Summer 6-4-2012

Development of Microbial Cell factories for Production of Biofuels and Bio-based Chemicals through Consolidated Bioprocessing

Akihiko Kondo
Kobe University

Recommended Citation

Akihiko Kondo, "Development of Microbial Cell factories for Production of Biofuels and Bio-based Chemicals through Consolidated Bioprocessing" in "Metabolic Engineering IX", E. Heinzle, Saarland Univ.; P. Soucaille, INSA; G. Whited, Danisco Eds, ECI Symposium Series, (2013). http://dc.engconfintl.org/metabolic_ix/4

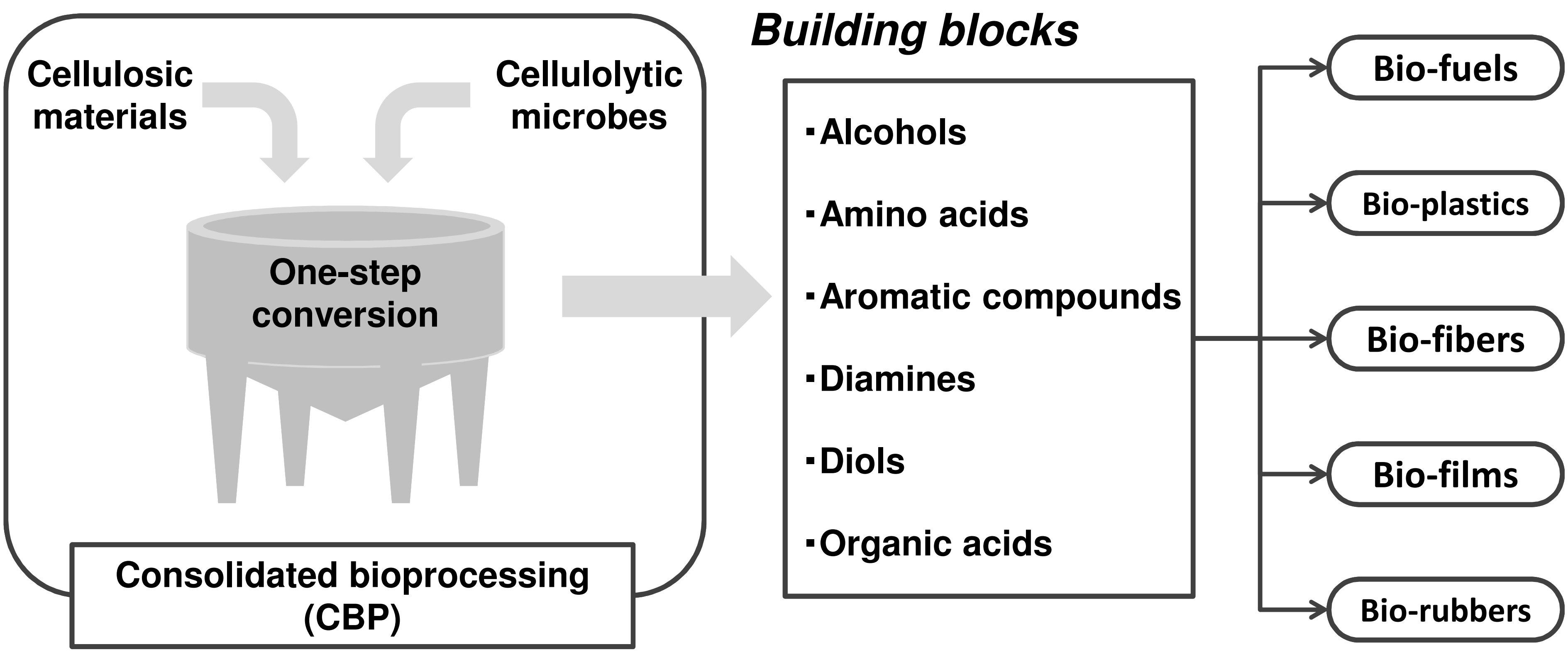
Metabolic engineering IX

June 3-7, 2012

Development of microbial cell factories for production of biofuels and bio-based chamicals through consolidated bioprocessing

