Synthetic pathways for aromatics production from biobased feedstock

Harald Ruijssenaars

BIRD Engineering

Delft-Schiedam, The Netherlands

BIRD Engineering

Contract research company in Delft, The Netherlands

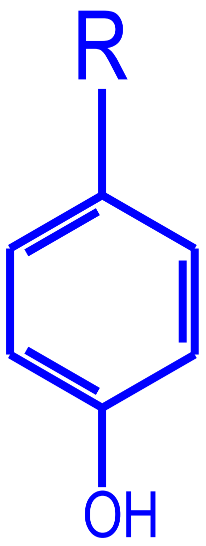
- fermentation / medium optimization services
- strain development services (yeast; bacteria)
- non-food market (biofuels, bio-based chemicals)

Collaboration / Customers

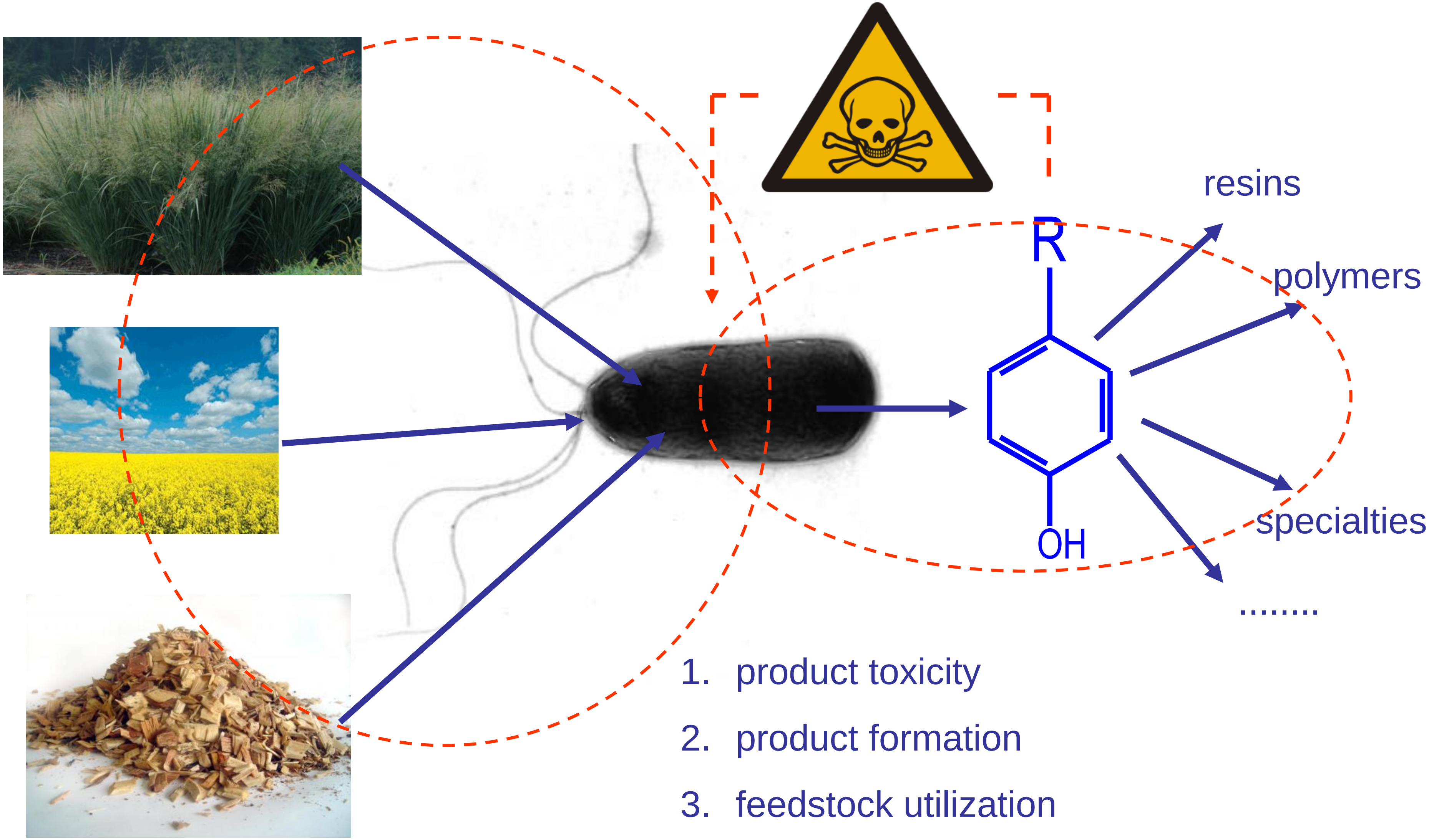
- (a.o.) Mascoma, Amyris, DSM, Tate&Lyle, Nedalco, Applikon, Heineken
- Delft University of Technology
- Knowledge networks: Kluyver Centre, BE-Basic, CLIB (Germany), SIM (USA)

Substituted (hydroxy-)aromatics

- many & diverse applications, *e.g.*, in plastics (LCP’s), resins, fibers
- fossil-based
- often difficult to synthesize chemically
  - improved / novel functionality with biotech



Biocatalytic production of aromatics - bioconversion



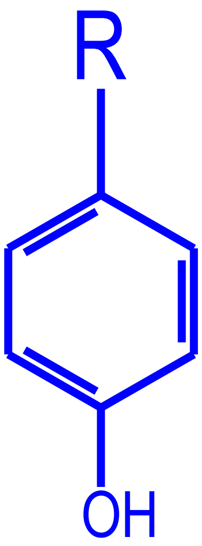
1. product toxicity
2. product formation
3. feedstock utilization

Product toxicity aromatics

Challenge for bioproduction:

•hydrophobic molecules (“solvents”)

•accumulation in cell membrane: cell death



Solvent tolerant host: *Pseudomonas putida* S12

Grows in presence of:

- 2^{nd} phase of toluene (7.2 mM in water phase)
- 2^{nd} phase of 1-octanol (4.2 mM)
- benzene (mutant up to 25 mM – near saturation)
- butanol (up to 6 %)

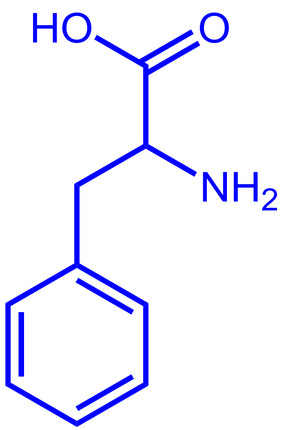
Volkers et al., Env. Microbiol. Rep. (2010) 456-60

Rühl et al., AEM 75 (2009) 4653-6

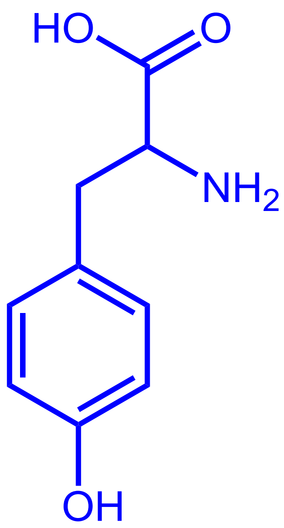
Synthetic pathways for aromatics synthesis in *P. putida* S12

Native pathways: aromatic amino acid synthesis

L-phenylalanine and L-tyrosine as ‘base compounds’ for production of non-native (‘synthetic’) aromatics

