by baker's yeast (*S. cerevisiae*). Baker's yeast (B.Y., 2.3 mg/mL) was added to the reaction medium, and the pH adjusted under stirring. The temperature of the experiment was maintained at 20°C during the period of observation. (A) Commercial strains from Holland and Japan; (B) commercial strains from Brazil.

induce an oxidative stress in living cells when the reserve of the reduced form of pyridine nucleotide is consumed. For a good performance in biotransformations, the addition of substrates after 1 h of incubation is necessary for the cells to reach a steady state in the reduction of the pyridine nucleotides, as observed in Fig. 1A.

For commercial strains from Holland and Japan, the reduction is dependent on temperature. Surprisingly, the Brazilian strains are not sensitive to this external factor, as depicted in Fig. 1B. To explain the cells' temperature sensitivity, this author proposed that molecules that act as "biological thermosensors" are present in the polysaccharide membrane surface. These "thermosensors" are not present in cells of the Brazilian strains (Fig. 1B).

Figure 2 shows the dependence of these reduction reactions on the external pH (from 1 to 9). At lower pH, the cells are not good reducers of [NAD(P)$^+$]. This phenomenon can be attributed to the extrusion of protons by ATPase (*18*). These cells are energized. Consequently, a net negative charge in the cell interior is observed that results in the transport of protons to the inside. The internal medium turns acid, which induces ATPase activity (*19*). This enzyme is responsible for protons extrusion. This phenomenon results in ATP depletion and accumulation of AMP, which inhibits phosphorylation of glucose (*18*). As a consequence, the glycolysis and pentose phosphate pathway, which are the paths that produce NAD(P)H, are inhibited as observed in Fig. 2A (pH = 1 and 3). Inter-

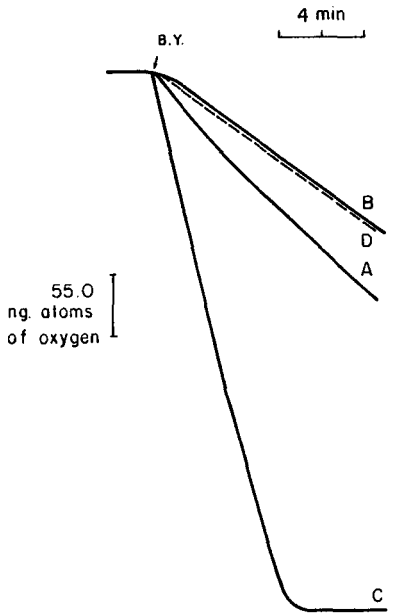


Fig. 3. Oxygen consumption by commerical strains of *S. cerevisiae*. Baker's yeast (B.Y., 2.3 mg/mL) was added to double-distilled and deionized water (pH = 6.0, temperature = 20°C). (A) Fermipan (Holland); (B) Itaiquara (Brazil); (C) Nishin Seifun Co. (Japan); and (D) Fleischmann (Brazil).

estingly, the Brazilian strains are not sensitive to the variations of pH. Possibly, these strains do not have ATPase in their membranes, or if presented, it is inactive under these conditions. It is noteworthy that when the external pH is varied 6 U, the internal pH in these cells varies by only 1.4 U (*20,21*).

Figure 3 shows that Japanese cells consume high quantities of oxygen, showing that these cells have an aerobic metabolism more active than those of the other cell types studied here (line c). The Dutch strain reduces the same quantities of NAD(P)H as the Japanese strain, but the consumption of O_2 is lower (line a). This indicates that the reduction occurs mainly through an anaerobic pathway. The O_2 consumption by Brazilian cells is very low (lines b and d), and consequently, their reduction of pyridine nucleotide is also low. As a consequence, their metabolism is slow. In practical terms, the cell with high quantities of NAD(P)H will reduce a substrate more rapidly. This is seen by comparing the two insets presented in Fig. 1 (addition of lithium acetoacetate). As can be seen in the second inset, lithium acetoacetate did not cause any detectable oxidation of NAD(P)H.

