

Pathway Reconstruction and Flux Quantification of Pentose Metabolism in Solventogenic Clostridia

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*Clostridium XII Conference, Nottingham UK
Sep. 10-12, 2012*

Comparative Genomic Reconstruction of Sugar Utilization Pathways

Important

- Central Machinery - main source of C and E
- Ecophysiology, adaptation, bioengineering
- Human microbiome, disease, nutrition

Challenging

- Variations - paralogs, NOGs
- Annotation nightmare

Feasible

- Genomic context - modules, operons
- Databases - SEED, RegPrecise
- Straightforward, testable

Transcriptional regulation

Central Carbon, Energy

Building Blocks

Sugar Utilization Pathways: knowledge from model bacteria; variations

Clostridium Genus

Strict anaerobes



Gram-positive endospore-forming Firmicutes

Various habitats



- Soil
- Aquatic sediments
- Intestinal tract of animals and humans

Diverse species



- Toxin-producing pathogens
- Species of biotechnological importance

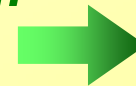
Genome resource



118 genomes sequenced

C. acetobutylicum

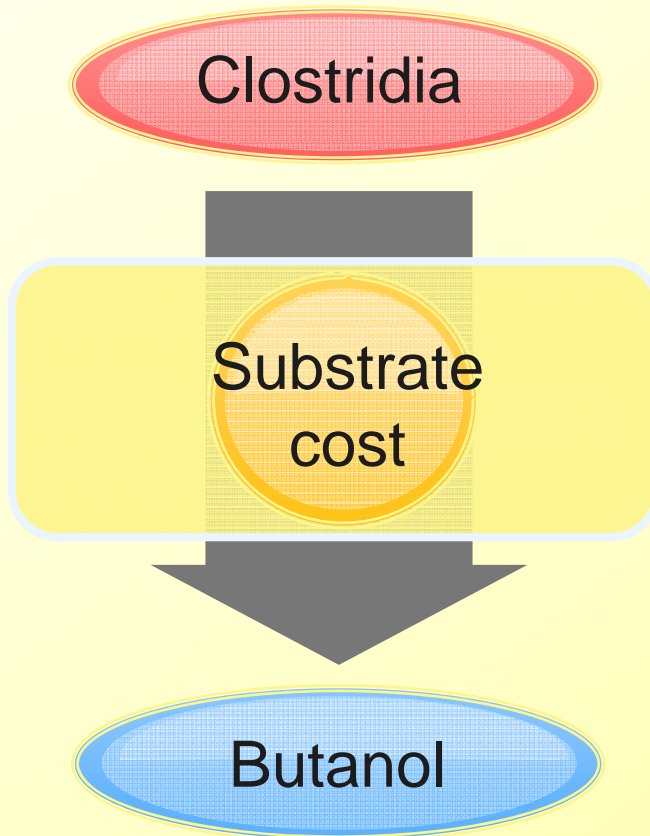
C. beijerinckii



Organic solvents:

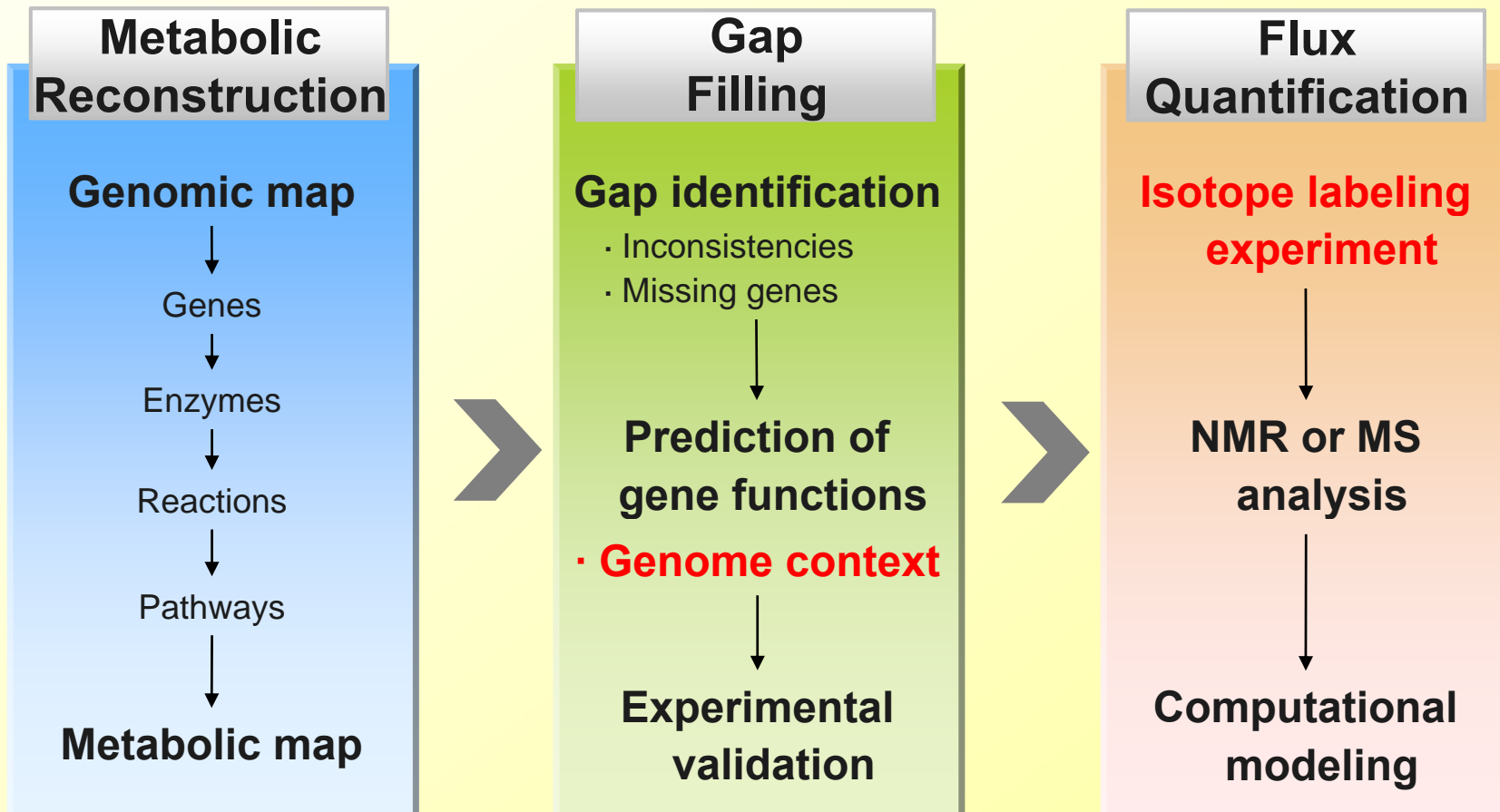
Butanol, acetone

Fermentative Butanol Production by Clostridia

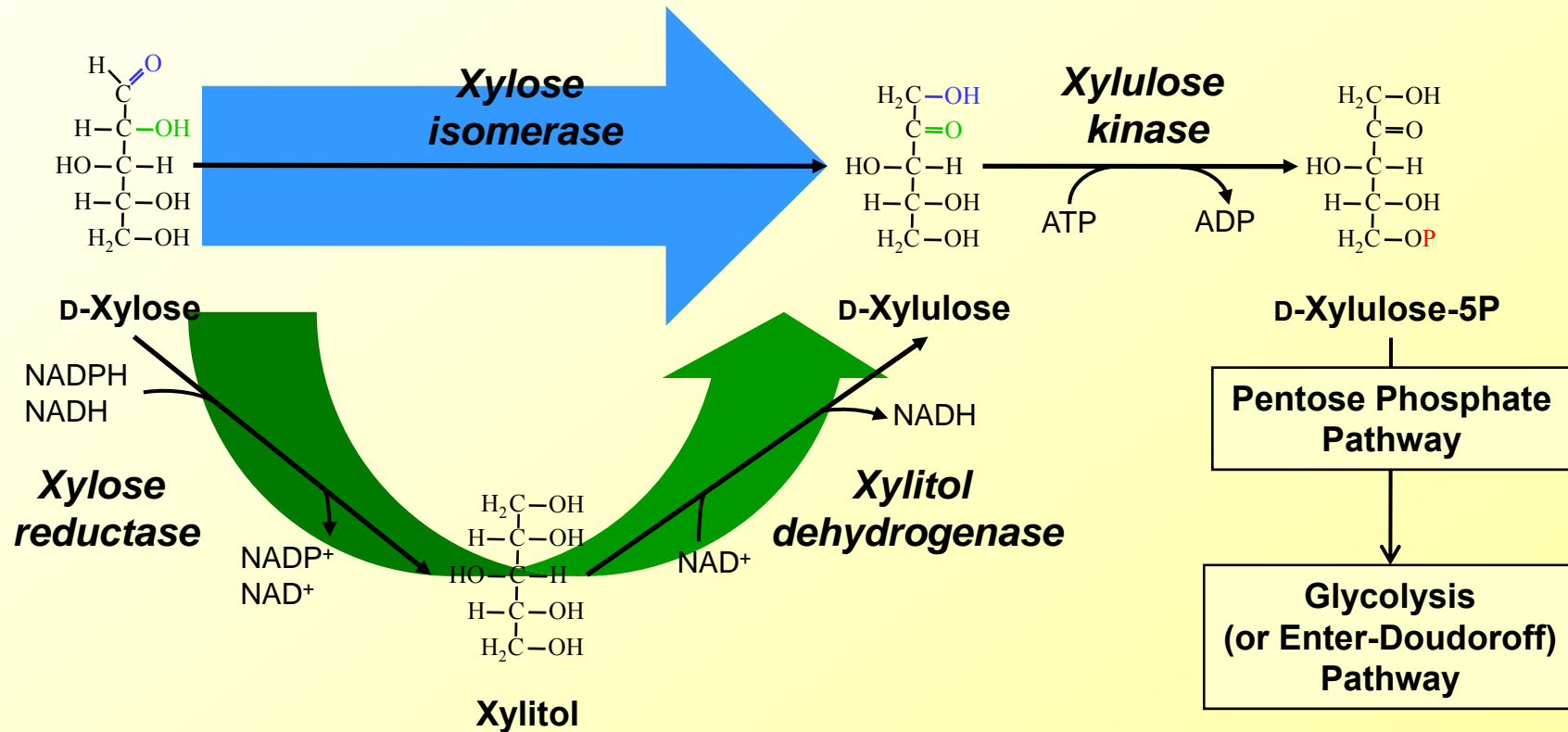


- *C. acetobutylicum*
C. beijerinckii
- Utilization of abundant and inexpensive lignocellulosic materials
- Pentose-rich hemicellulose
- A solvent for a wide variety of industrial applications
- A potential fuel