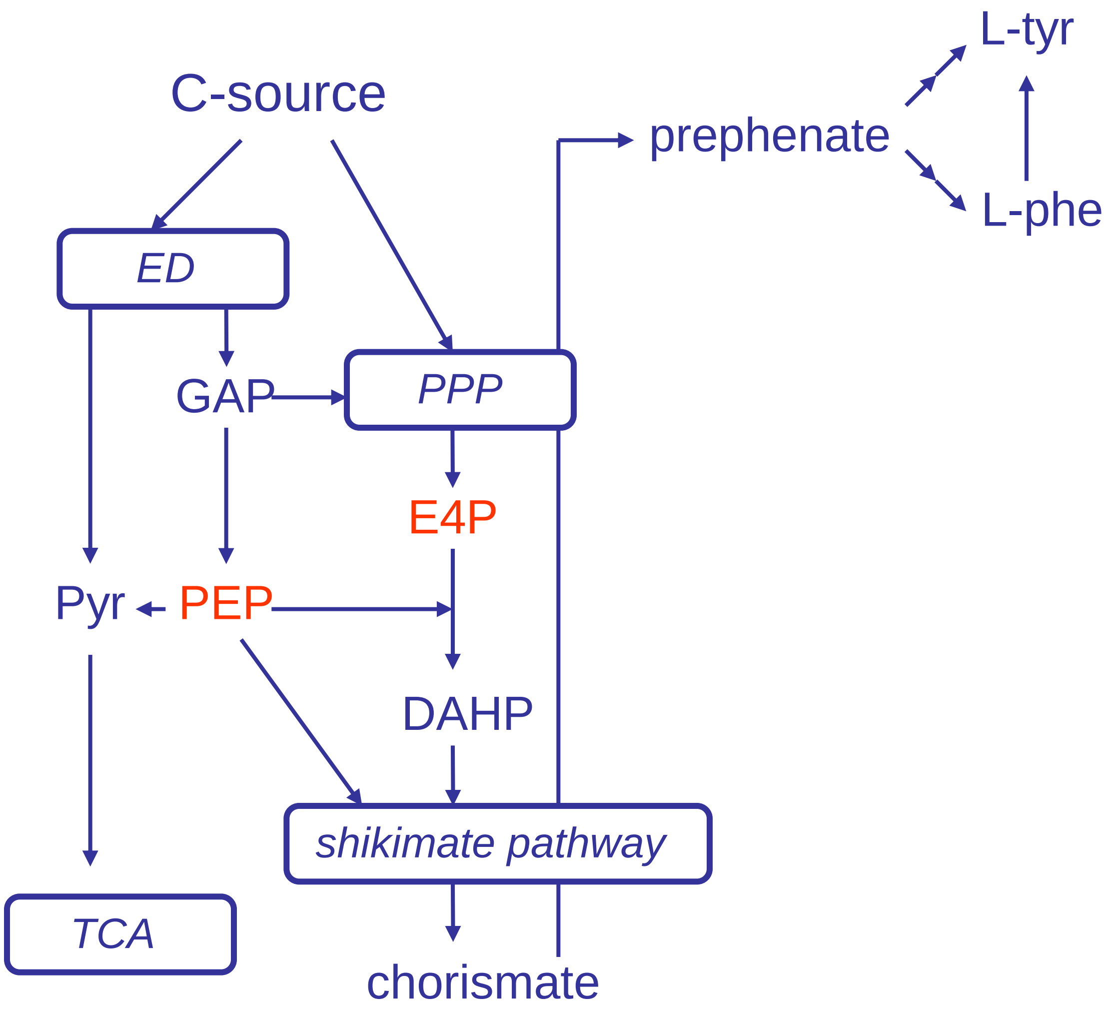


L-phe

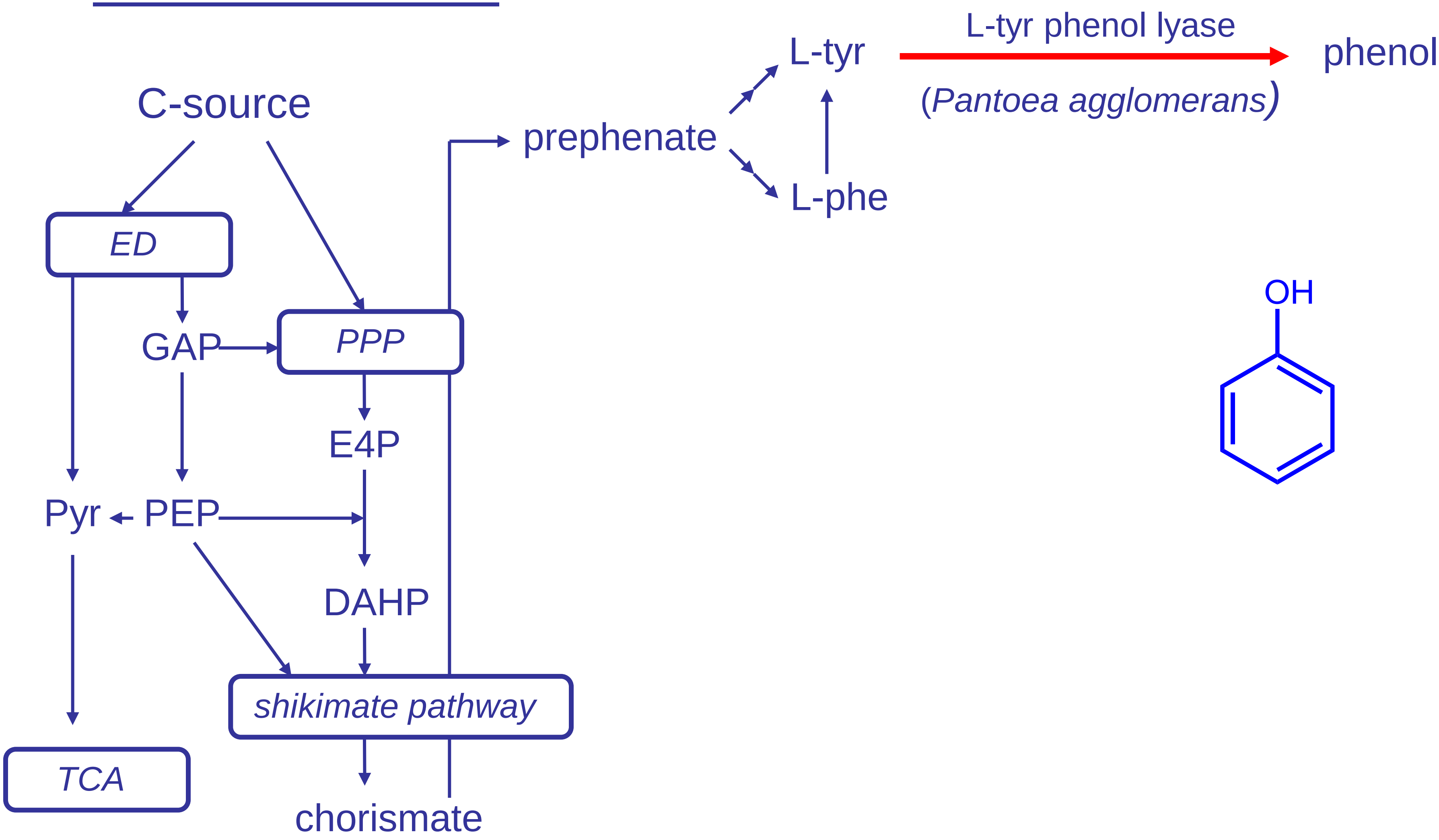


L-tyr

Product formation

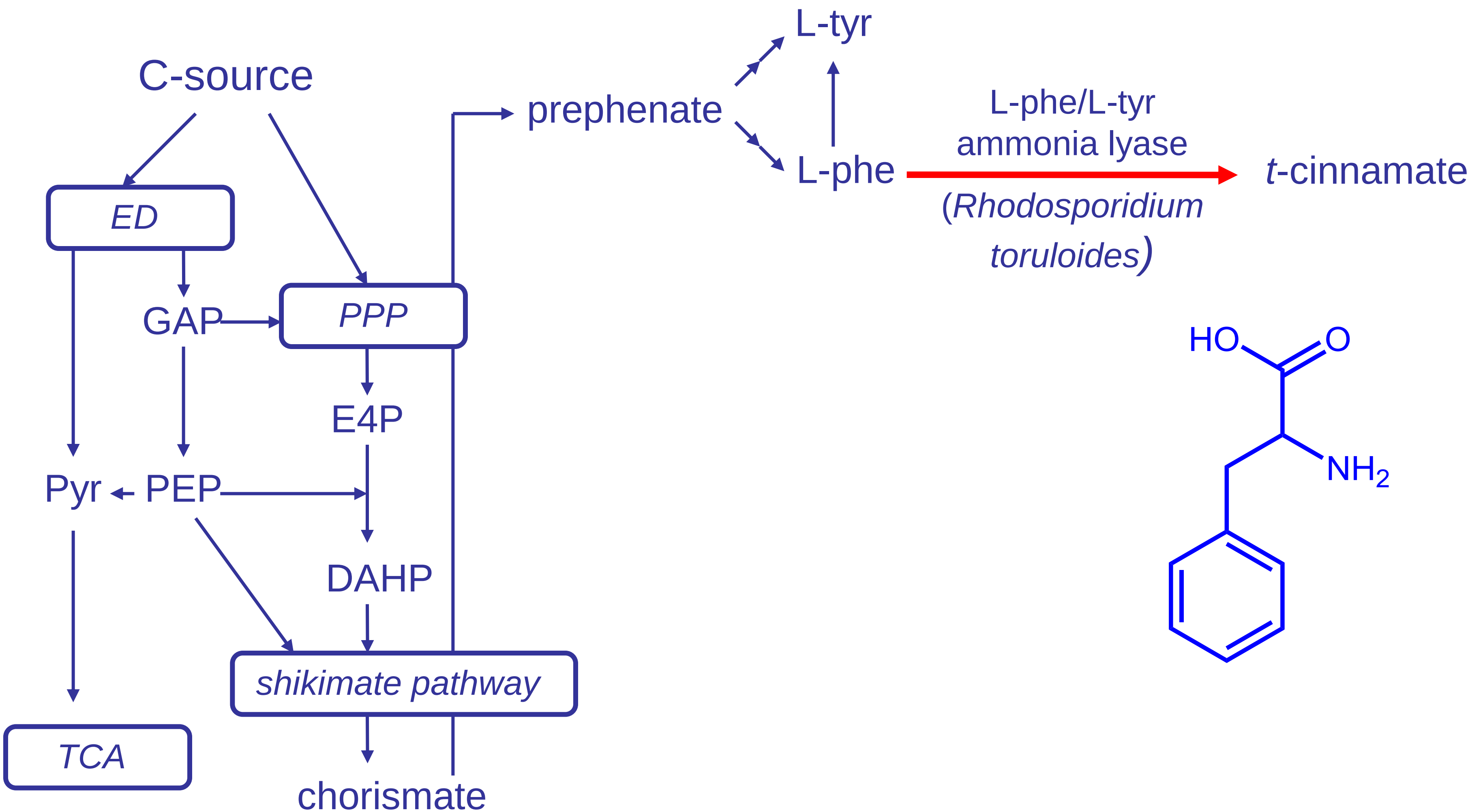


Product formation



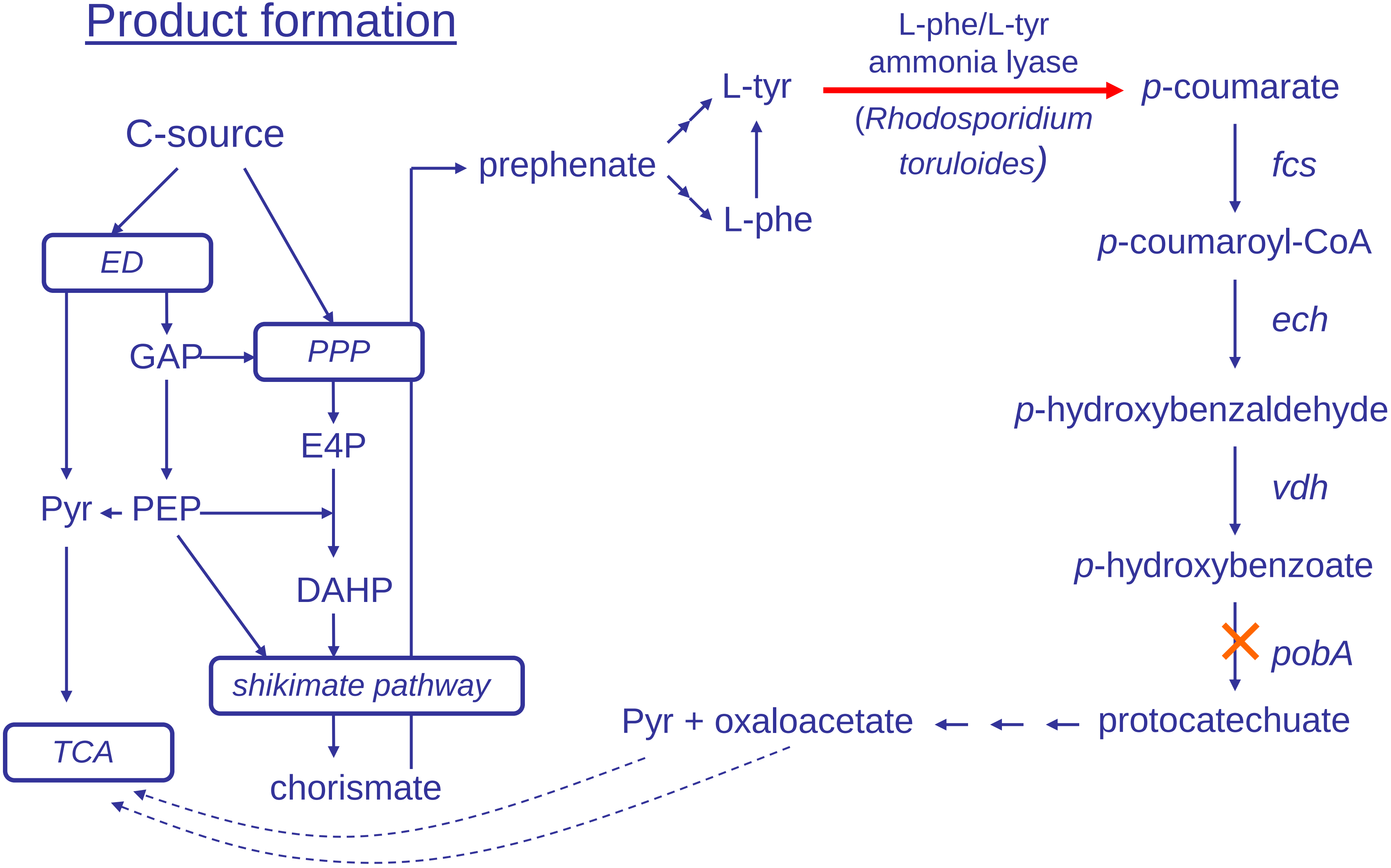
Wierckx et al., J. Bacteriol. 2008

Product formation

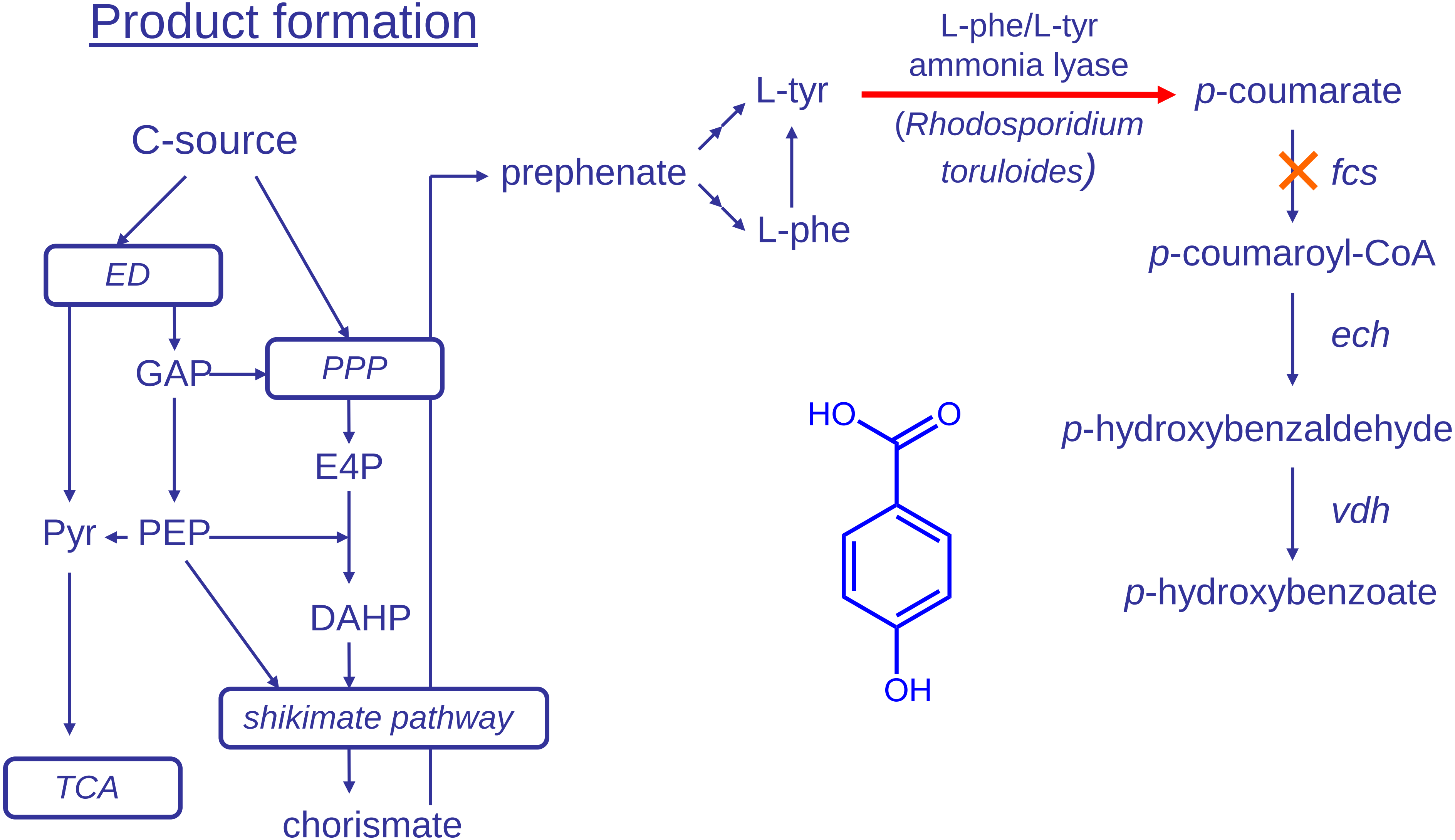


Nijkamp et al., Appl. Microbiol. Biotechnol. 2005

Product formation

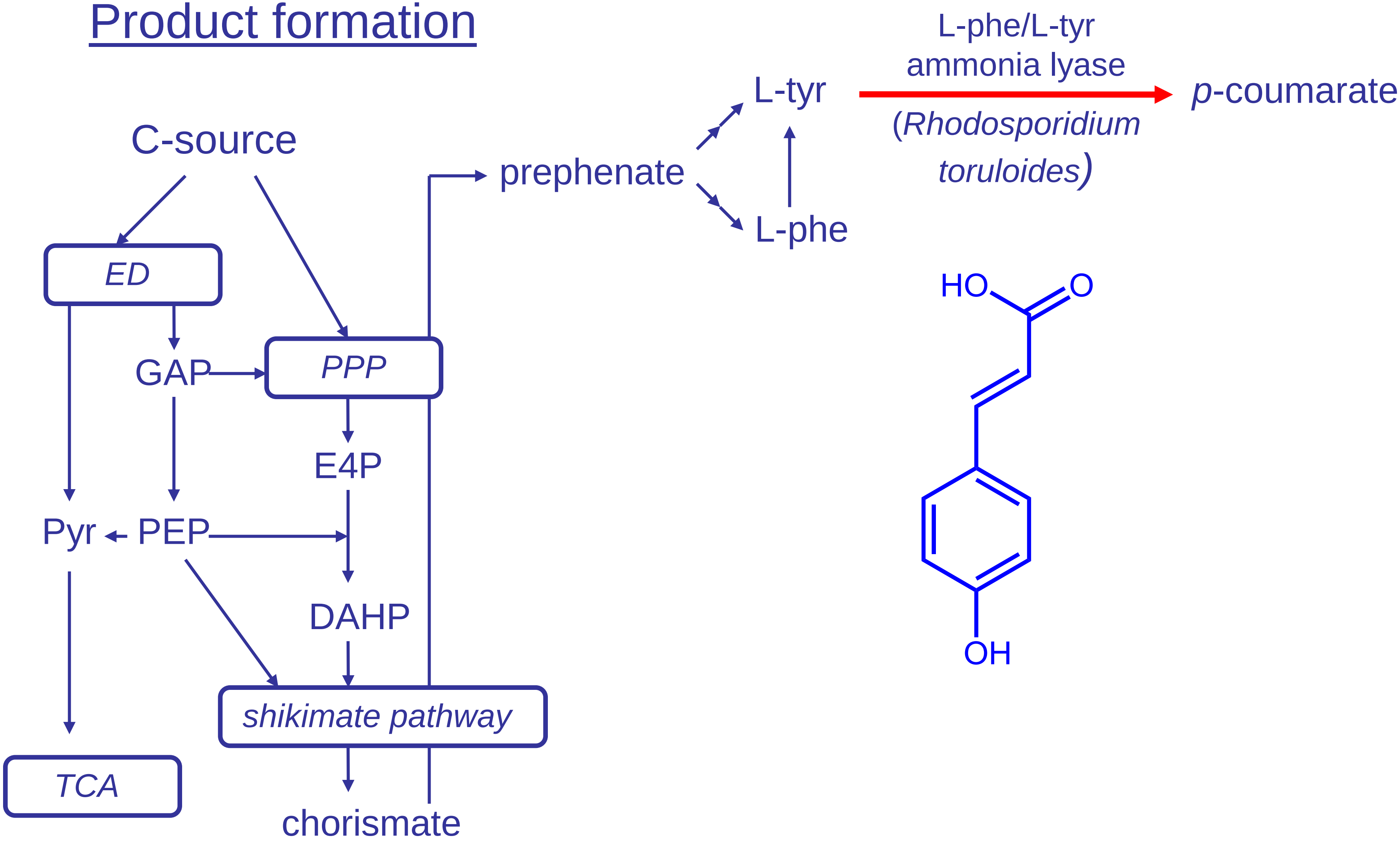


Product formation