Allyl alcohol (a probable inhibitor of the L-lactate dehydrogenase, used by Nakamura and coworkers [*12,14*]) was added in experiments shown in Fig. 4. This substrate is transformed to acrolein by the action of the alcohol dehydrogenase (*22*). Acrolein induces lipoperoxidation at

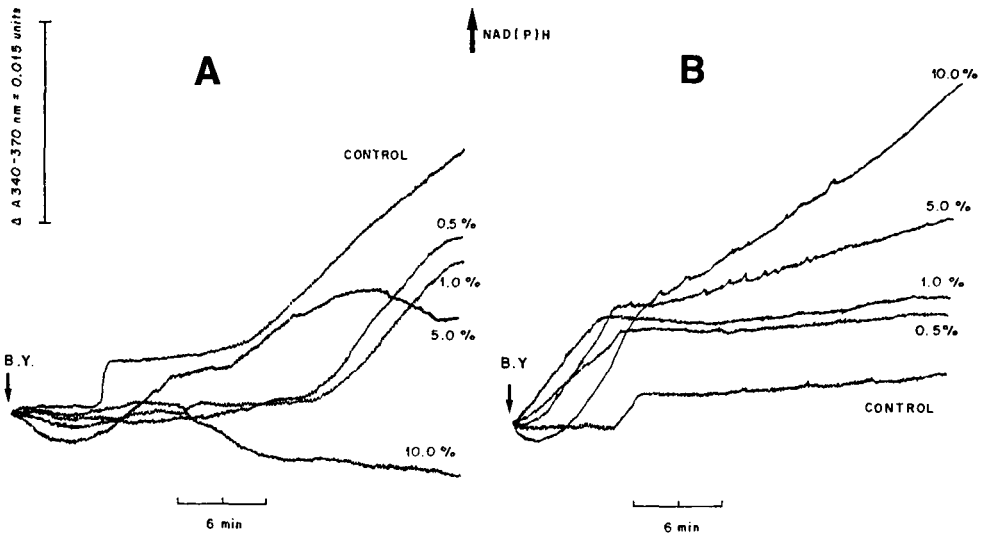
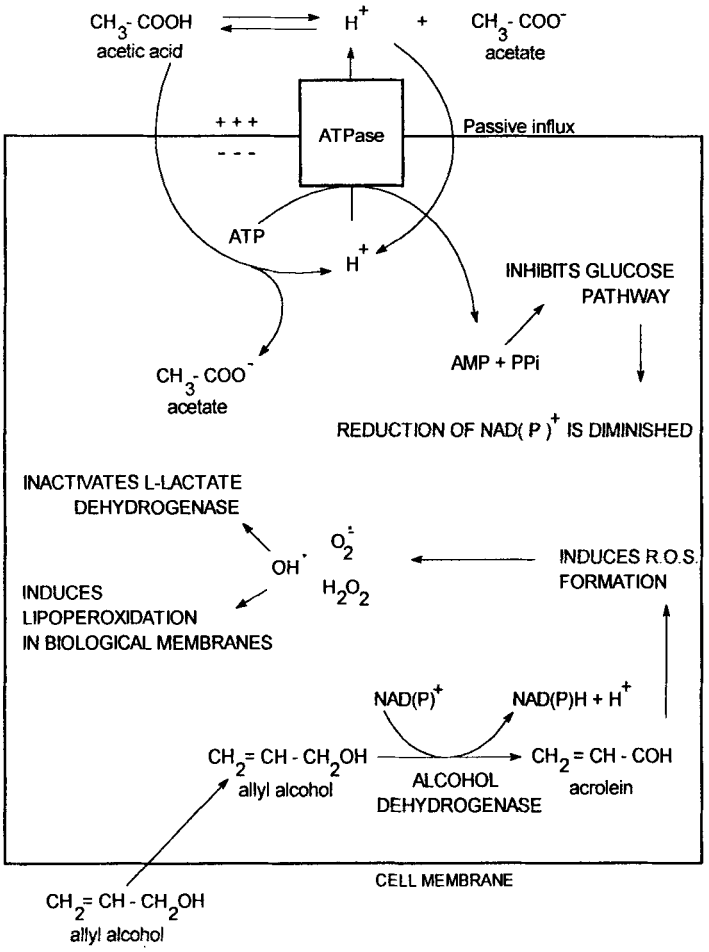


