Biotechnological Applications of Metabolic Network Analysis

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Outline

- Maximal Yields of Biotechnological Processes
- Pathway Design for Improved Yield
- Channelling Metabolism into Desired Routes

Maximal Yields of Biotechnological Processes

Amino Acid Production

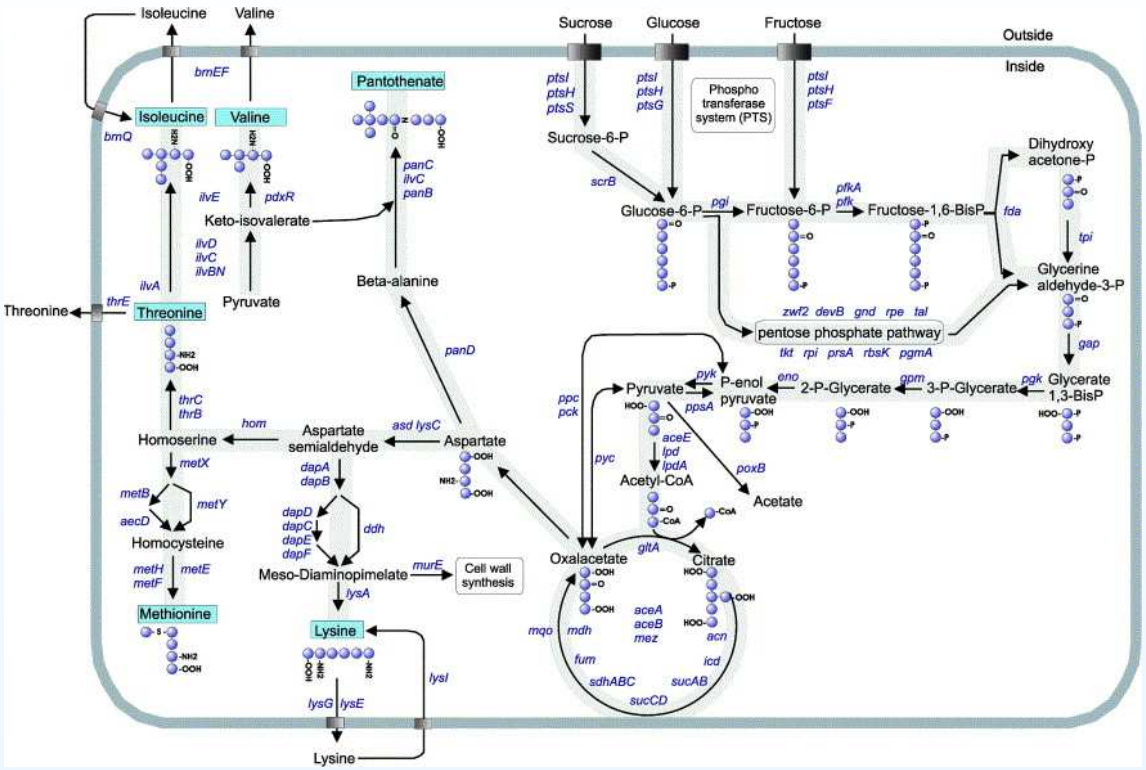
aa	Annual producn. tons	Use
glu	1,500,000 (2001)	flavouring
lys	300,000 (1996)	animal feed
thr	1000	animal feed
met	10,000	animal feed
ile	400	infusion solutions
		dietary products
val		ditto
leu		ditto
phe	12,000 (1997)	aspartame sweetener
trp	600 (1997)	animal feed
his		pharmaceutical
tyr	150 (1997)	pharmaceutical

Producing Strains

1. *Corynebacterium glutamicum*. Isolated as glu producer by Kinoshita et al, 1957. *Corynebacteria* are rod-shaped Gram +ve, not usually motile, generally aerobic, oxidising a wide range of organics. Many are soil organisms, some animal parasites (*C. diptheriae*). Principal organism (inc. ssp. *flavum* and *lactofermentum* for: glu, lys.