Verhoef et al., J. Biotechnol. 2007

Product formation



Nijkamp et al., Appl. Microbiol. Biotechnol. 2007

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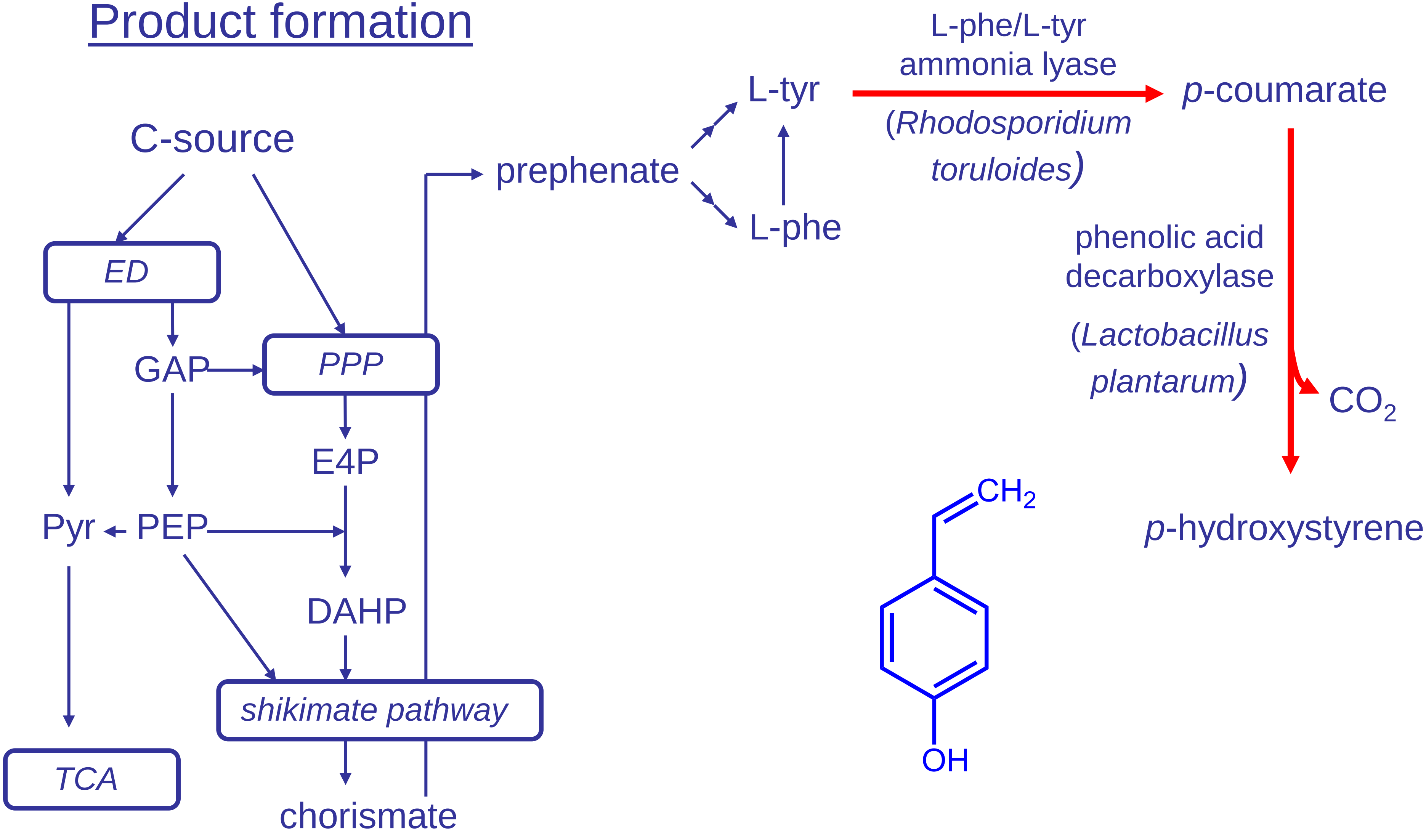
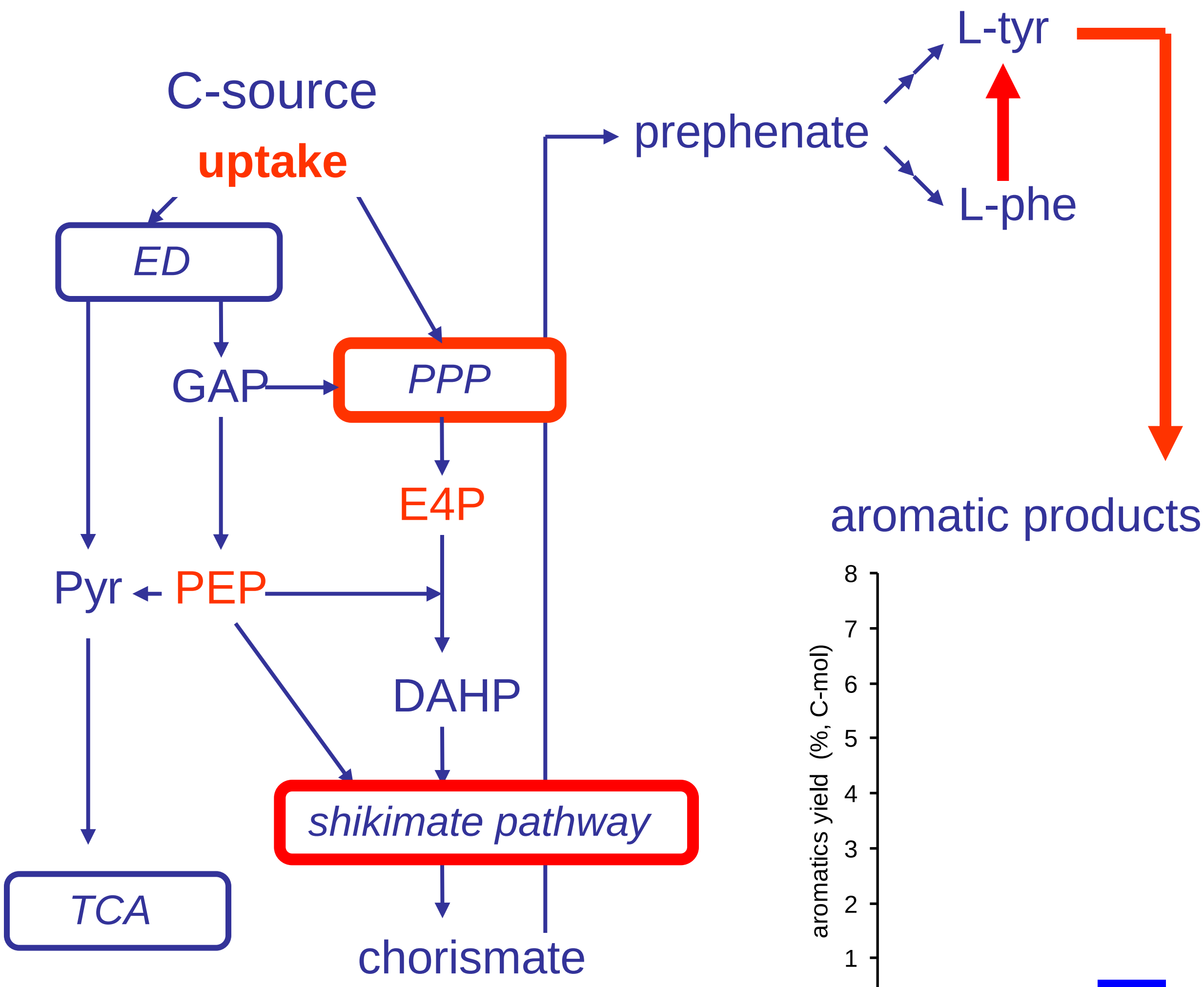


Verhoef et al., AEM. 2009

Product formation – synthetic network improvement

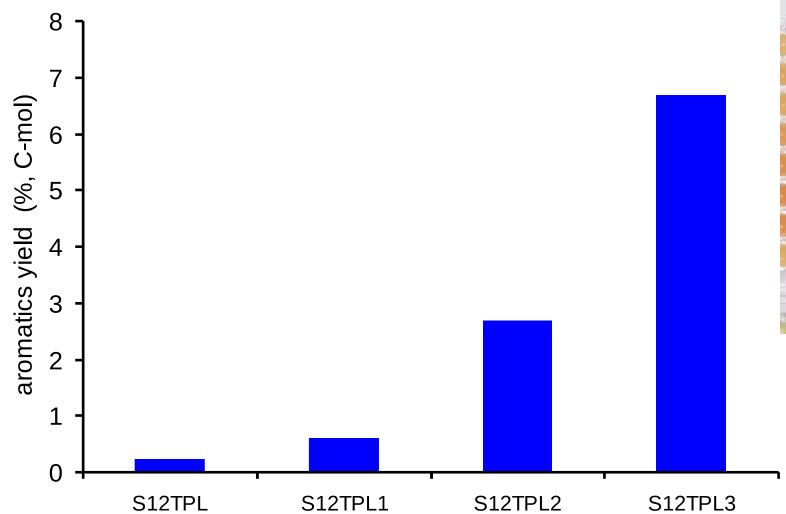


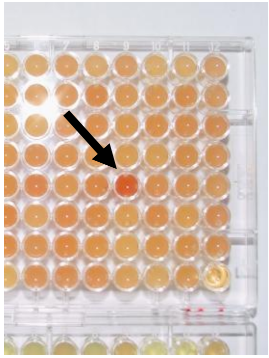
targeted and random mutagenesis