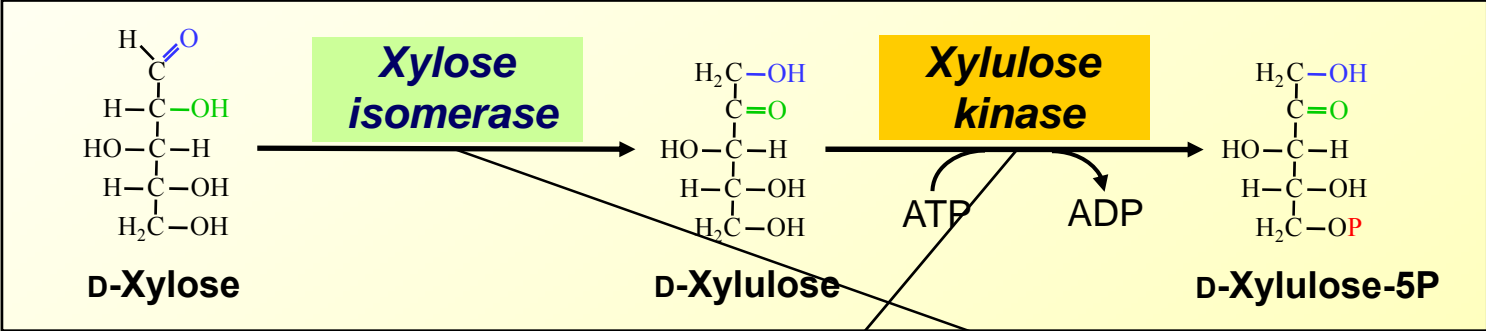
Approach



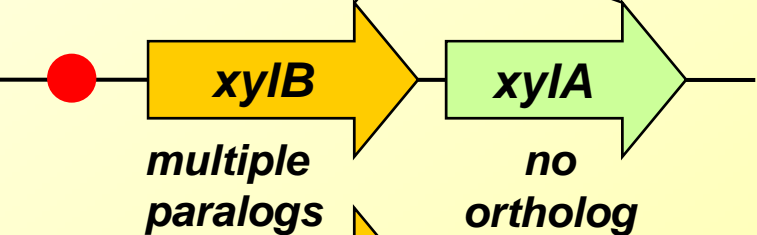
Xylose Utilization Pathway



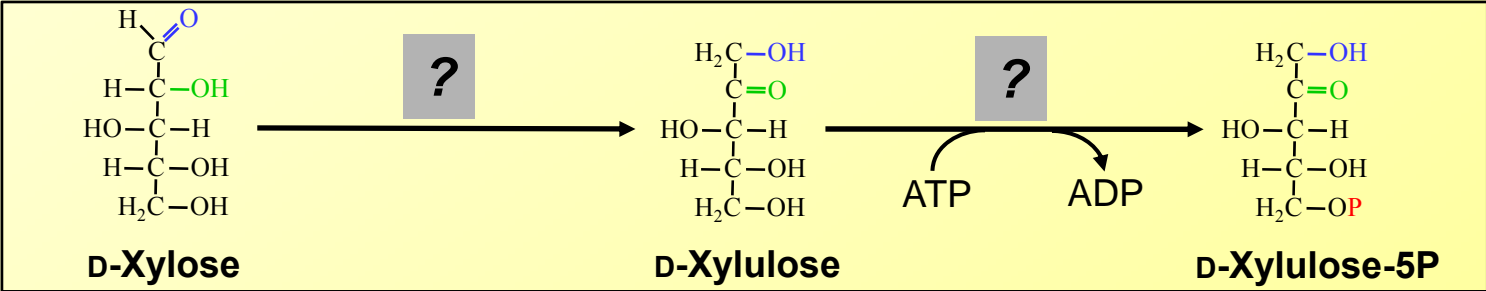
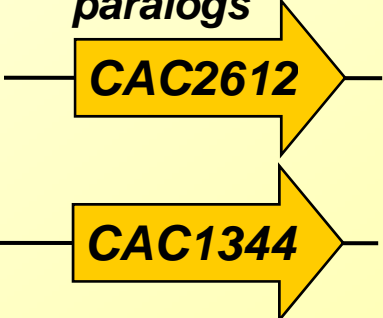
Reconstruction of Xylose Utilization Pathway in *Clostridium*



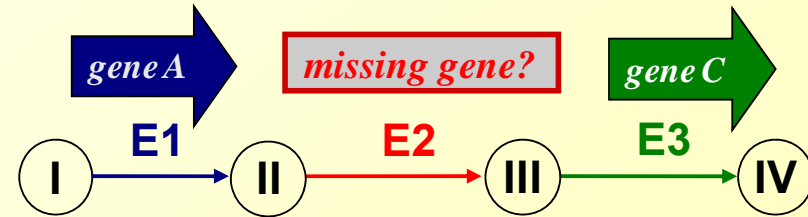
Bacillus subtilis



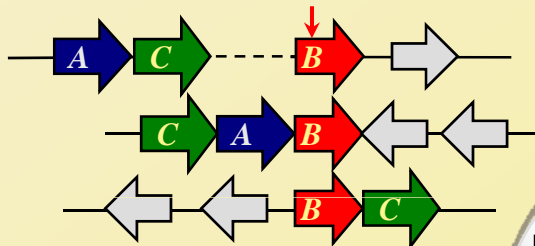
Clostridium acetobutylicum



Genome Context

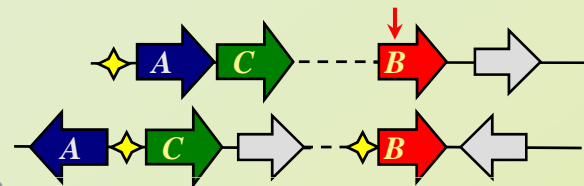


Clustering on the chromosome



Conserved operons

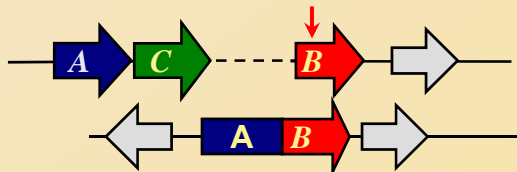
Shared regulatory sites



Conserved regulons

Evidence of functional coupling

Protein fusion



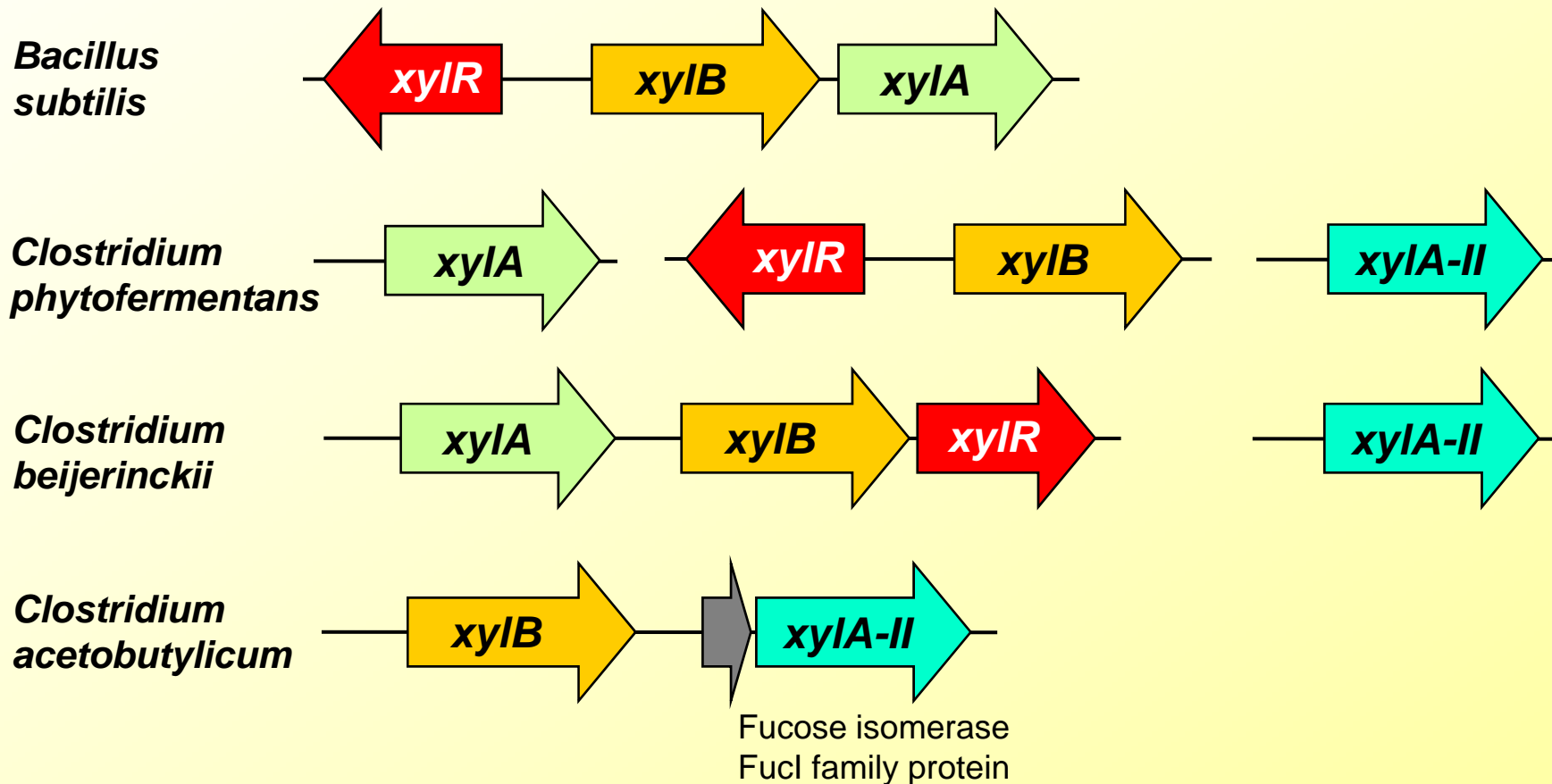
“Rosetta stone”