2. *E. coli*. Main advantage is well–studied, with good genetic manipulation systems. But amino acid metabolism has more complex regulation, and forms acetate even in aerobic conditions. Organism for thr and aromatic aas.

Lysine Production



- Not produced by wild–type *C. glutamicum*. Producer isolated by mutagenesis and selection for resistance to inhibitory analogues (e.g. S-2-aminoethyl-L-cysteine).
- Some strains possess feedback–resistant aspartate kinase.
- *C. glutamicum* has an inducible, energy–dependent lysine exporter (Kramer & Broer, 1991).

Maximum Attainable Yield of Lysine

Approaches to calculating this have included:

- Input–output stoichiometry
- Pathway tracing and accounting
- Structural analysis of the network, by elementary modes analysis and/or linear programming

Input–Output Stoichiometry

If we assume that glucose, oxygen and ammonia are available to make lysine, and water and CO_2 can be products, then a possible equation of lysine synthesis is:

$$-a.C_6H_{12}O_6 - b.O_2 - c.NH_3 + C_6H_{14}N_2O_2 + d.CO_2 + e.H_2O = 0$$

The values of a—e must produce a net zero for each of the elements in the equation.

For example, balancing N requires:

$$-c + 2 = 0$$

The H balance leads to:

$$a = \frac{e+4}{6}$$

Input–Output Stoichiometry 2

The C balance gives d in terms of a.

Substituting a, c and d in the O balance leads to:

$$b = e - 3$$

Assuming b cannot be negative, i.e. no oxygen evolution, then $e \geq 3$.

The largest theoretical molar yield of lysine per glucose is then $\frac{1}{a}$:

$$\frac{1}{a} = \frac{6}{e+4} = \frac{6}{7} = 86\%$$

But can this be implemented in the *C. glutamicum* metabolic network?

Example from “Metabolic Engineering”, Nielsen et al.