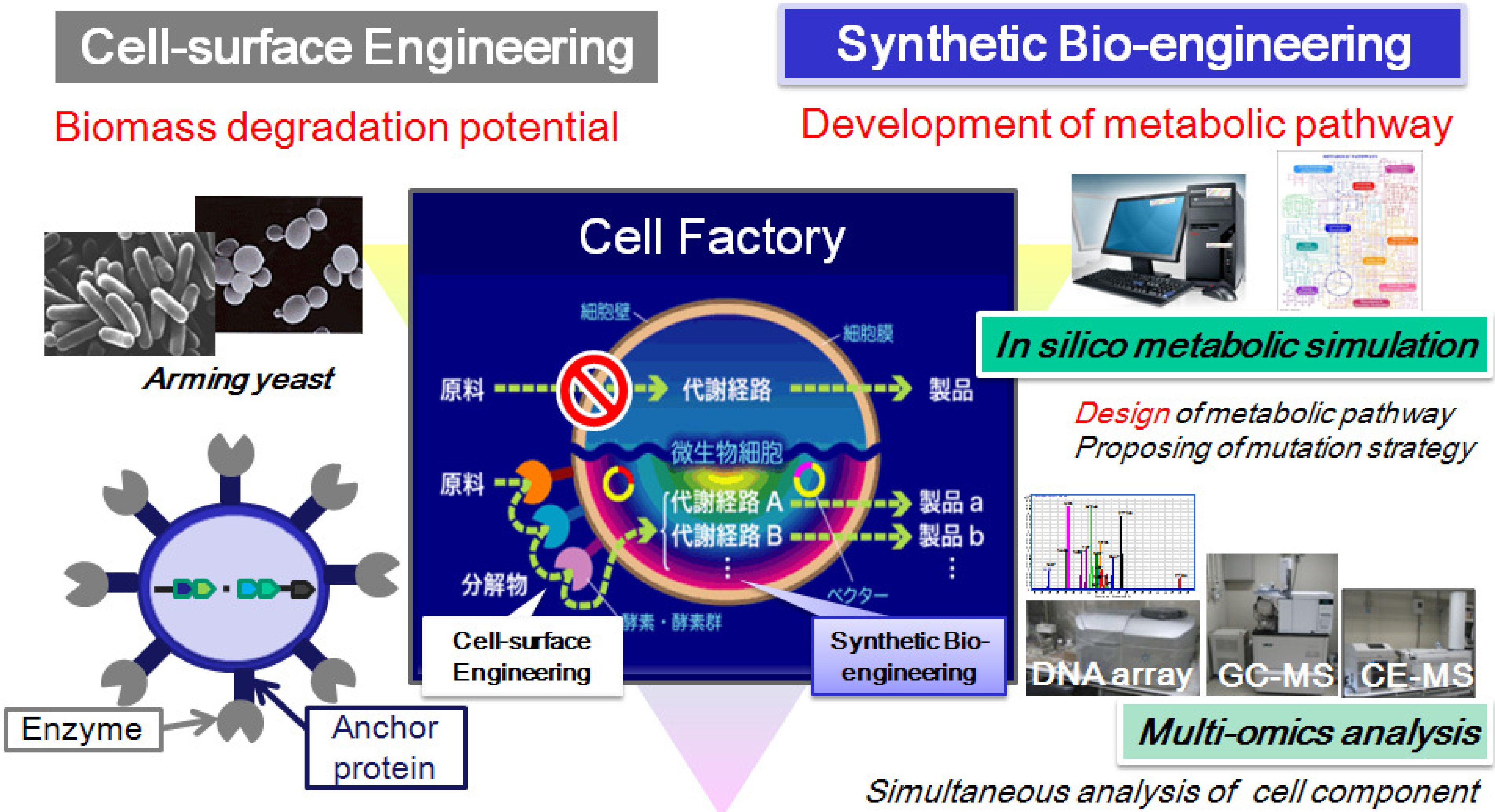
Kobe University
Professor, Department of Chemical Science and Engineering
Director, Biorefinery Center
RIKEN Institute, Team Leader, BMEP,
Akihiko Kondo