screening and selection

improved flux to L-tyr?

transcriptomics and flux analysis





B-Basic
Bio-based Sustainable Industrial Chemistry

Wierckx et al., J. Bacteriol. 2008

Wierckx et al., J. Biotechnol. 2009

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Feedstock use

Biobased feedstock

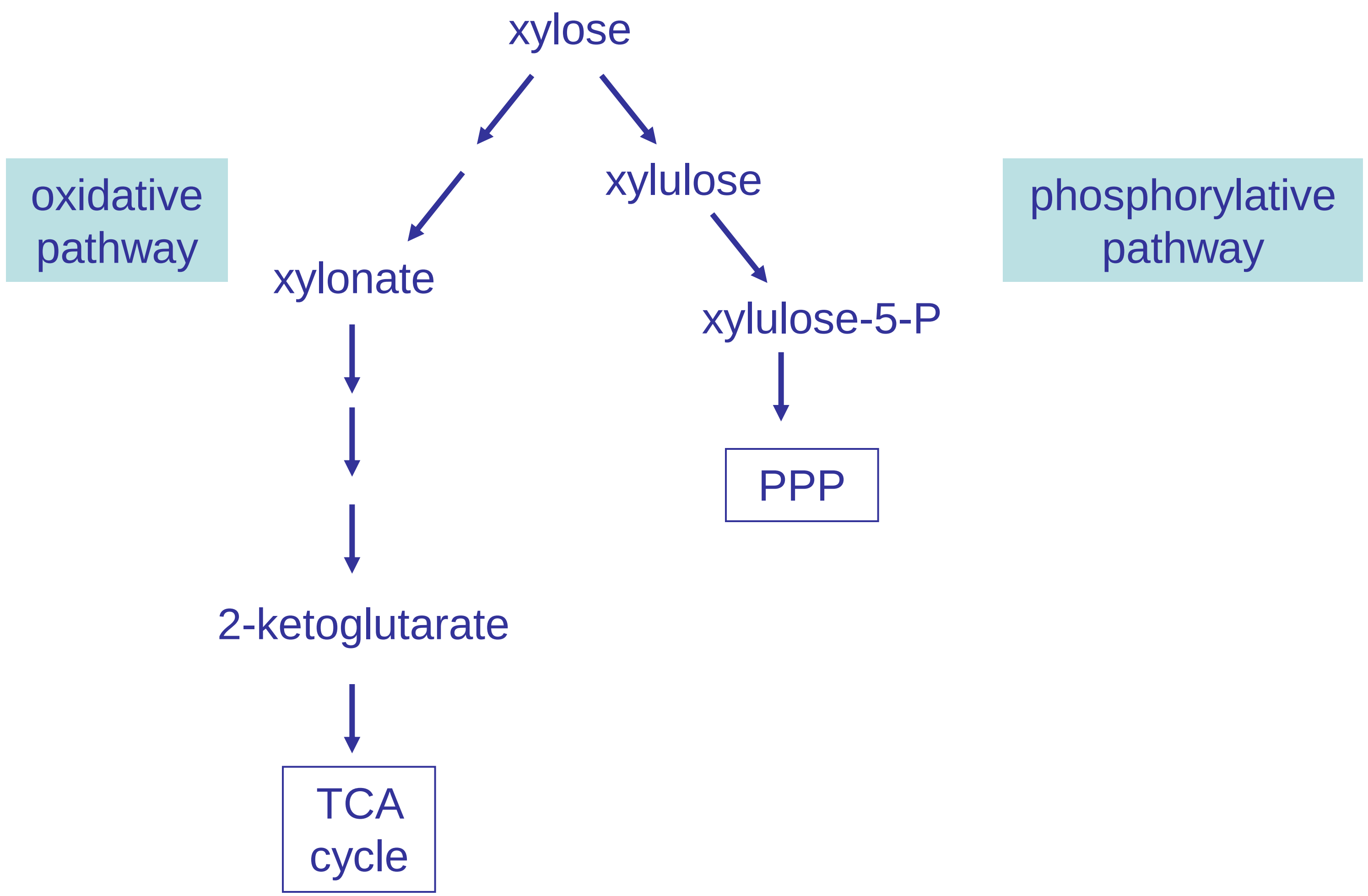
- multiple components: LC hydrolysate -> glc, xyl, ara, other sugars
- inhibitors: acetate, furaldehydes, aromatics

