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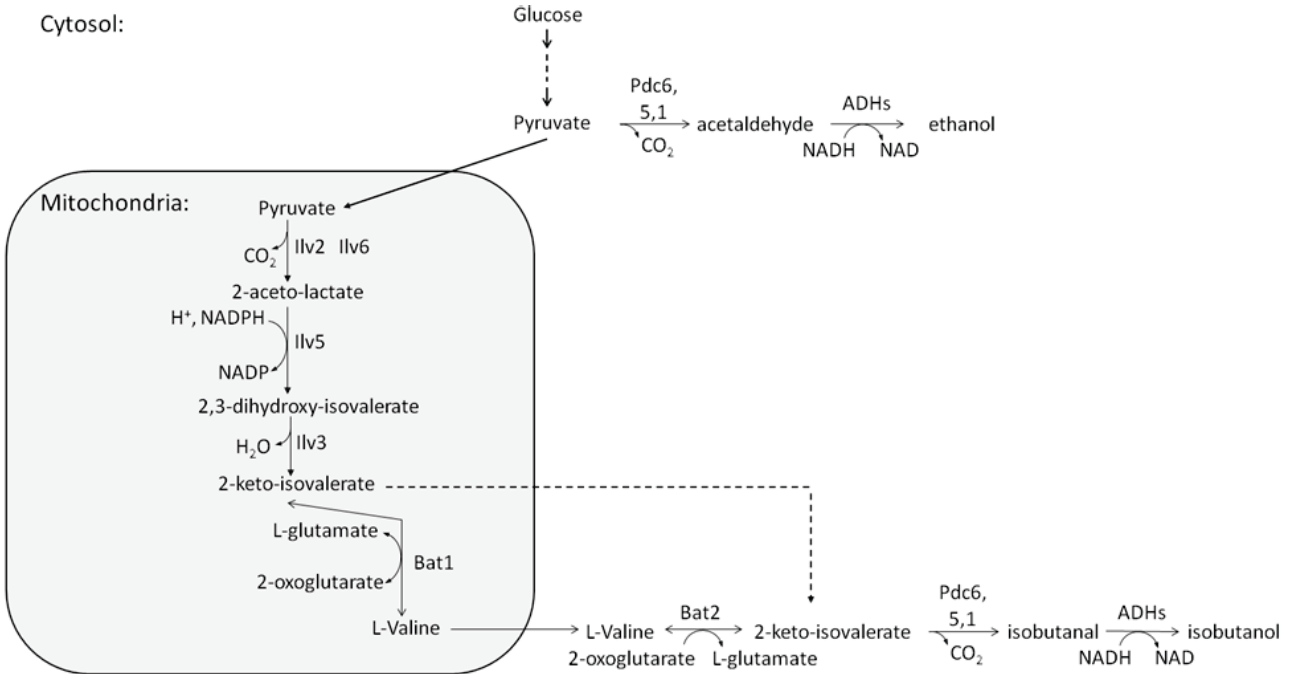
Project for PhD studies

MODULATION OF ISOBUTANOL PRODUCTION IN *SACCHAROMYCES CEREVISIAE* BY MITOCHONDRIA

Biofuels have become the rapidly growing branch of science due to a great demand for fuels and the need to protect the environment. It is well known that microorganisms such as *Clostridia* produce isobutanol, however, recently *Saccharomyces cerevisiae* gained attention in this field due to the simplicity of genetic modulations in these organisms.

Saccharomyces cerevisiae are well known for ethanol production. However, isobutanol seems better liquid biofuel than ethanol because it can be blended up with gasoline at grater scale. Moreover isobutanol is less hygroscopic, has longer chain and higher octane number than ethanol, which is much needed characteristic considering biofuel production.

Mitochondria are the organelles in *Saccharomyces cerevisiae* that are involved in isobutanol production. Isobutanol biosynthesis pathway consists of three major steps:
- transformation of glucose to pyruvate (cytosol)
- anabolic synthesis of ketoisovalerate- an intermediate in valine biosynthesis (mitochondria)
- catabolism of ketoacid into isobutanol- Ehrlich pathway (cytosol)



Source: Chen et al. Biotechnology for Biofuels 2011, 4:21

The production of isobutanol in *Saccharomyces cerevisiae* is on a very small scale. There are continuous attempts to potentiate the pathway of isobutanol production by many genetic manipulations. However, little is known how manipulation of mitochondria can affect this process.

In my project, I would like to focus on the role of mitochondria in isobutanol production by *Saccharomyces cerevisiae* and the influence of this product on the yeast. The project would include among others:
- isobutanol and ethanol production assessment by HPLC
- respiration and mitochondrial potential measurements
- reactive oxygen species and calcium changes measurements as the signal transmitters
- measurements of isobuthanol toxicity

Changes of these parameters would be evoked by modulation of mitochondrial activity by such compounds as: inhibitors of respiratory complexes, uncouplers of oxidative phosphorylation and mitochondrial ion channels activators.

The research will hopefully answer the question how the activity of mitochondria can modulate the isobutanol production in *Saccharomyces cerevisiae*, which is of a great importance for biofuel production and utility.

Related literature:

1. Compartmentalization of metabolic pathways in yeast mitochondria improves the production of branched-chain alcohols. Avalos JL, Fink GR, Stephanopoulos G. *Nat Biotechnol.* 2013 Apr;31(4):335-41.

2. Construction of an artificial pathway for isobutanol biosynthesis in the cytosol of Saccharomyces cerevisiae. Matsuda F, Kondo T, Ida K, Tezuka H, Ishii J, Kondo A. *Biosci Biotechnol Biochem*. 2012 Nov 23;76(11):2139-41.

3. Cytosolic re-localization and optimization of valine synthesis and catabolism enables inseased isobutanol production with the yeast Saccharomyces cerevisiae. Brat D, Weber C, Lorenzen W, Bode HB, Boles E. *Biotechnol Biofuels*. 2012 Sep 6;5(1):65.