Yield by Network Analysis

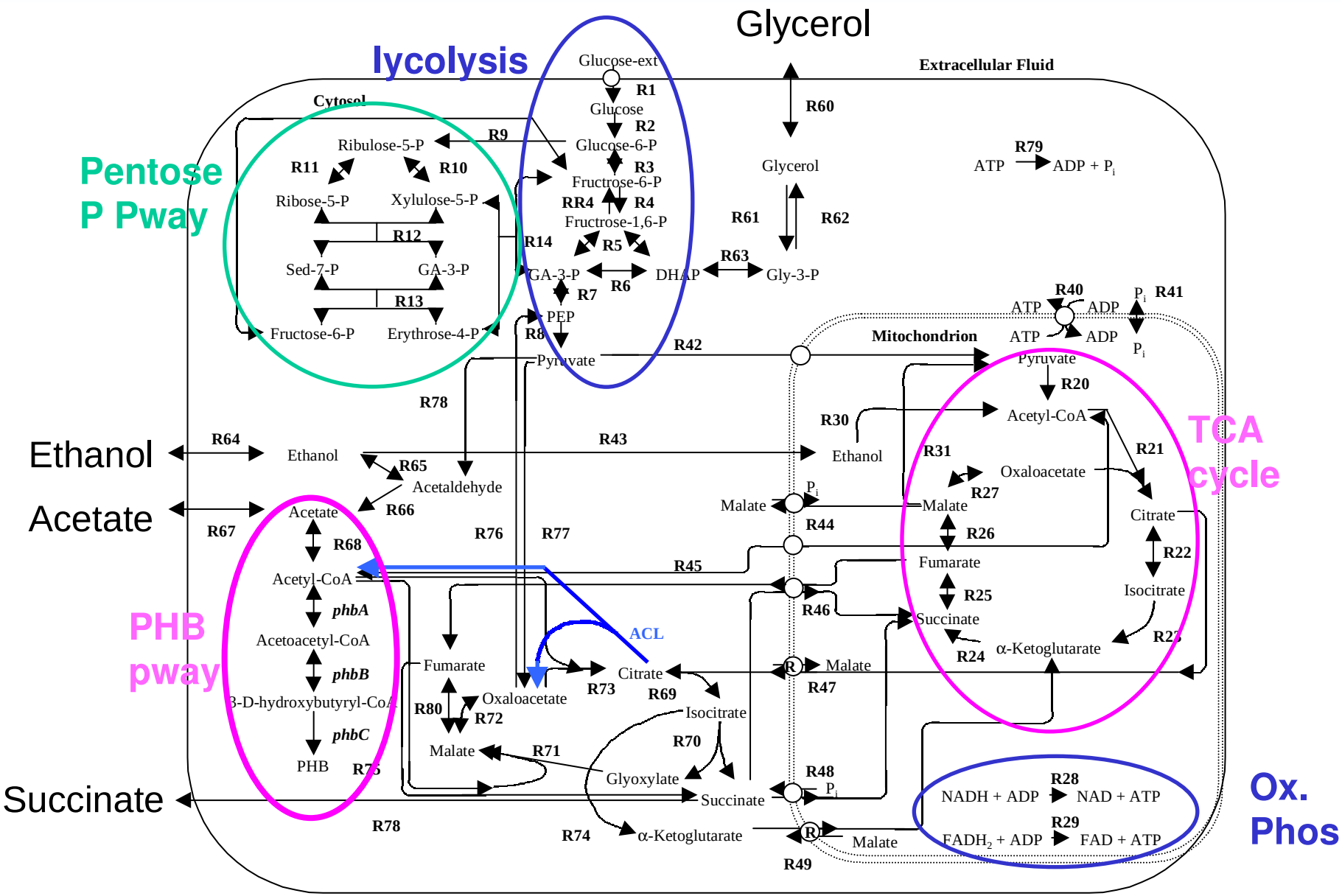
- Elementary modes analysis or Linear Programming (Flux Balance Analysis) show a variety of possible molar yields from 60%, to 75% to 86%.
- The highest value is only achievable if there is a transhydrogenase to exchange NADH for NADPH, and this is not thought to be present in *C. glutamicum*.
- In the absence of transhydrogenase, the yield of 75% requires that no PEP is lost as pyruvate through the pyruvate kinase reaction, and in fact excess pyruvate (formed in the PTS system of glucose uptake) is used by pyruvate carboxylase or PEP synthase that would need to be added to the native metabolic network.

Pathway Design for Improved Yield

Biodegradable Plastics

Polyhydroxybutyrate or polyhydroxyalkanoates

Polyhydroxybutyrate Synthesis in Yeast



Optimal yields of PHB synthesis

Based on highest–yielding elementary modes of the network:

Wild-type yeast + PHB pathway

1. 2 Acetate + EtOH $\rightarrow$ PHB + 2 CO_2 0.67
2. 65 Ac. + 31 EtOH $\rightarrow$ 30 PHB + 72 CO_2 0.63

(Number following each mode is the fractional carbon conversion.)

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Wild-type yeast + ATP–citrate lyase + PHB pathway

3. 12 EtOH $\rightarrow$ 5 PHB + 4 CO_2 0.83
4. 77 EtOH + 31 Glycerol $\rightarrow$
   48 PHB + 4 Ac. + 47 CO_2 0.78

(Number following each mode is the fractional carbon conversion.)

