Universitat Autònoma de Barcelona

Isobutanol production by genetically modified organisms

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Isobutanol is a high-energetic alcohol with a promising potential in the fuel industry.

Despite the fact that ethanol is still by far the most used liquid biofuel, isobutanol presents several advantages compared to it. Isobutanol can be naturally produced by some yeasts in the mitochondria from L-valine or from its precursors. Metabolic engineering techniques have been performed in order to improve this biosynthetic route or to introduce it into other microorganisms.

Working with bacteria

Introduce and overexpress the genes of isobutanol's biosynthetic pathway

Escherichia coli, *Corynebacterium glutamicum* and *Bacillus subtilis* are, among others, well implemented bacteria in industrial processes.

Bacteria generally present higher product yields than yeast. *E. coli*, for instance, is currently able to produce isobutanol at a concentration of 50g/L, nearly 1000 times over the concentration reached producing isobutanol on *S. cerevisiae*'s cytosol.

The cyanobacteria *Synechocystis* sp. strain PCC 6803 allows us to transform CO_2 and glucose into isobutanol.

Clostridium cellulolyticum allows us to produce isobutanol from lignocellulosic material.

Other methods to increase isobutanol's production

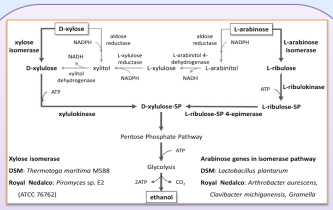

Figure 6. Increase microorganism's substrate range to make it able to metabolise pentose sugars, which represent a high portion of lignocellulosic materials (up to more than 30%).

Site-directed or random mutagenesis to generate overproducing strains.

Main Isobutanol Biosynthetic Route

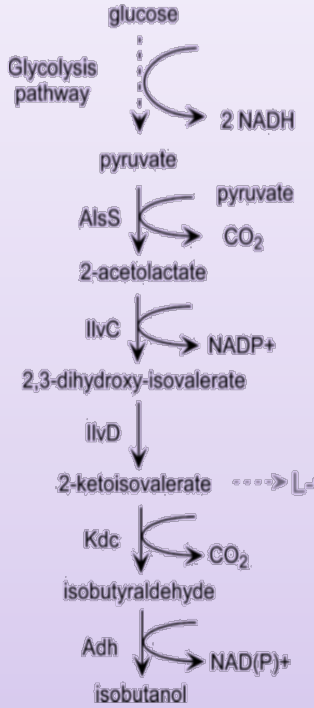

Figure 1 . Biosynthetic route of isobutanol.

Working with yeast (usually *Saccharomyces cerevisiae*)

Keeping the route in the mitochondria

Main problems to solve:

-Cofactor balancing - Regulatory hindrances

Proposed Solutions:

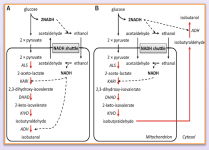

Figure 2. Engineering mitochondrial NADPH-dependent enzymes to make them become NADH-dependent.

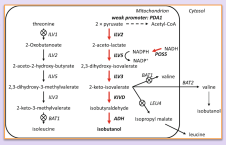

Figure 3. Deleting the genes involved in competing reactions.

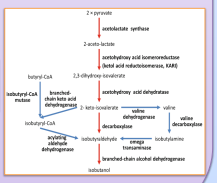

Figure 4. Using compounds from other metabolic routes as isobutanol precursors.

Creating the route in the cytosol

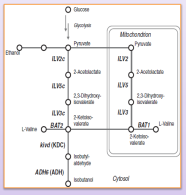

Figure 5. Construction and overexpression of a homologous pathway in the cytosol.

Supression of competing genes (such as Pyruvate decarboxylase).

Using genes to ensure balancing of the cofactors (such as Ilv5 and *GAPDH*).

Remarkable Data

Comparison between Ethanol and Isobutanol

	Ethanol	Isobutanol
Octane rate	112	102
Energy content (% of gasoline)	65%	82%
Water solubility	Fully miscible (100%)	Limited miscibility (8.5%)
Oxygen content	35%	22%
Maximum blend with gasoline	85%	99.9%
Blend recommended in current engines	10%	16.1%
Pipeline corrosion	Yes	No
Need for engine modification	Yes	No

Different names for the enzymes participating in the synthesis of isobutanol

- Acetolactate synthase: *ALS*, *Ilv2* or *AHAS*
- Acetohydroxyacid isomeroreductase: *AHAIR*, *Ilv5*, *IlvC* or *KARI*
- Dihydroxy acid dehydratase: *DHAD*, *Ilv3* or *IlvD*
- 2-ketoacid decarboxylase: *KDC* or *Kivd*
- Alcohol dehydrogenase: *ADH*

Conclusions

- It is possible to obtain isobutanol using genetically modified organisms containing isobutanol's biosynthetic route (Figure 1).
- It is also possible to improve isobutanol's production by means of using some of the aforementioned techniques.
- Both bacteria and yeast are able to produce isobutanol. Nevertheless, bacteria present higher yields, being able to reach higher isobutanol concentrations.

References

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