		WT *P. putida* S12
glucose	35.4 %	**+**
xylose	20.2 %	**-**
arabinose	2.4 %	**-**
uronic acid	2.5 %	**+**
org. acids (Ac^-, formate)	4.8 %	**+**
furaldehydes (HMF, furfural)	0.7 %	**-**
aromatics (lignin)	19.2 %	**+/-**

Synthetic biology: optimize efficient feedstock use

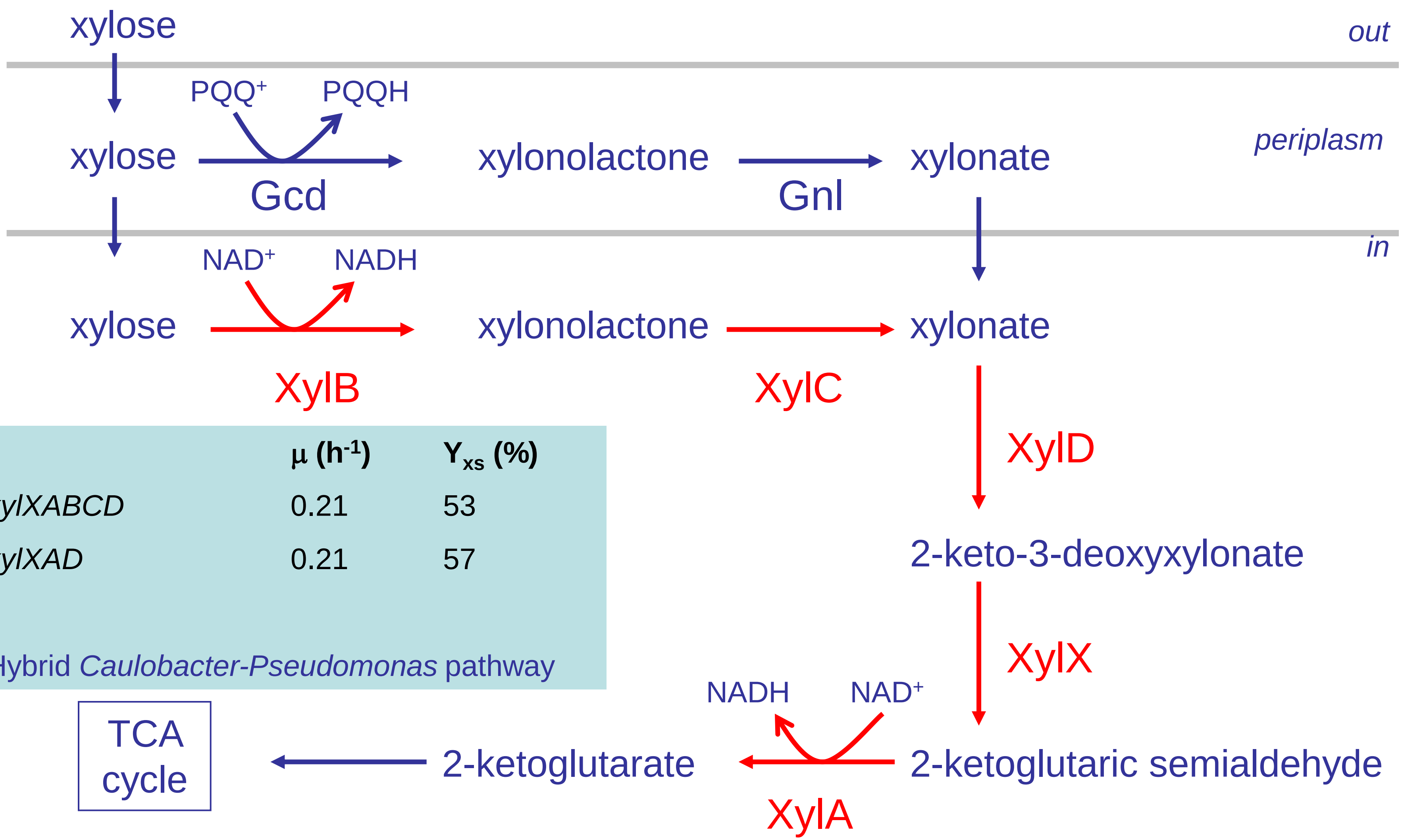
minimize effects inhibitors

Oxidative / phosphorylative xylose utilization



Xylose utilization - 1

Oxidative xylose pathway *Caulobacter crescentus*



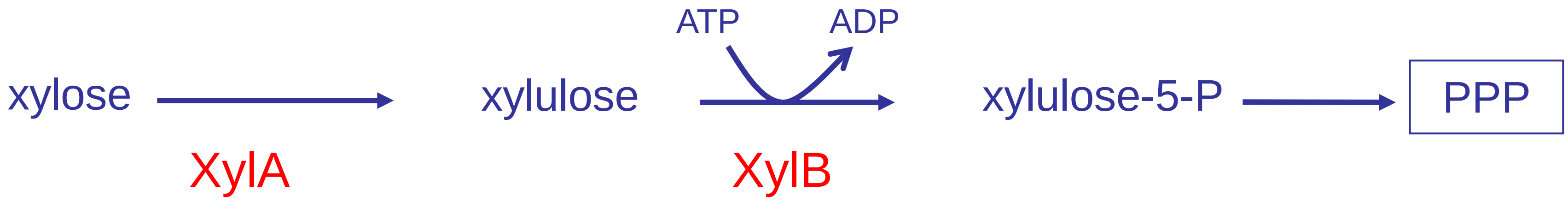
	μ (h^{-1})	Y_{xs} (%)
xylXABCD	0.21	53
xylXAD	0.21	57

Hybrid *Caulobacter-Pseudomonas* pathway

Meijnen et al., AEM 75 (2009) 2784-2791

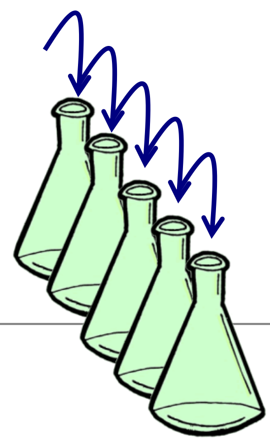
Xylose utilization - 2

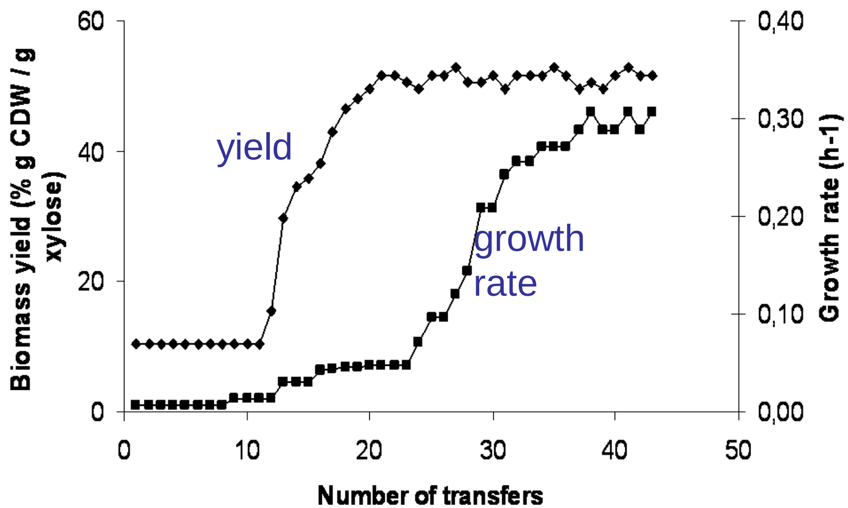
Phosphorylative xylose pathway *E. coli*