Phylogenetic profiles

→	A	B	C
-	+	+	+
+	-	-	-
+	-	-	-

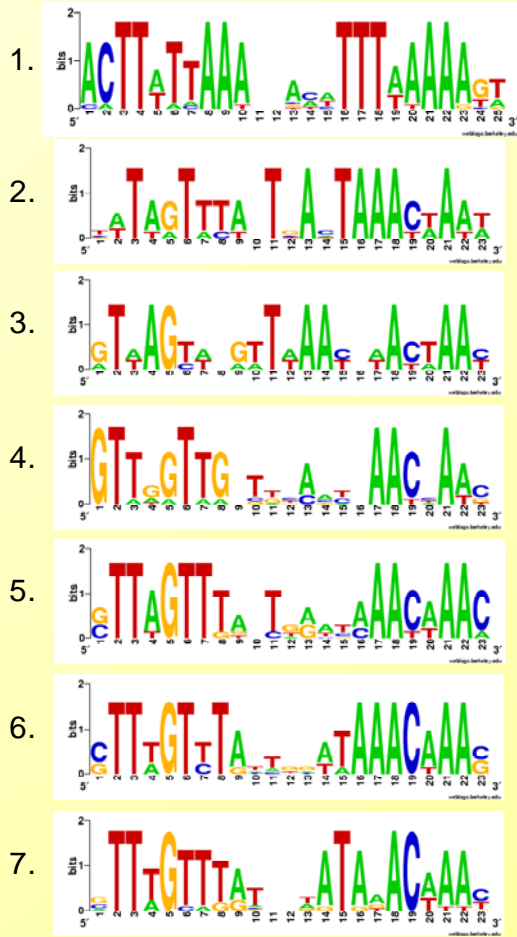
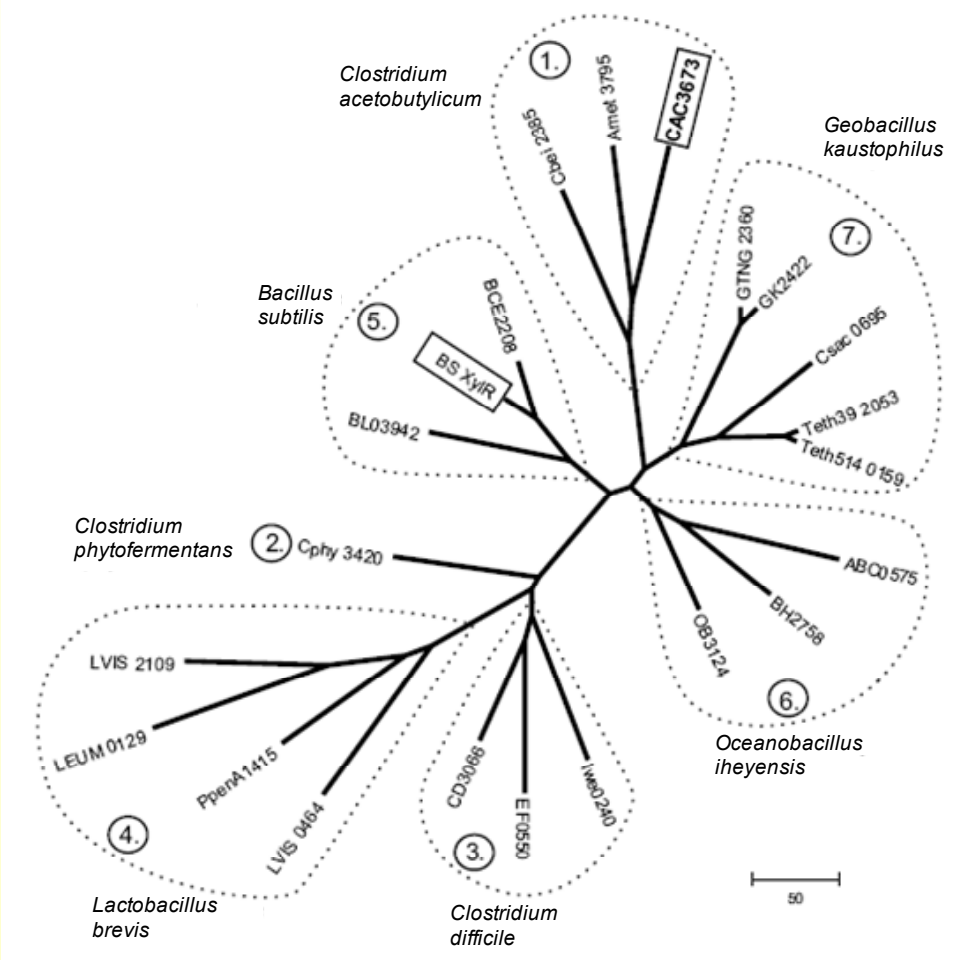
Co-evolution

Reconstruction of Xylose Utilization Pathway and Regulons

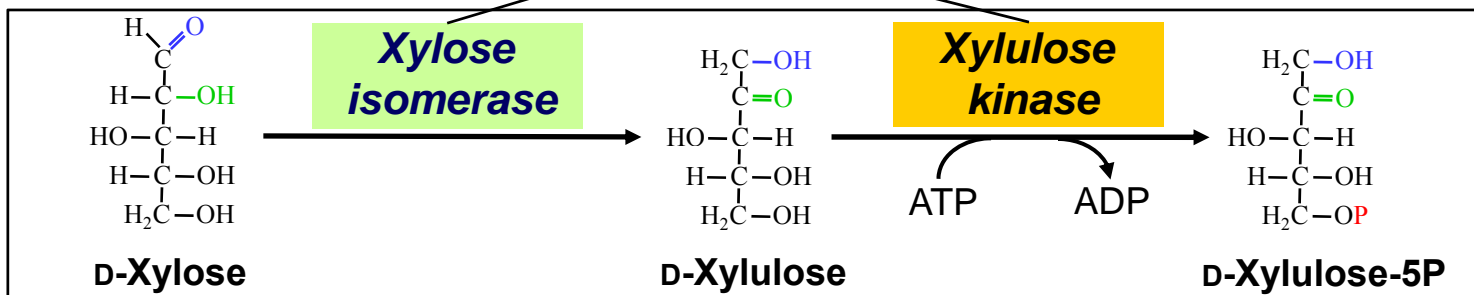
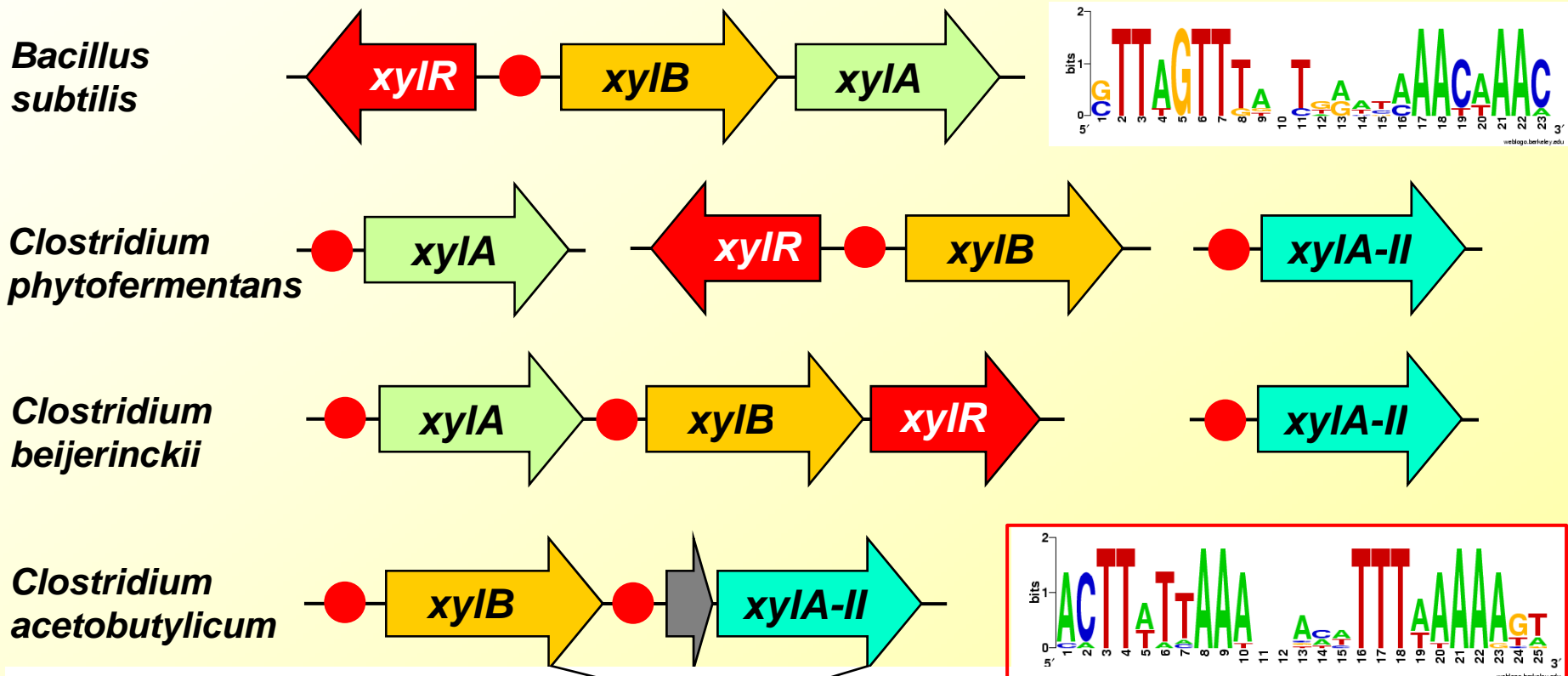