Bio-refinery through CBP



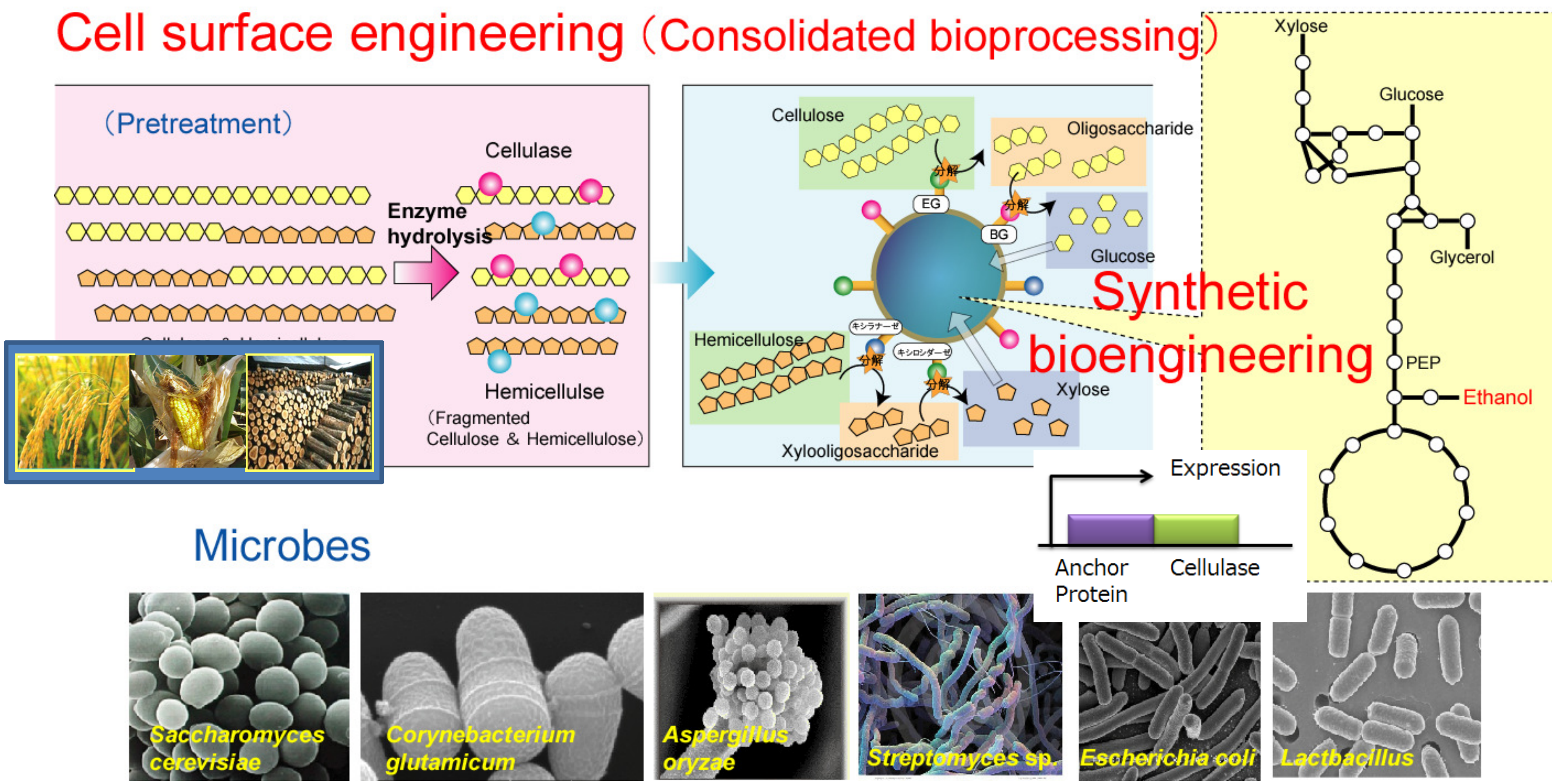
Ishii J, Poster 37 : Isobutanol production

Construction of cell factories for biorefinery



Breeding of super microbial cells(cell factory) for direct production of fuels and chemicals from biomass