Channelling Metabolism into Desired Routes

Ethanol from Plant Waste

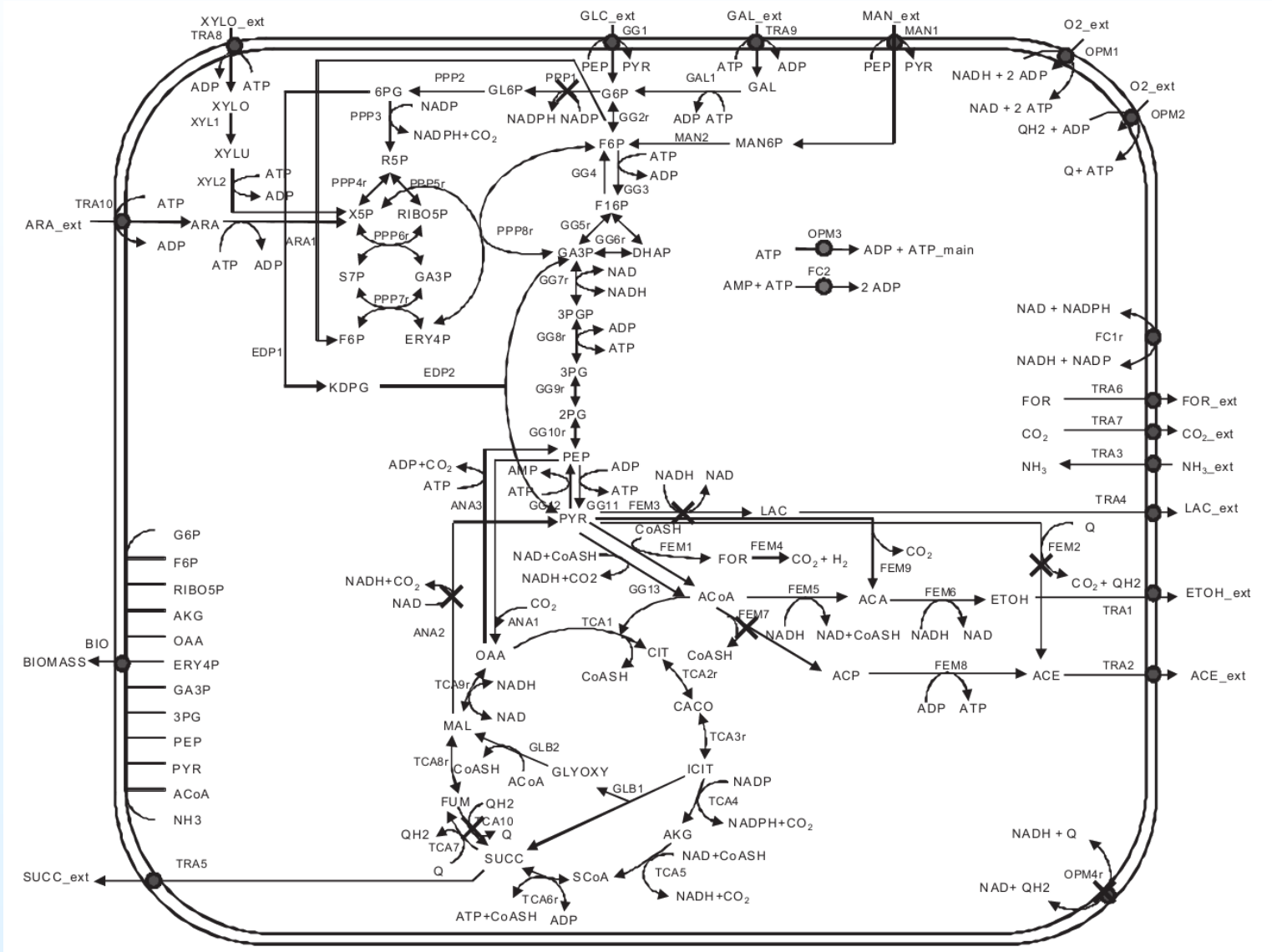
Some of the issues:

- Plant wastes (e.g. straw) contain cellulose and hemicellulose which can be hydrolysed to glucose and pentose sugars.
- Yeasts convert glucose to ethanol, but don't readily use the pentoses.
- *Escherischia coli* can use pentoses as well as glucose, but ethanol is not its preferred product.
- E. coli is easy to engineer, but can it be modified to make ethanol from pentoses in such a way that it cannot mutate back to its original state?