	μ (h^{-1})	Y_{xs} (%)
P. putida S12xylAB	0.01	10
P. putida S12xylAB2	0.35	67

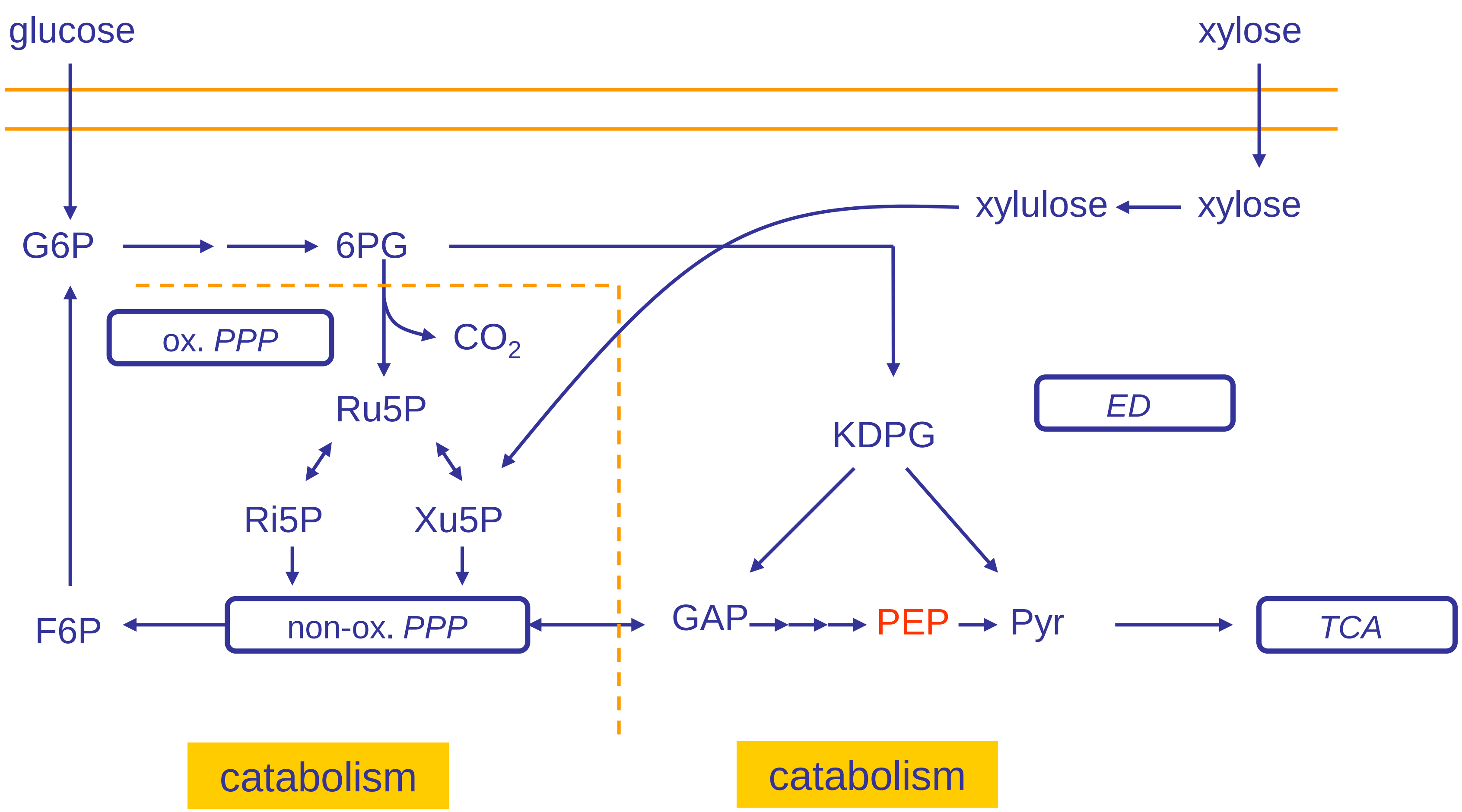
Strain improvement via evolutionary selection





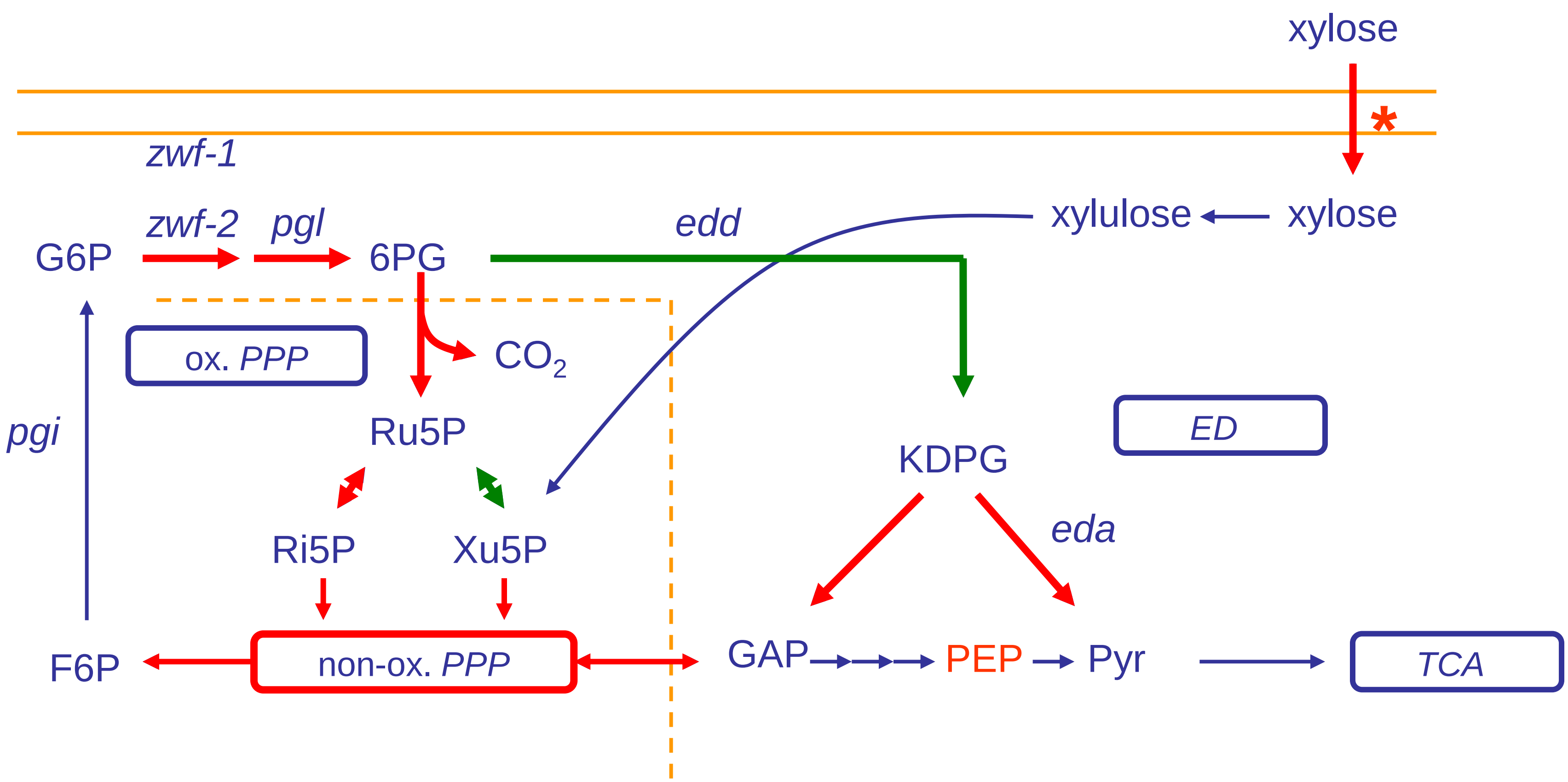
Meijnen *et al*. AEM 74 (2008) 5031-7

Molecular basis improved xylose utilization phenotype?



phosphorylative xylose metabolism requires extensive “rewiring” of the metabolic network!

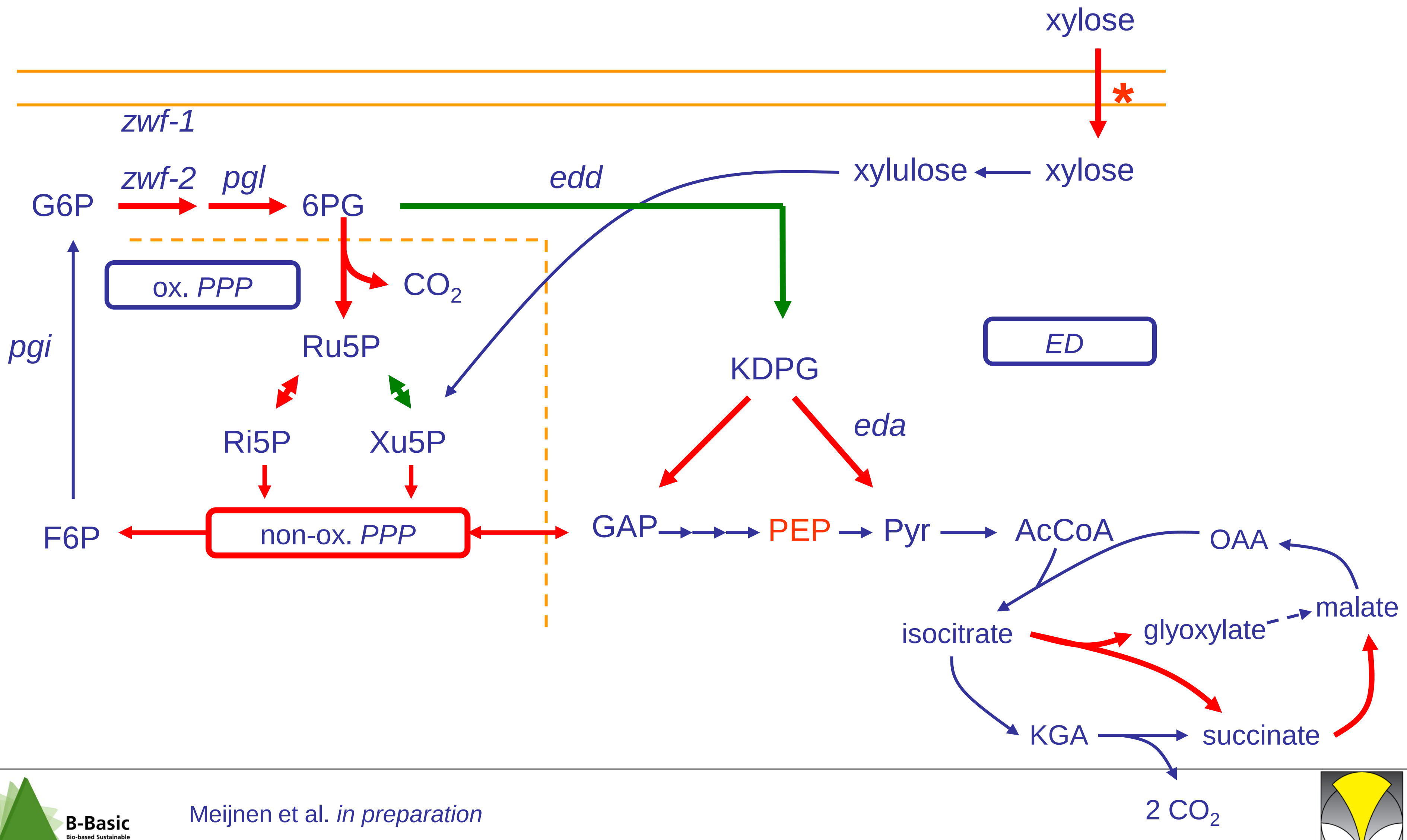
Transcriptomics evolved xylose utilizing phenotype