- Conserved chromosomal cluster: *xyIA-II* and *xyIB*

Reconstruction of XylR Regulons

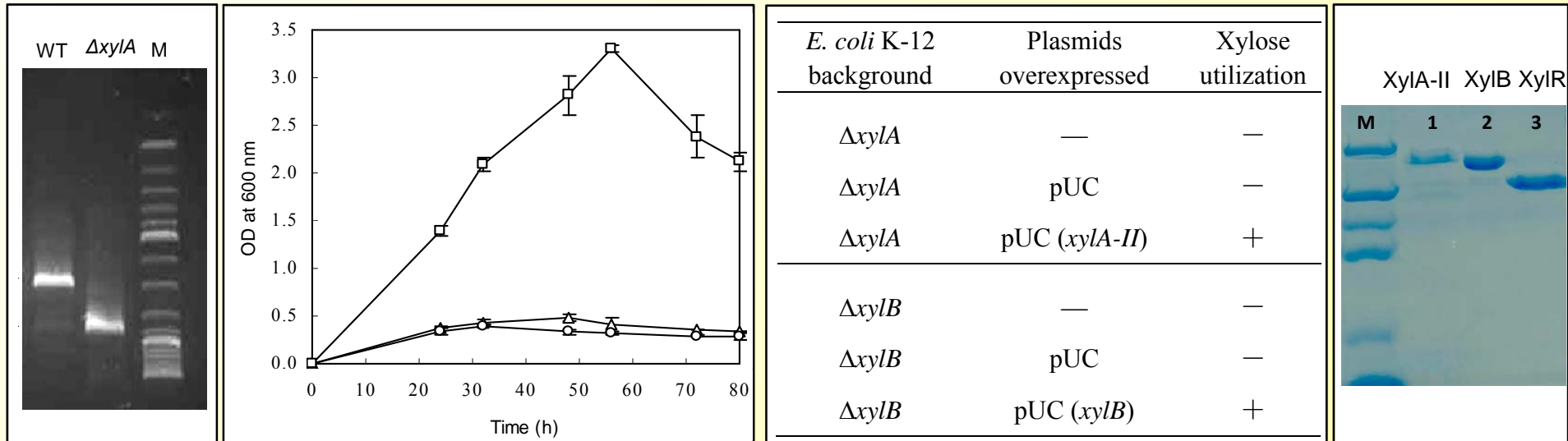


Reconstruction of Xylose Utilization Pathway and Regulons

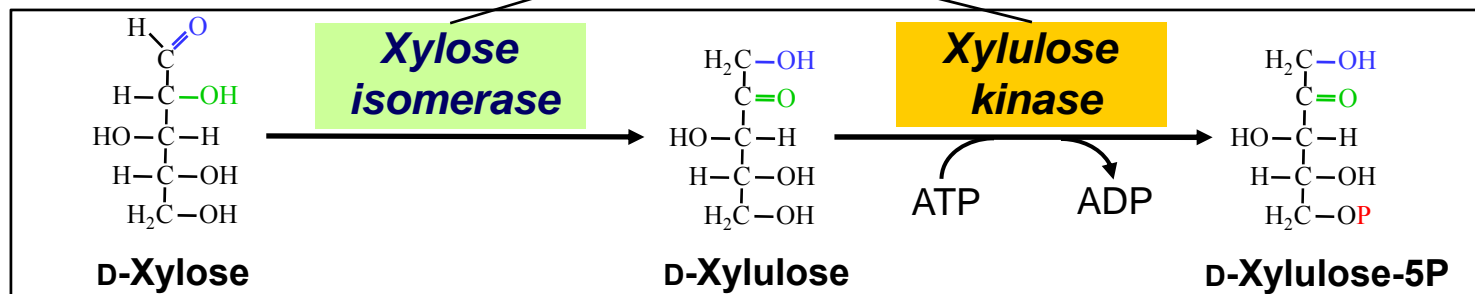
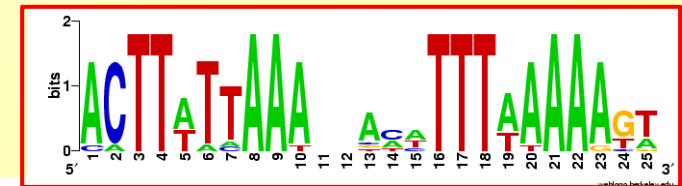
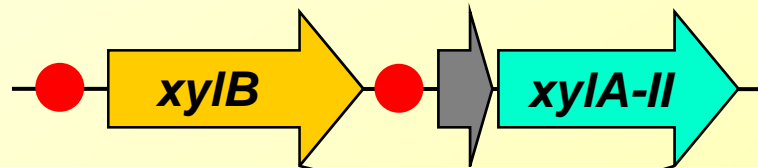


Experimental Validation

Gene inactivation, genetic complementation in *E. coli*, enzymatic assay, EMSA



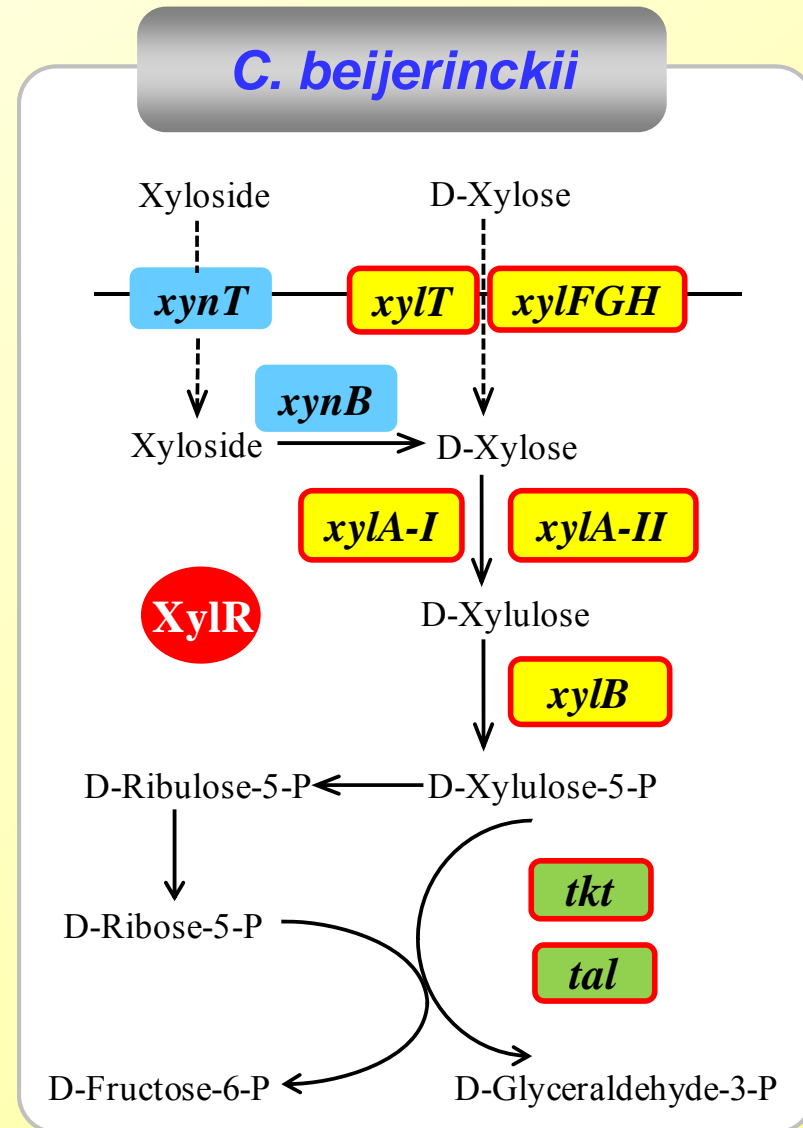
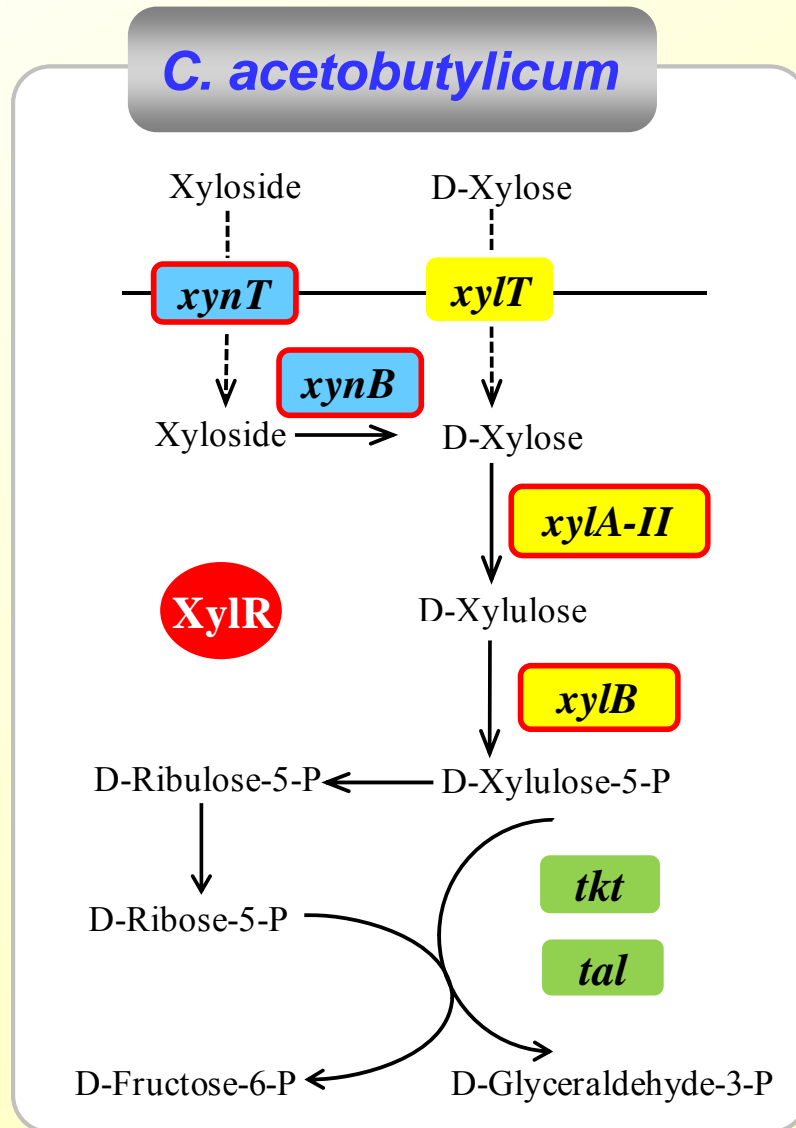
Clostridium acetobutylicum