Fig. 4. Influence of allyl alcohol addition on the reduction of $NAD(P)^+$ inside cells of *S. cerevisiae*. Baker's yeast (B.Y., 2.3 mg/mL) was added to double-distilled and deionized water (pH = 6.0, temperature = 20°C) containing allyl alcohol in the indicated percentages. (A) Commercial strains from Holland and Japan; (B) commercial strains from Brazil.

biological membranes through reactive oxygen species (ROS) production (*23*). These oxygen species can inactivate L-lactate dehydrogenase (*24*) and, if present in higher concentrations, can oxidize the thiol proteins present at organelles (*25*) and cell membranes (*26*) (Scheme 1). This produce a homeostasis alteration of the cells and organelles, and a loss of cell viability (*26*). To avoid this, the cells need a good antioxidant defense mechanism that is made up of such enzymes as calalase and superoxide dismutase.

Figure 4A shows that additions of allyl alcohol cause a decrease in the reduction of $NAD(P)^+$ in the Dutch and Japanese strains. On the other hand, this phenomenon is inverted with Brazilian strains (Fig. 4B). Presumably, the latter has a good defense mechanism as mentioned above. The large increase in the production of NAD(P)H by the Brazilian strains observed may be the result of the fact that when allyl alcohol is transformed into acrolein, 1 molecule of NAD^+ is reduced (*22*) (Scheme 1). This probably also occurs in the other strains, but the toxicity of acrolein destroys a great deal of cells owing to the absence of these antioxidant enzymes.

Another additive tested was acetic acid (Fig. 5). Acetic acid is believed to improve the enantiomeric excess owing to an inhibition of the active site of selective intracellular enzymes in *S. cerevisiae* (*15*). The increase of acetic acid concentration makes the reduction of $NAD(P)^+$ decrease (Fig. 5A and B). The mechanism for explaining this is believed to be the same



Scheme 1. A schematic illustration of the possible action mechanism of acetic acid on passive H^+ influx and allyl alcohol on generation of ROS and inhibition of L-lactate dehydrogenase on *S. cerevisiae*.

as that for Fig. 2, when the external pH is low (Scheme 1). Measurements in another laboratory of the internal pH through ^{31}P NMR of cells before and after acetic acid addition found that this and other organic acids diminish internal pH (27). From this, it is possible to extrapolate that acetic acid acts as a "selector" of enzymes owing to diminished internal pH. Some enzymes isolated from *S. cerevisiae* can act better in one range of pH than others (*12*).

The principle problem in reactions conducted with baker's yeast is the solubility of the organic compounds to be biotransformed. Compounds with detergent capacity, such as dodecanol, have been used to improve this solubility. However, detergents and emulsifiers are generally very poisonous for living cells. Decanol was added in the reaction medium containing baker's yeast. Figure 6 (A and B) shows that all cells react in