* mutated glc transporter

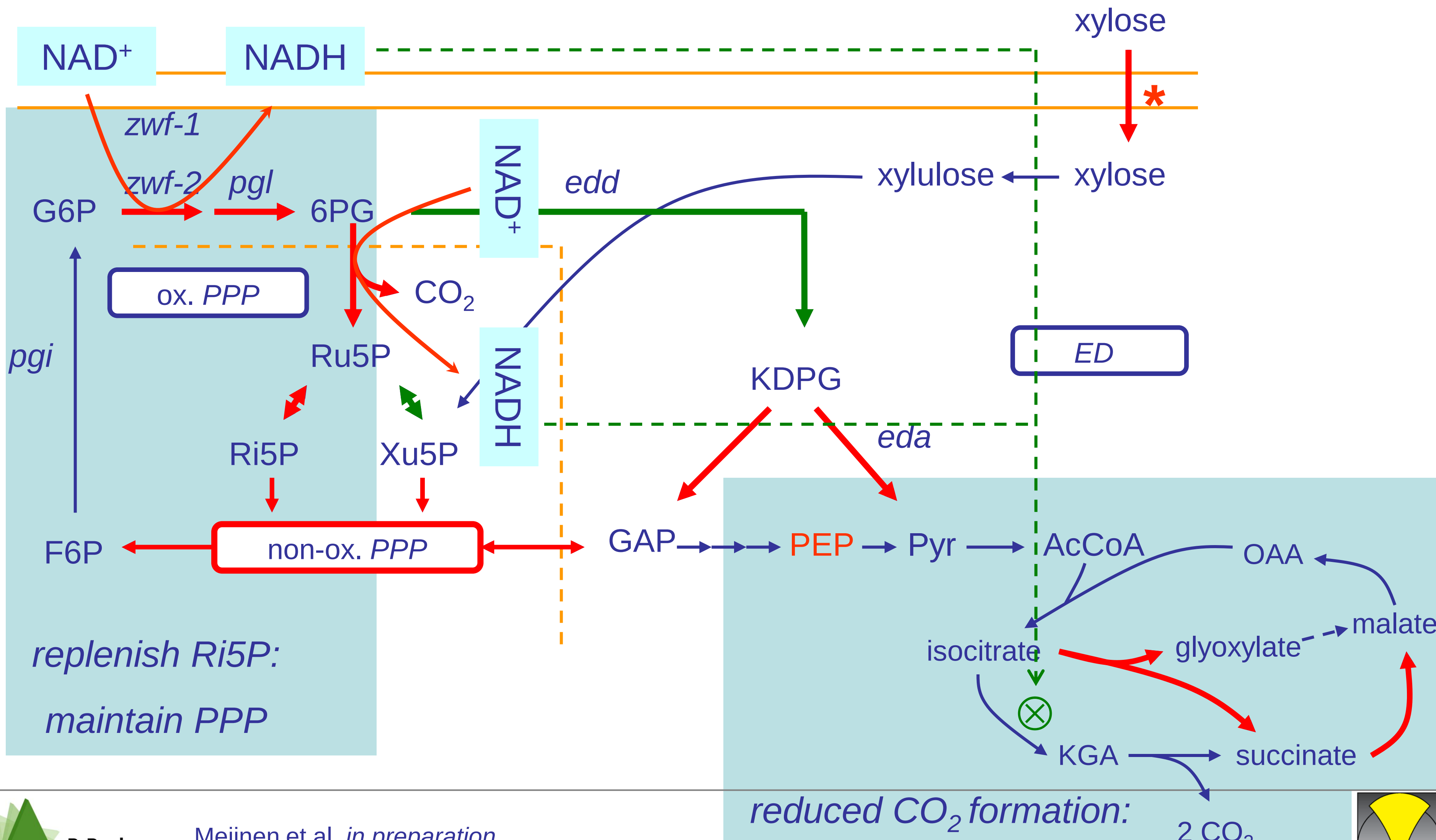
Meijnen et al. *in preparation*

Transcriptomics evolved xylose utilizing phenotype



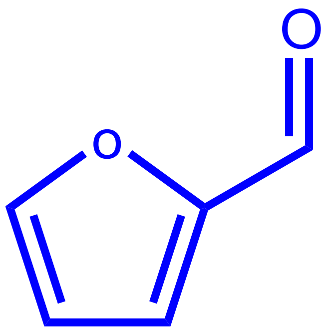
Meijnen et al. *in preparation*

Transcriptomics evolved xylose utilizing phenotype

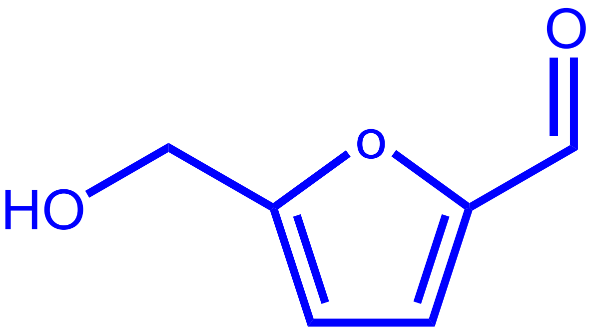


Meijnen et al. *in preparation*

Furaldehyde metabolism



furfural



hydroxymethyl
furfural (HMF)

Degradation products of pentoses (furfural) or hexoses (HMF)

Toxic fermentation inhibitors / carbon loss

No (genetic) characterization of catabolic pathways

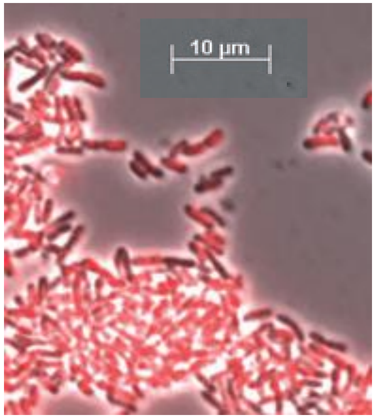
Few microorganisms known to degrade furaldehydes

Wierckx et al. (2010) Microb
Biotechnol 3: 336-343

Furaldehyde metabolism

Novel furfural / HMF degrading bacterium isolated: *Cupriavidus basilensis* HMF14

- Gram^{-} bacterium
- Mesophilic (<38 ºC), neutrophilic aerobe
- Growth on HMF, furfural, aromatics, NO sugars
- PHA production



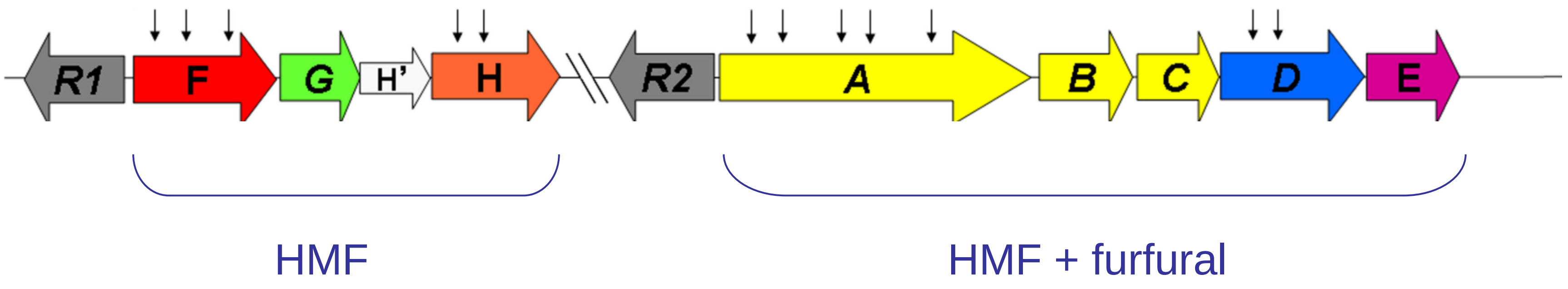
Identify HMF-furfural degradation genes by transposon mutagenesis

Wierckx et al. (2010) Microb Biotechnol 3: 336-343

Furaldehyde metabolic pathways of *C. basilensis* HMF14

Transposon mutagenesis

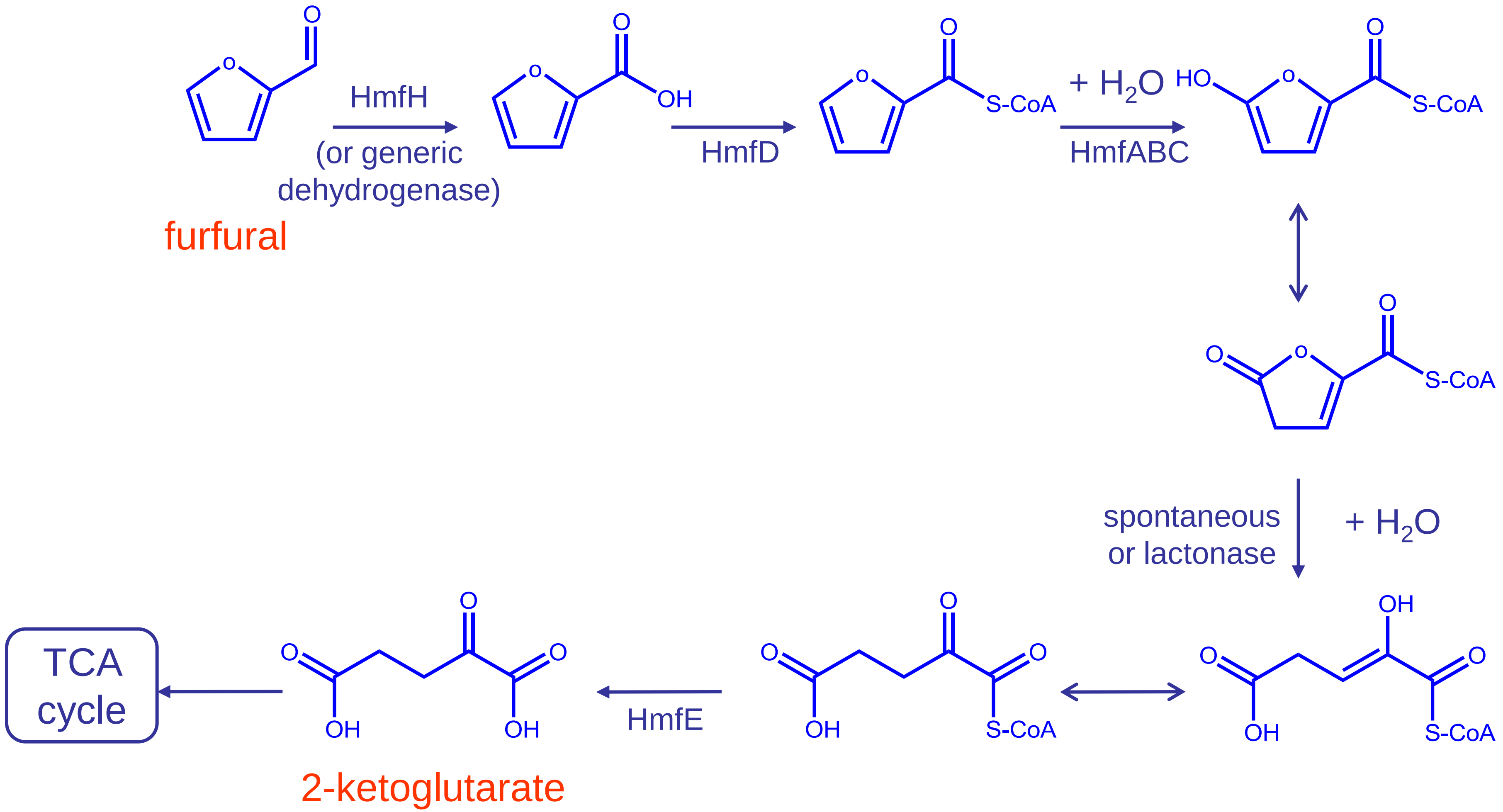
- Random insertions in genome: 14.000 clones
- Screen for HMF^{-} or furfural^{-} mutants: 25 clones
- Identify transposition loci: 8 genes in 2 clusters