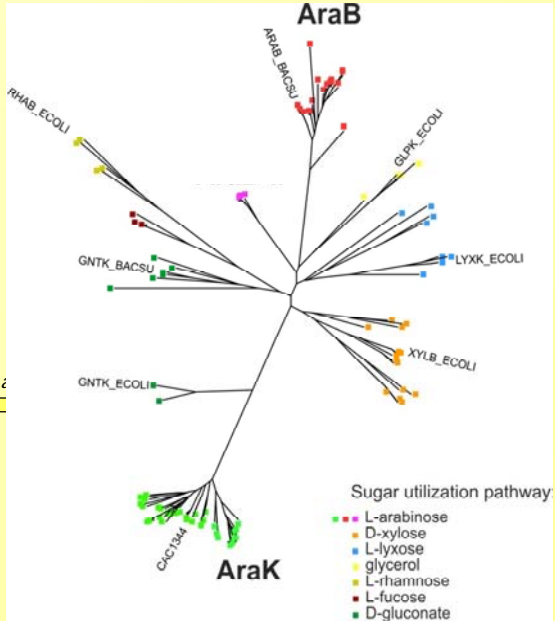
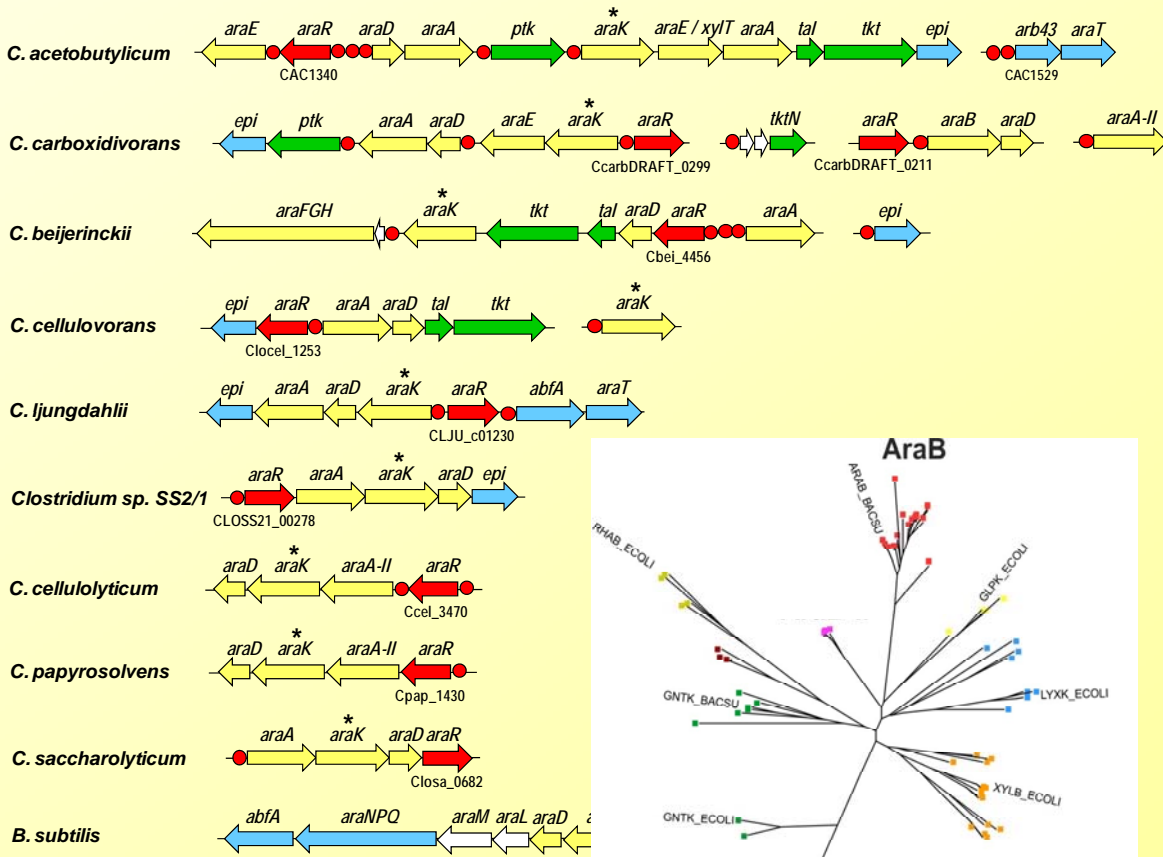
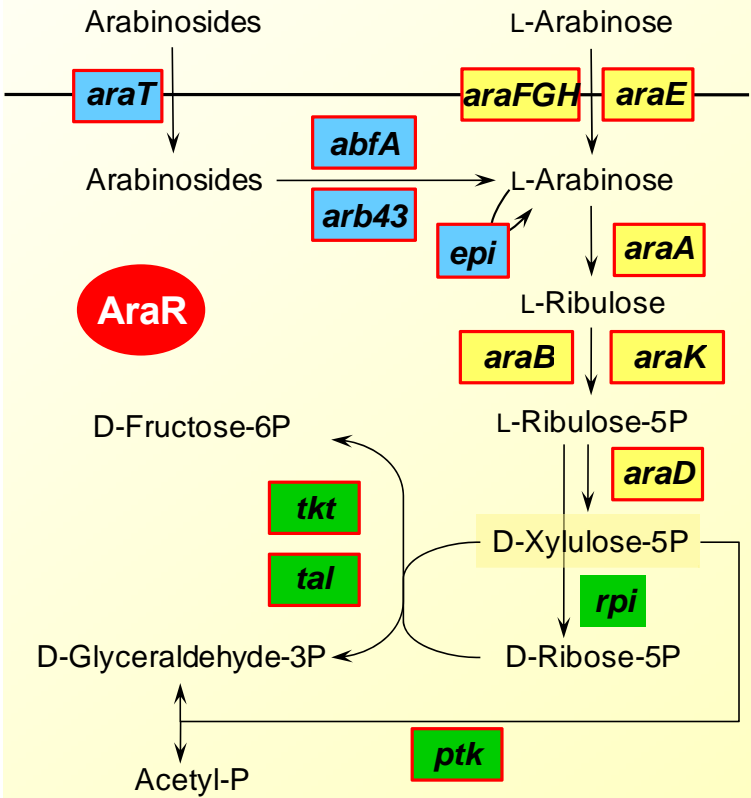
Reconstruction of Xylose Utilization Pathway and Regulons

Taxonomic group ^b Organism	Xylose pathway			Xylose transporter		Regulators			Xyloside transport and degradation				
	XylA-I	XylA-II*	XylB	XylFGH	XylT*	XylR ^{ROK}	XylR ^{LacI*}	XylR ^{AraC}	XynB	XylS	XynT*	Xyn ^{ABC*}	Xyn ^{PTS*}
Clostridiales (5/16)													
<i>Clostridium acetobutylicum</i>		+	+		+	+			+	+	+		
<i>Clostridium beijerincki</i>	+	+	+	+	+	+	+		+	+	+	+	
<i>Clostridium difficile</i>	+		+			+			+	+	+		+
<i>Clostridium phytofermentans</i>	+	+	+			+			+	+			
<i>Alkaliphilus metalliredigens</i>	+		+	+		+							
Thermoanaerobacterales (4/6)													
<i>Moorella thermoacetica</i>	+		+	+			+						
<i>Thermoanaerobacter sp.X514</i>	+		+	+		+							
<i>T. pseudethanolicus</i>	+		+			+			+			+	
<i>C. saccharolyticus</i>		+	+	+		+			+	+		+	
Lactobacillales (6/41)													
<i>Lactococcus lactis Il1403</i>	+		+		+			+	+		+		
<i>Lactococcus lactis cremoris</i>	+		+					+	+				
<i>Leuconostoc mesenteroides</i>	+		+		+	+			+	+	+		
<i>Pediococcus pentosaceus</i>	+		+			+			+	+	+		
<i>Lactobacillus brevis</i>	+		+		+	+			+	+	+	+	
<i>Enterococcus faecalis</i>	+		+			+			+				+
Bacillales (9/57)													
<i>Listeria welshimeri serovar</i>	+		+			+				+	+		
<i>Oceanobacillus iheyensis</i>	+		+			+			+			+	
<i>G. thermodenitrificans</i>	+		+			+			+			+	
<i>Geobacillus kaustophilus</i>	+		+	+		+							
<i>Bacillus clausii</i>	+		+	+		+			+				
<i>Bacillus cereus</i>	+		+		+	+							
<i>Bacillus halodurans</i>	+		+			+			+			+	
<i>Bacillus licheniformis</i>	+		+			+			+	+		+	
<i>Bacillus subtilis</i>	+		+			+			+		+		

Reconstruction of Xylose Utilization Pathway and Regulons



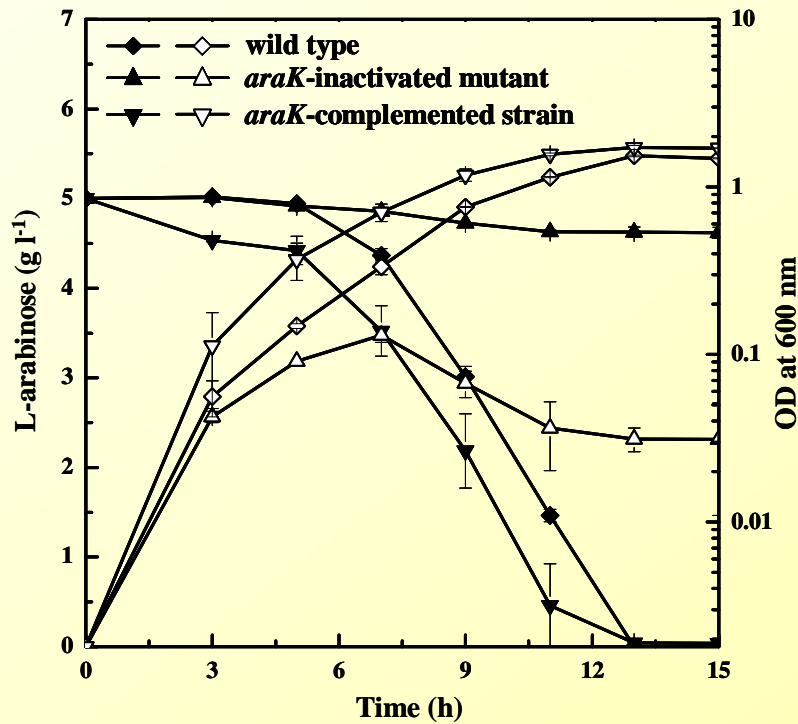
Reconstruction of Arabinose Utilization Pathway and Regulons



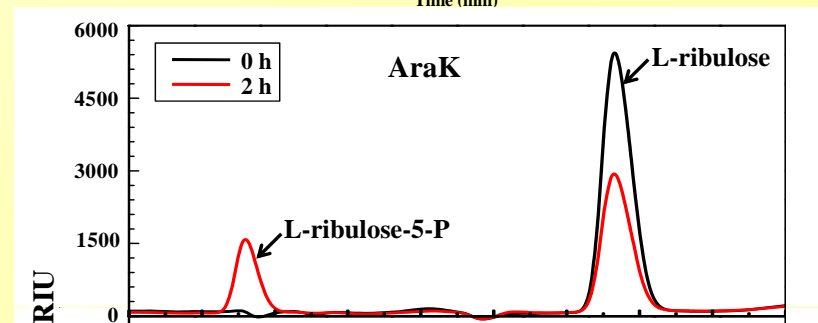
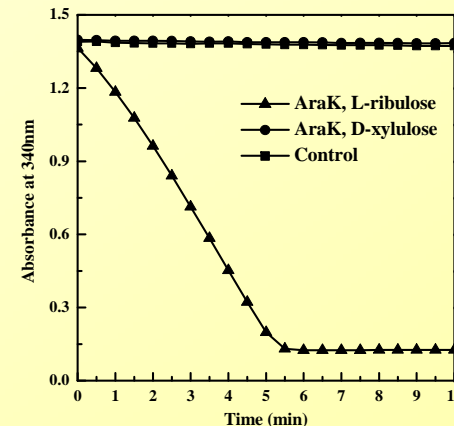
Identification of a novel ribulokinase AraK and extension of AraR regulon