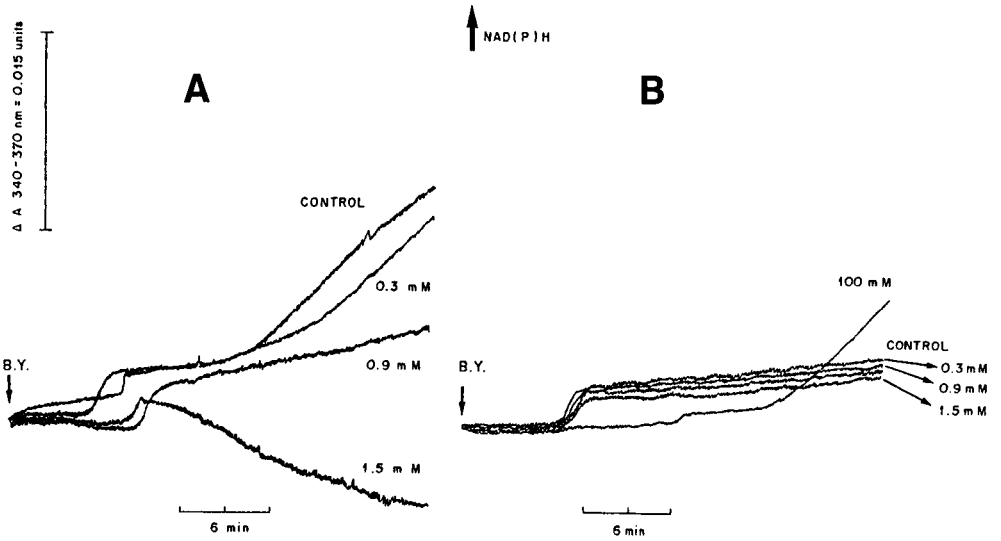


Fig. 5. Influence of acetic acid addition on the reduction of NAD(P)$^+$ inside cells of *S. cerevisiae*. Baker's yeast (B.Y., 2.3 mg/mL) was added to double-distilled and deionized water (pH = 6.0, temperature = 20°C) containing acetic acid in the described concentrations.

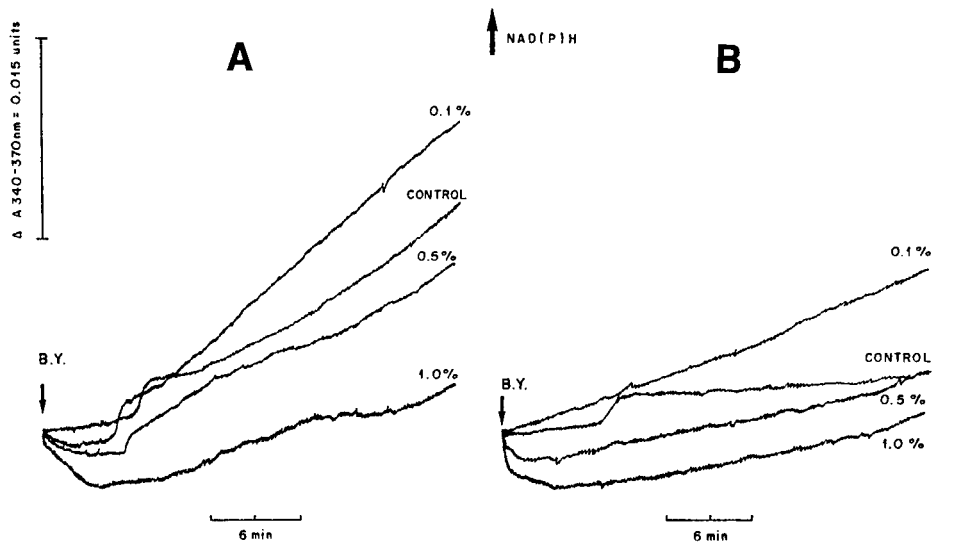


Fig. 6. Effect of decanol addition on the reduction of NAD(P)$^+$ inside cells of *S. cerevisiae*. Baker's yeast (B.Y., 2.3 mg/mL) was added to double-distilled and deionized water (pH = 6.0, temperature = 20°C) containing decanol in the indicated percentages. (A) Commercial strains from Holland and Japan; (B) commercial strains from Brazil.

same way. Low concentrations of decanol improve NAD(P)$^+$ reduction, whereas high concentrations have a toxic effect (detergent action in membrane lipids).

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