Koopman et al. (2010)
PNAS 107: 4919-4924

Furaldehyde metabolic pathways of *C. basilensis* HMF14

functional analysis of gene clusters



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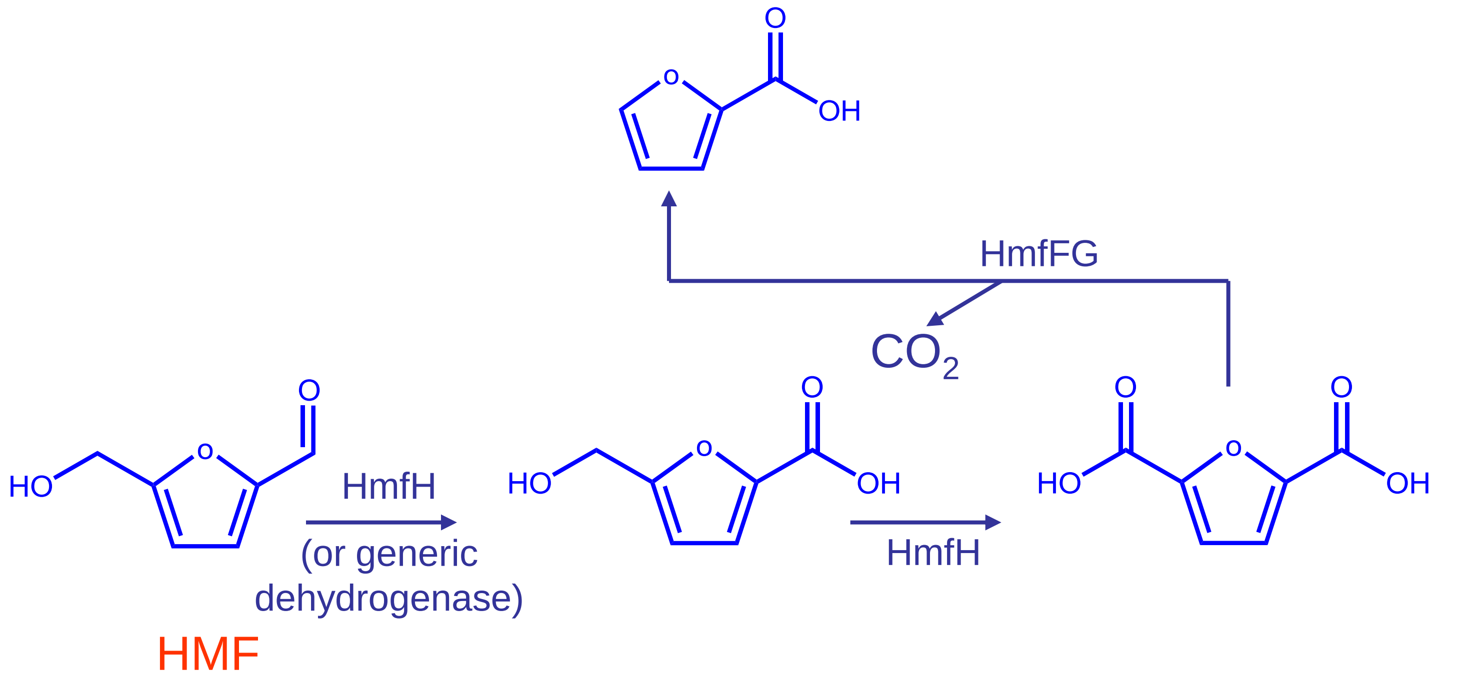

Koopman et al. (2010)

PNAS 107: 4919-4924

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Furaldehyde metabolic pathways of *C. basilensis* HMF14

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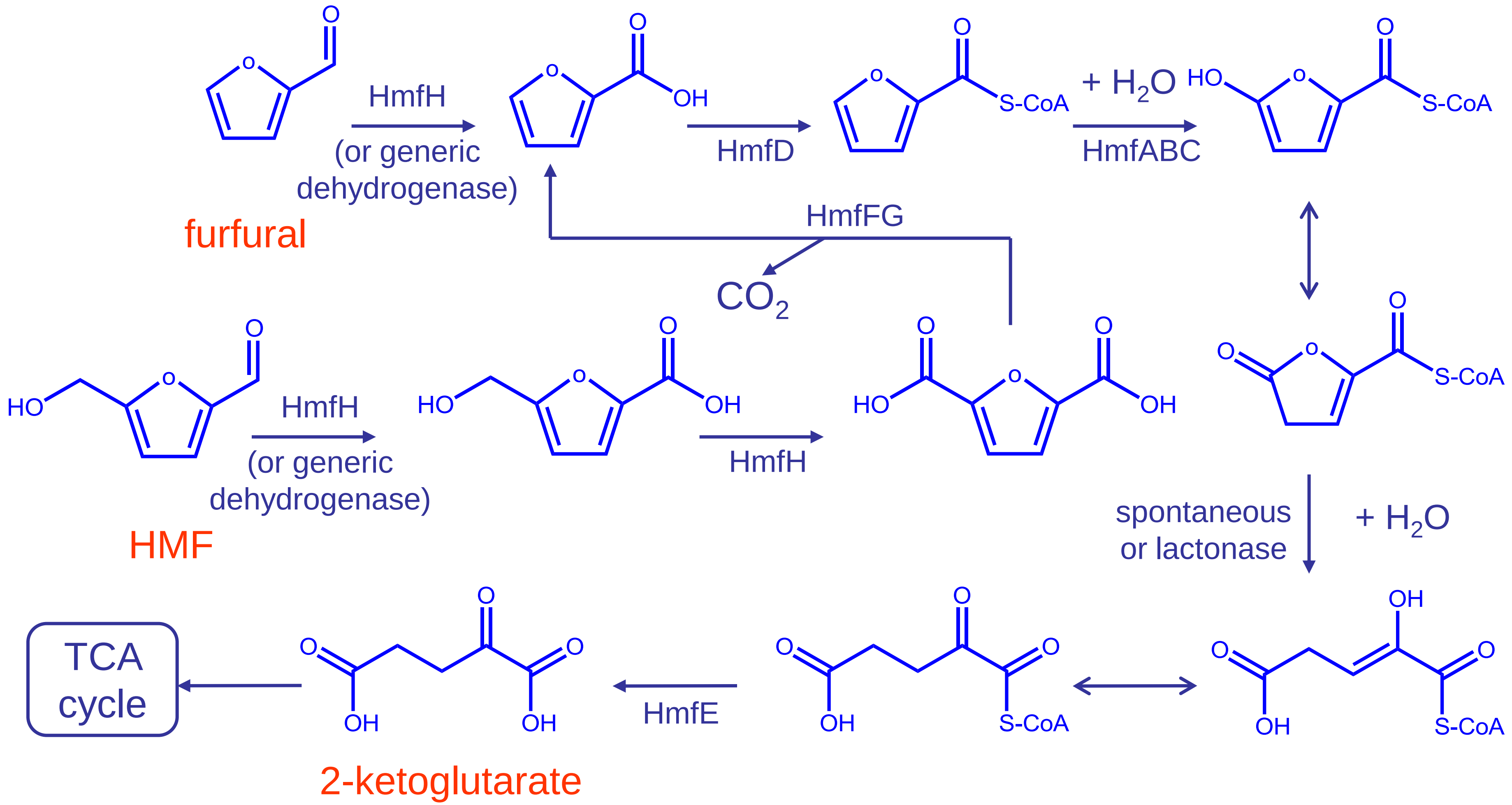

Koopman et al. (2010)

PNAS 107: 4919-4924

Furaldehyde metabolism in *P. putida* S12

	μ (h^{-1})	Y_{xs} (%)	
hmfABCDE	0.30	51	growth on furfural
hmfABCDE + *hmfFGH*	0.23	40	growth on HMF (and furfural)

		WT *P. putida* S12	**engineered *P. putida* S12**
glucose	35.4 %	+	+
xylose	20.2 %	-	+
arabinose	2.4 %	-	+
uronic acid	2.5 %	+	+
org. acids (Ac^-, formate)	4.8 %	+	+
furaldehydes (HMF, furfural)	0.7 %	-	+
aromatics (lignin)	19.2 %	+/-	+/-

Koopman et al. (2010)
PNAS 107: 4919-4924

Summary and conclusions - 1

Synthetic pathways constructed in *P. putida* S12 for:

- production of various aromatic products
  - *combined heterologous / endogenous activities / gene deletions*
- utilization of xylose (+ arabinose)
  - *oxidative / phosphorylative*
- utilization (detoxification) of furaldehydes
  - *novel pathway / genes isolated and characterized from environmental isolate*

Summary and conclusions - 2

Synthetic pathways may be:

•complete heterologous pathways

•hybrid heterologous / endogenous pathways

•“short-circuited” endogenous pathways

Synthetic pathways commonly need optimization

•improve metabolic flux towards (unnatural) product

•“rewiring” primary metabolic network

System-wide disturbance: optimization requires system-wide approach

•targeted / rational: extensive systems biology input (still underdeveloped)

•semi-targeted / random; classical strain improvement / evolutionary selection combined with system-wide analysis: pragmatic

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