Experimental Validation of Predicted Ribulokinase

(Mutant construction and phenotype analysis)

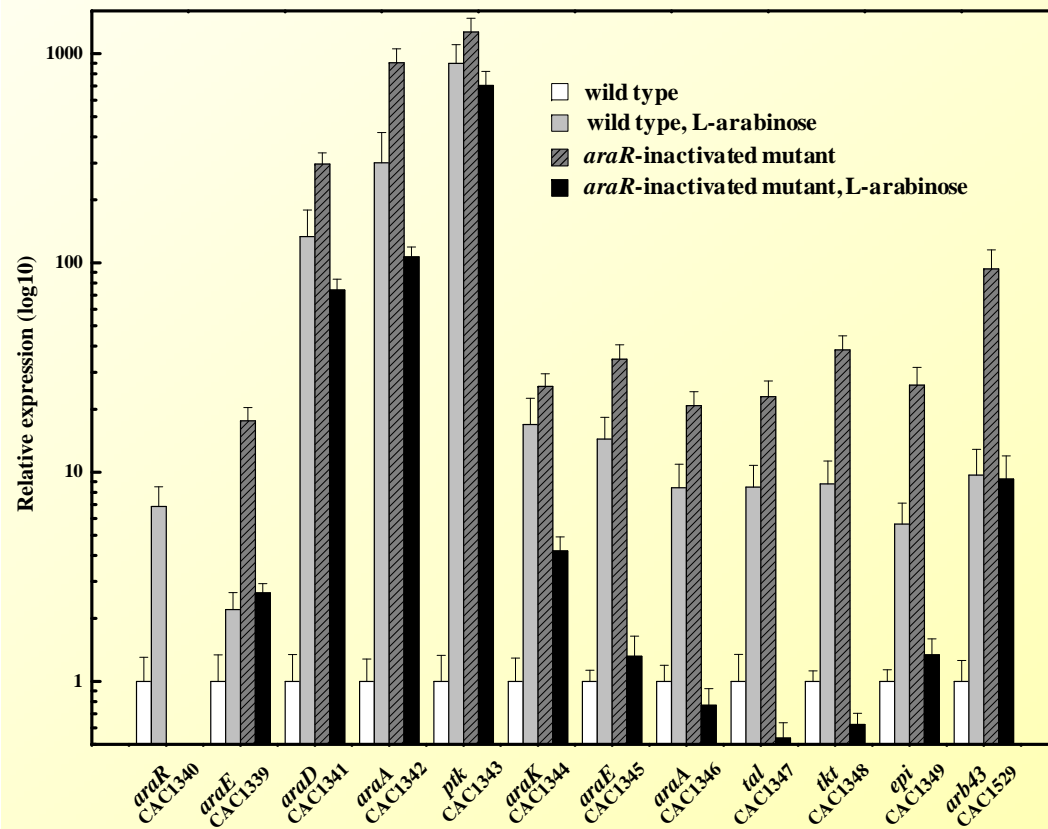


(Enzymatic assay and product identification)

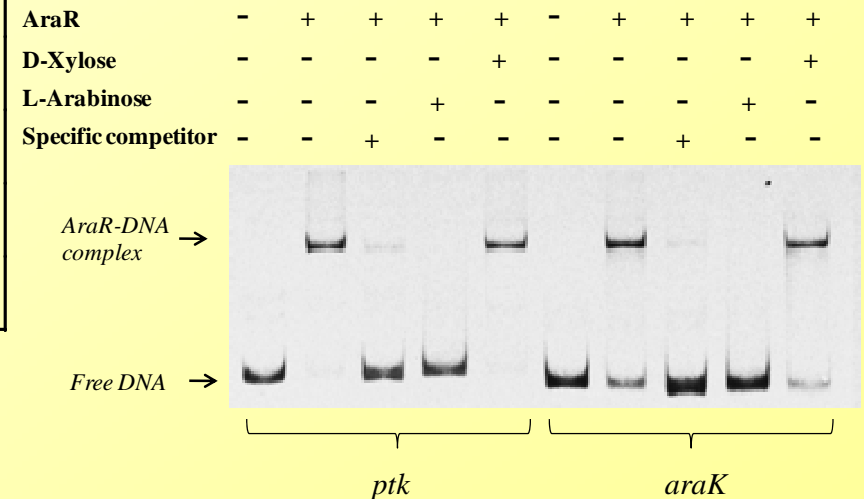
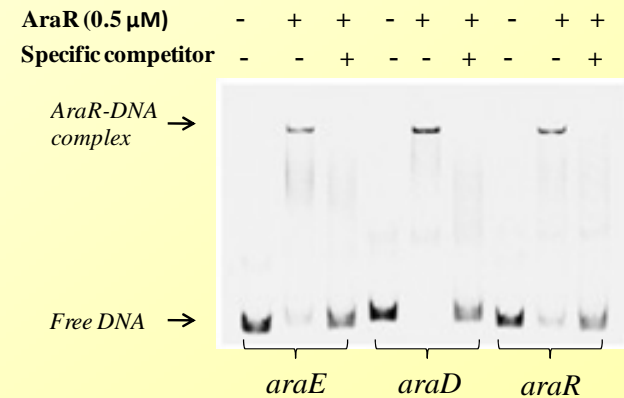