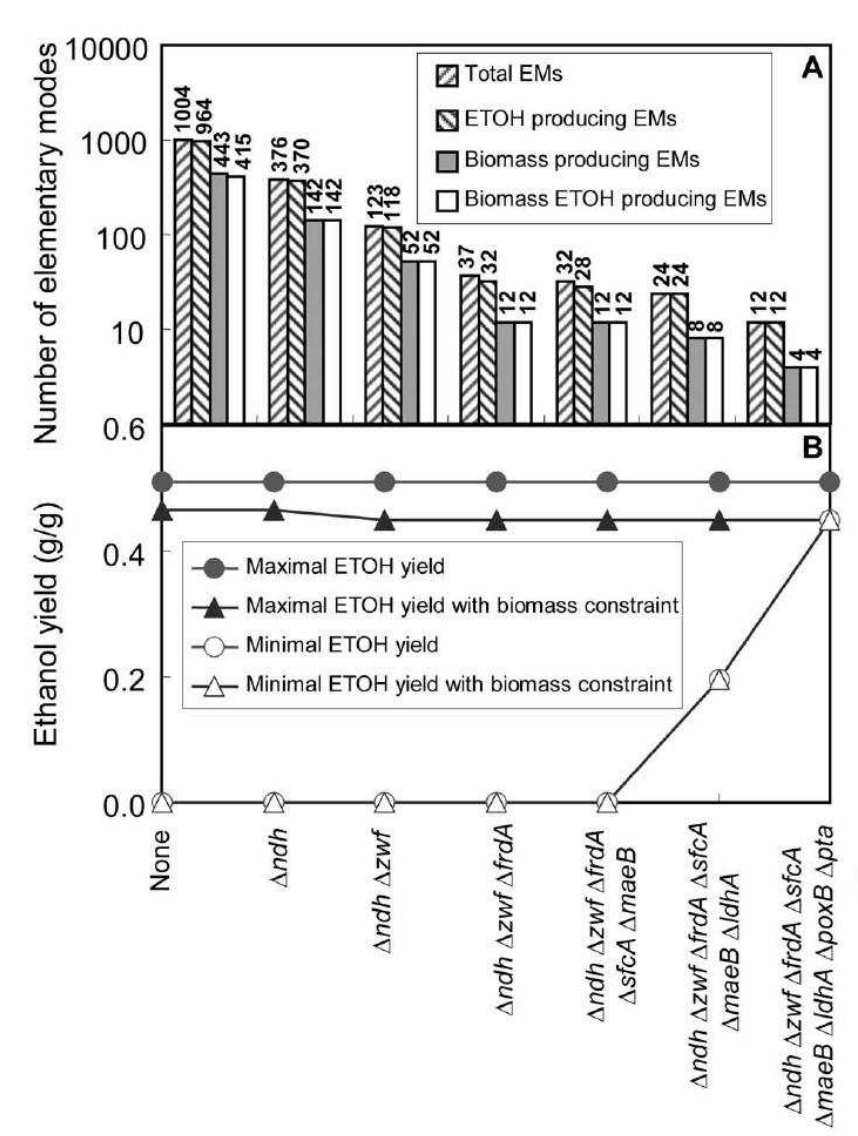
A Demonstration Solution

- Friedrich Srienc's group (Trinh et al, Appl. Env. Microbiol, 74, 3634-3643, 2008)built a medium-sized structural model of E coli central carbon metabolism.
- They computed the elementary modes leading from glucose and pentoses to products including ethanol and biomass.
- They searched for reactions that were *needed* for modes leading to other products but which were *not needed* for *some* of the routes to biomass and ethanol.
- They found a set of *eight* reactions that between them disabled all the modes except those leading to either ethanol alone or biomass and ethanol.
- They made a the deletion mutants and obtained close to the theoretically-predicted yields of ethanol.

The Model



The Analysis



Redirecting Metabolism into a Synthetic Pathway

3–HydroxyPropionate Production in *C. necator*

Nicole's slides

Conclusion

- Don’t overlook the value of an atomically-balanced stoichiometric equation for an overall metabolic process, though it only works if you already know the identities — and elemental composition — of the inputs and outputs involved.
- Elementary modes analysis can be used to design metabolic network modifications to obtain improved yields.
- Strategies can include both addition of heterologous enzymes to provide new routes, or deletion of native enzymes to block unproductive routes.