Experimental Characterization of Predicted AraR Regulon

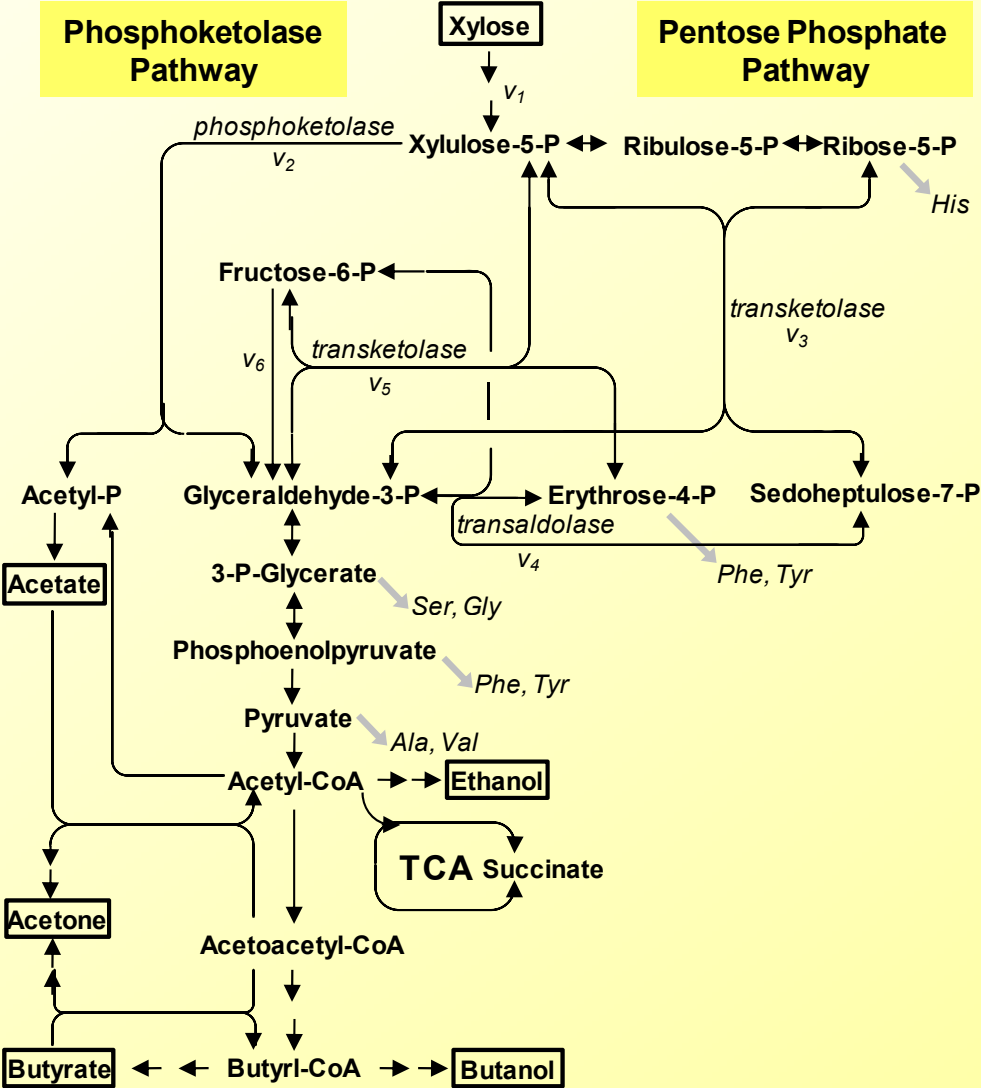
Transcriptional analysis



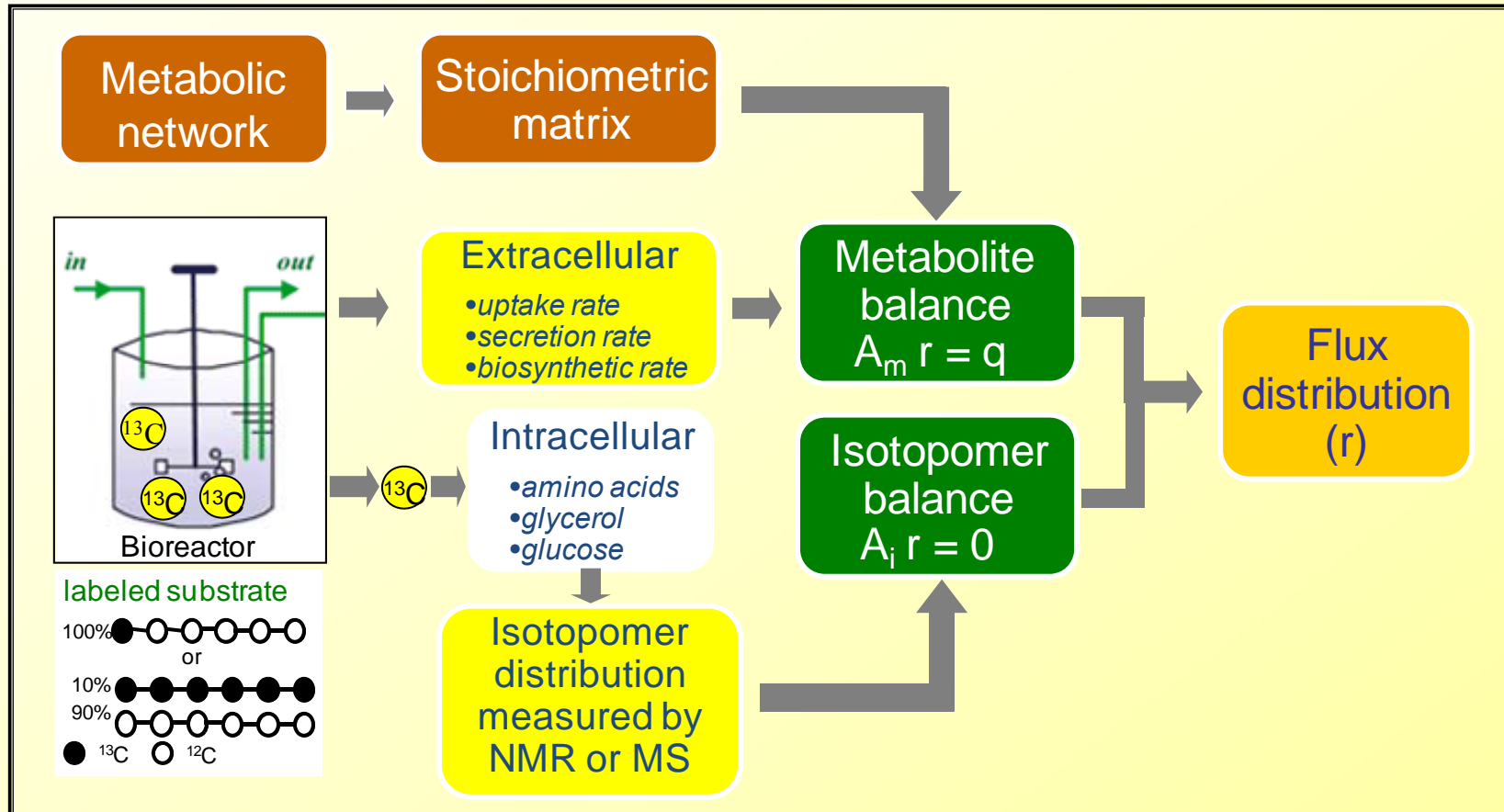
Electrophoretic mobility shift assay



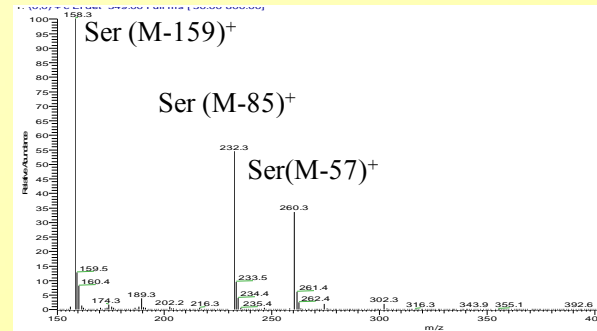
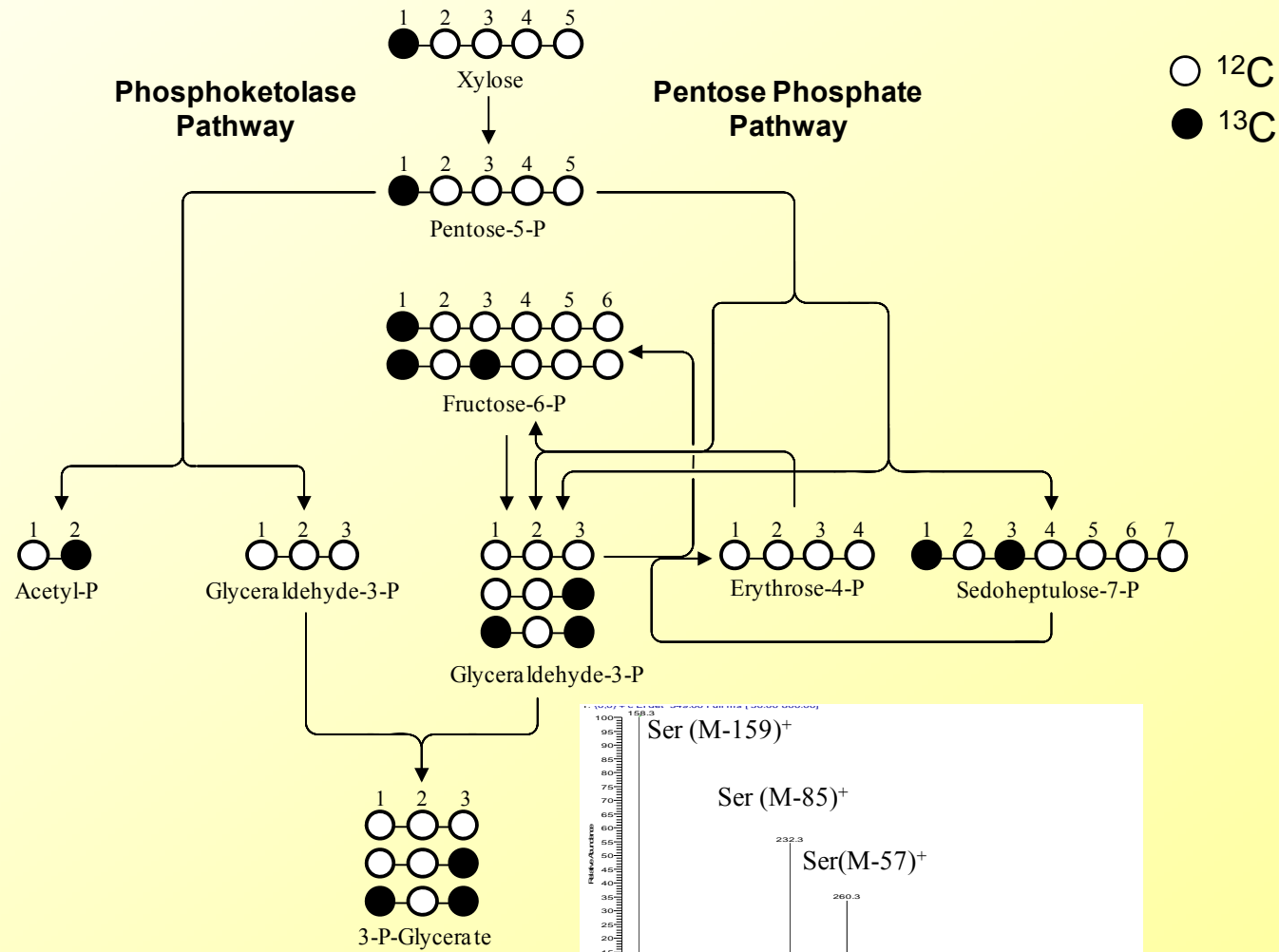
Pathway of Xylose Metabolism in *C. acetobutylicum*



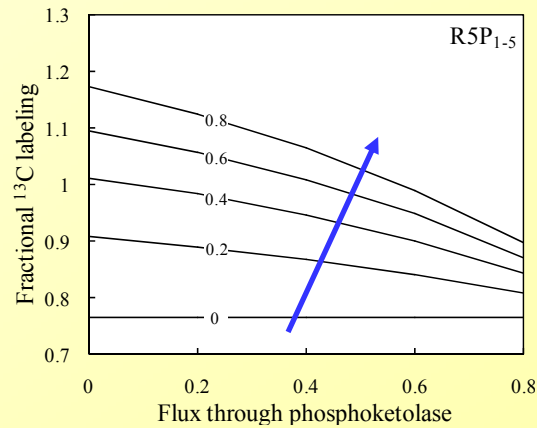
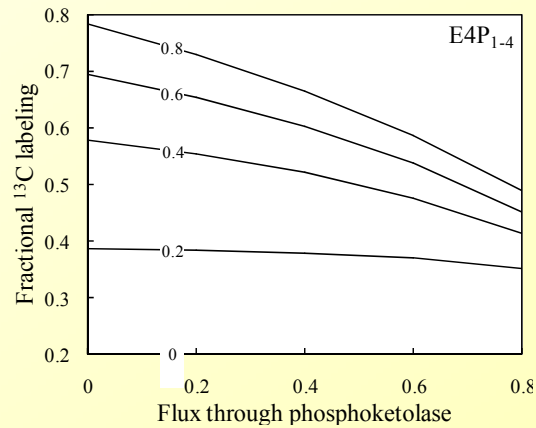
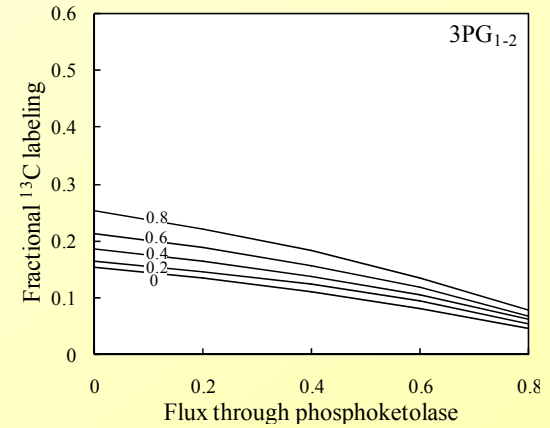
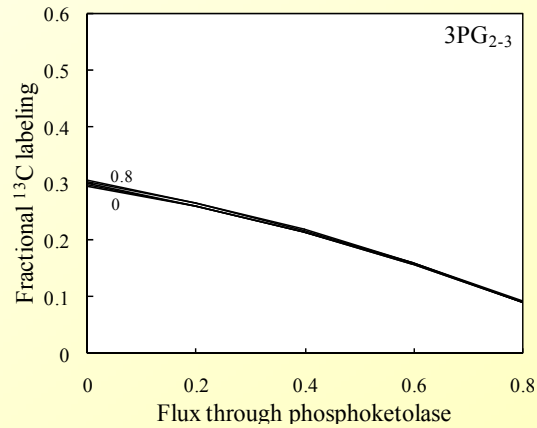
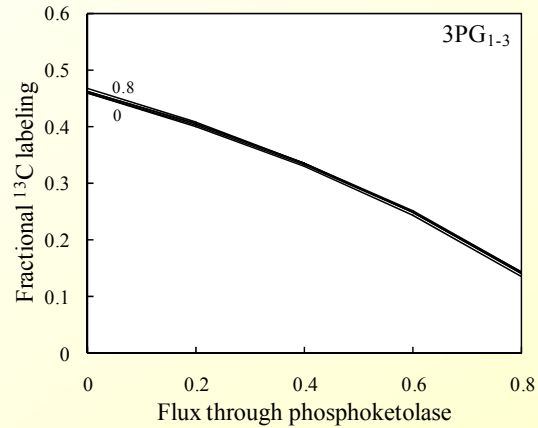
¹³C-Based Metabolic Flux Analysis



¹³C Flux Analysis of Xylose Metabolism

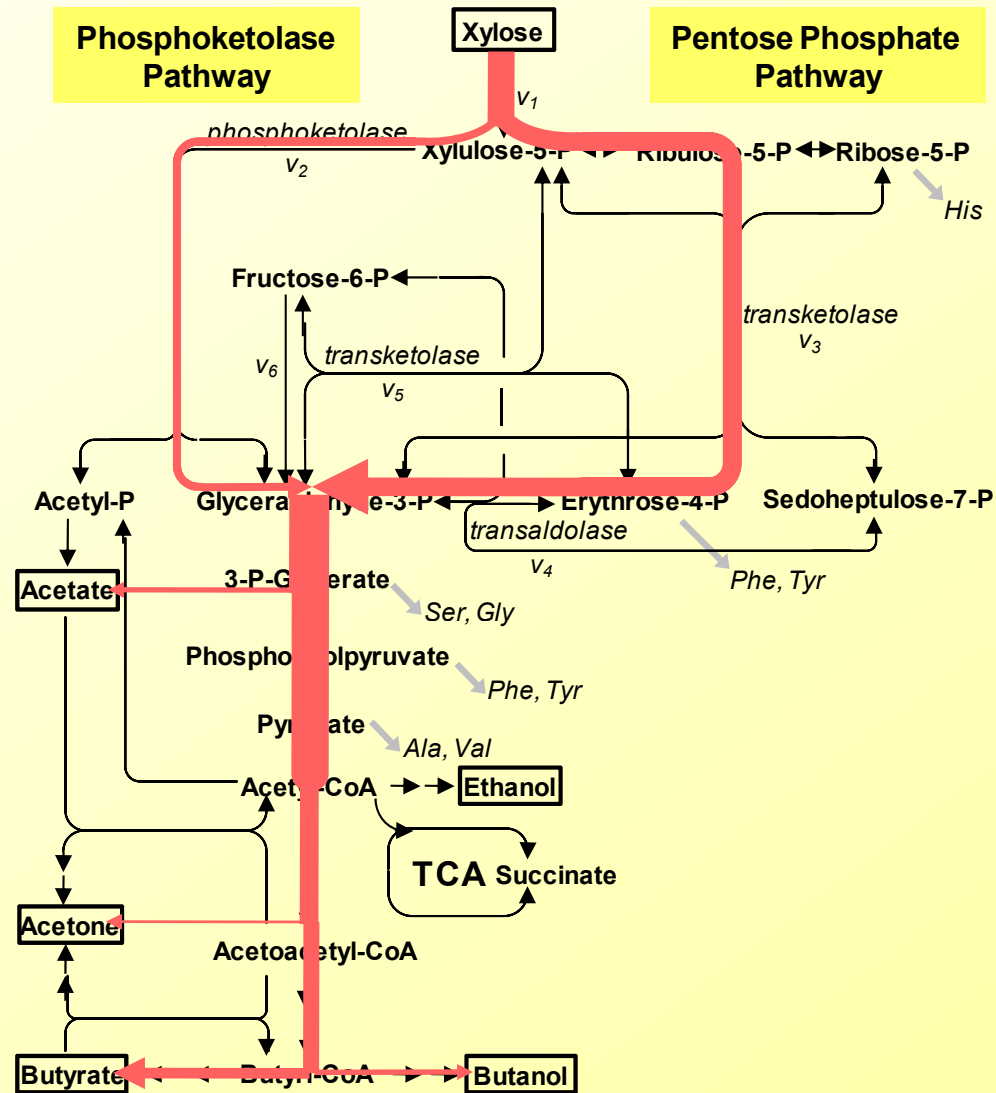


Simulations of ^{13}C Labeling Experiments

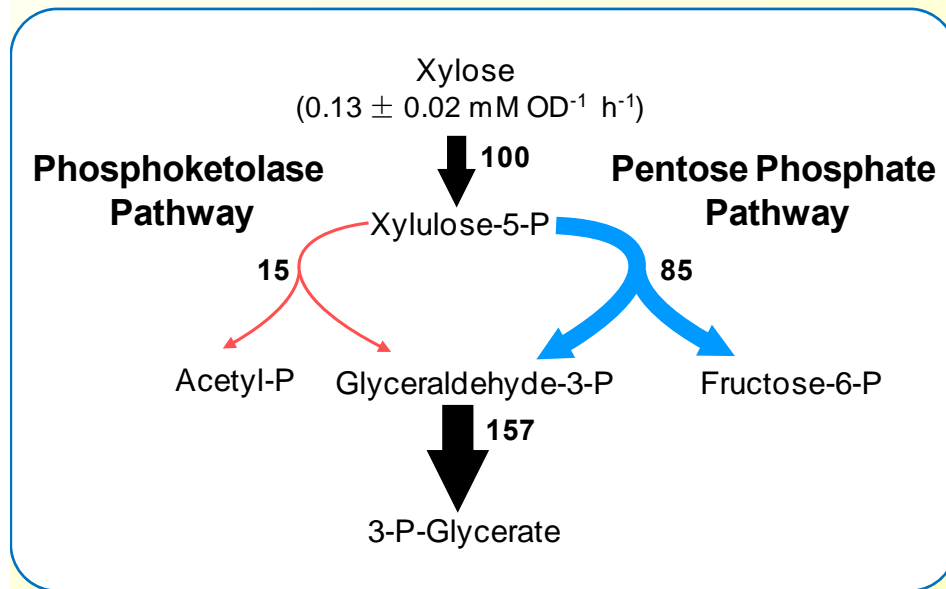


Extent of reversibility of transketolase and transaldolase reactions was set at 0, 0.2, 0.4, 0.6, and 0.8.

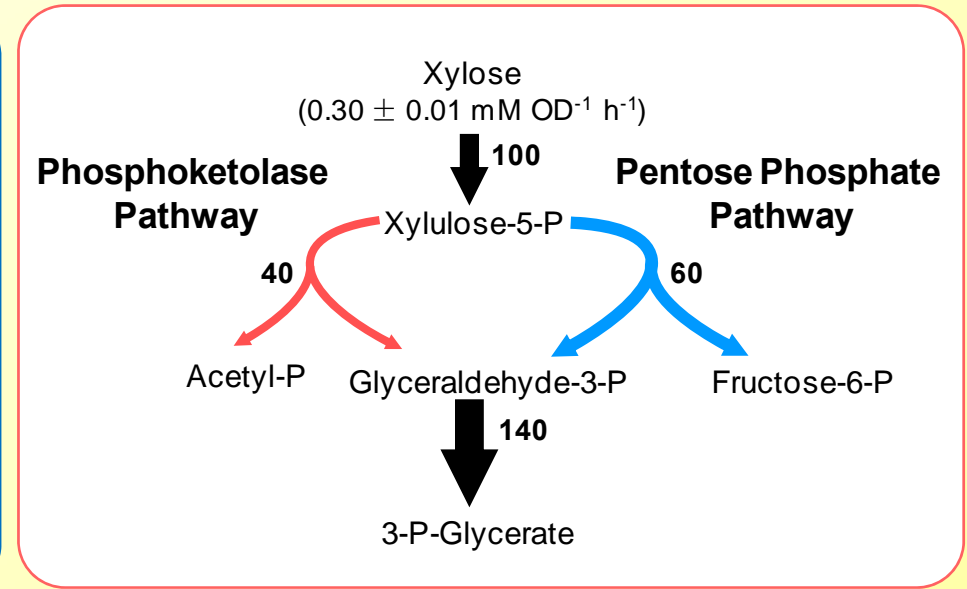
Xylose Catabolic Flux in *C. acetobutylicum*



Xylose Catabolic Flux in *C. acetobutylicum*



10 g l⁻¹ xylose



20 g l⁻¹ xylose

Summary

1

A novel xylose isomerase (XylA-II) was identified and the gene coding for xylulokinase was unambiguously assigned in clostridia. A new XylR-binding DNA motif was identified in several *Clostridium* species.

2

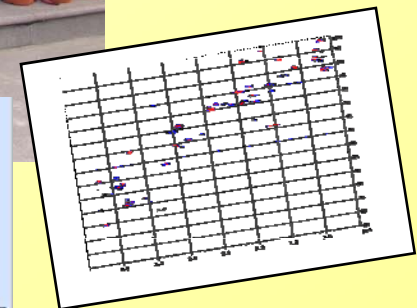
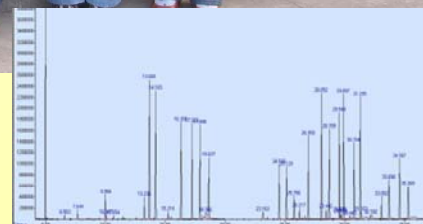
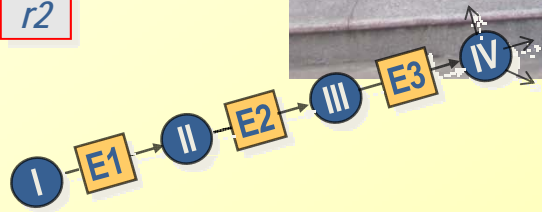
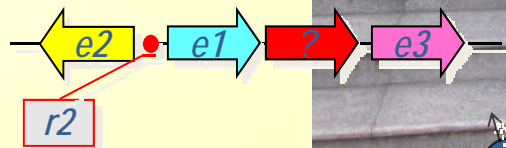
A novel ribulokinase (AraK) was identified in clostridia. In addition to the genes involved in arabinose utilization and arabinoside degradation, extension of the AraR regulon to the pentose phosphate pathways genes in several *Clostridium* species was revealed.

3

The use of the phosphoketolase pathway for xylose catabolism in *C. acetobutylicum* was revealed. The split ratio of the phosphoketolase pathway to the pentose phosphate pathway was increased when cells were grown at a higher concentration of xylose.

THE TEAM

Microbial Metabolic Engineering & Comparative Genomics



Acknowledgements

Collaborators

SIBS, CAS

Weihong Jiang

Sheng Yang

Yang Gu

Burnham Institute
for Medical Research

Dmitry Rodionov

Andrei Osterman

Sponsors

- ★ National Science Foundation of China (NSFC)
- ★ National Basic Research Program, Ministry Of Science & Technology (MOST) of China