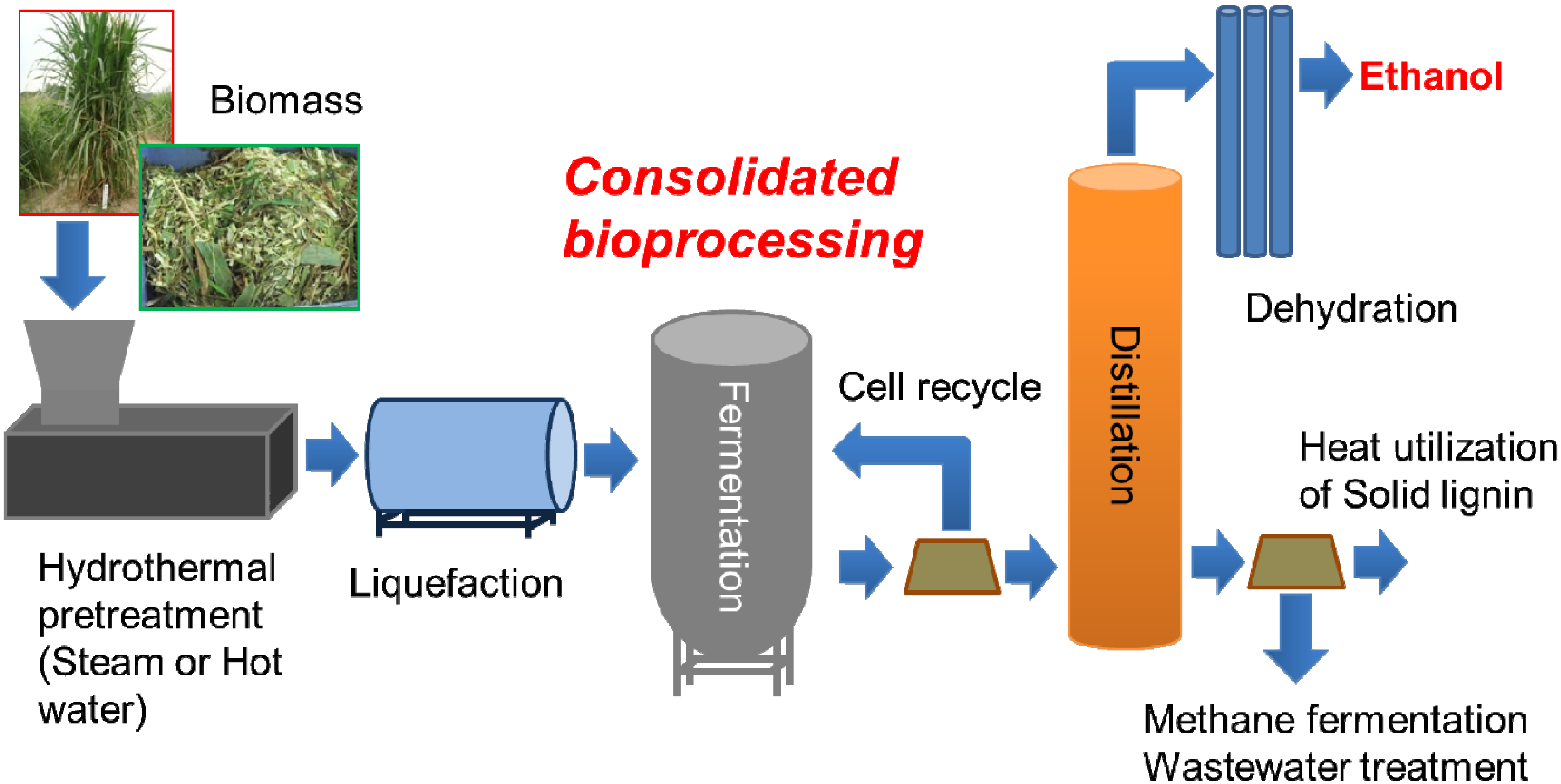
Cell factories to industrially important processes



A combination of cell surface engineering and synthetic bioengineering will be a very effective approach to develop cells with novel metabolic ability for industrial applications.

Process Development

Environmentally benign and consolidated process



Process Development

Bench-scale plant in Kobe University


50 kg/h presser


100 kg/batch steamer

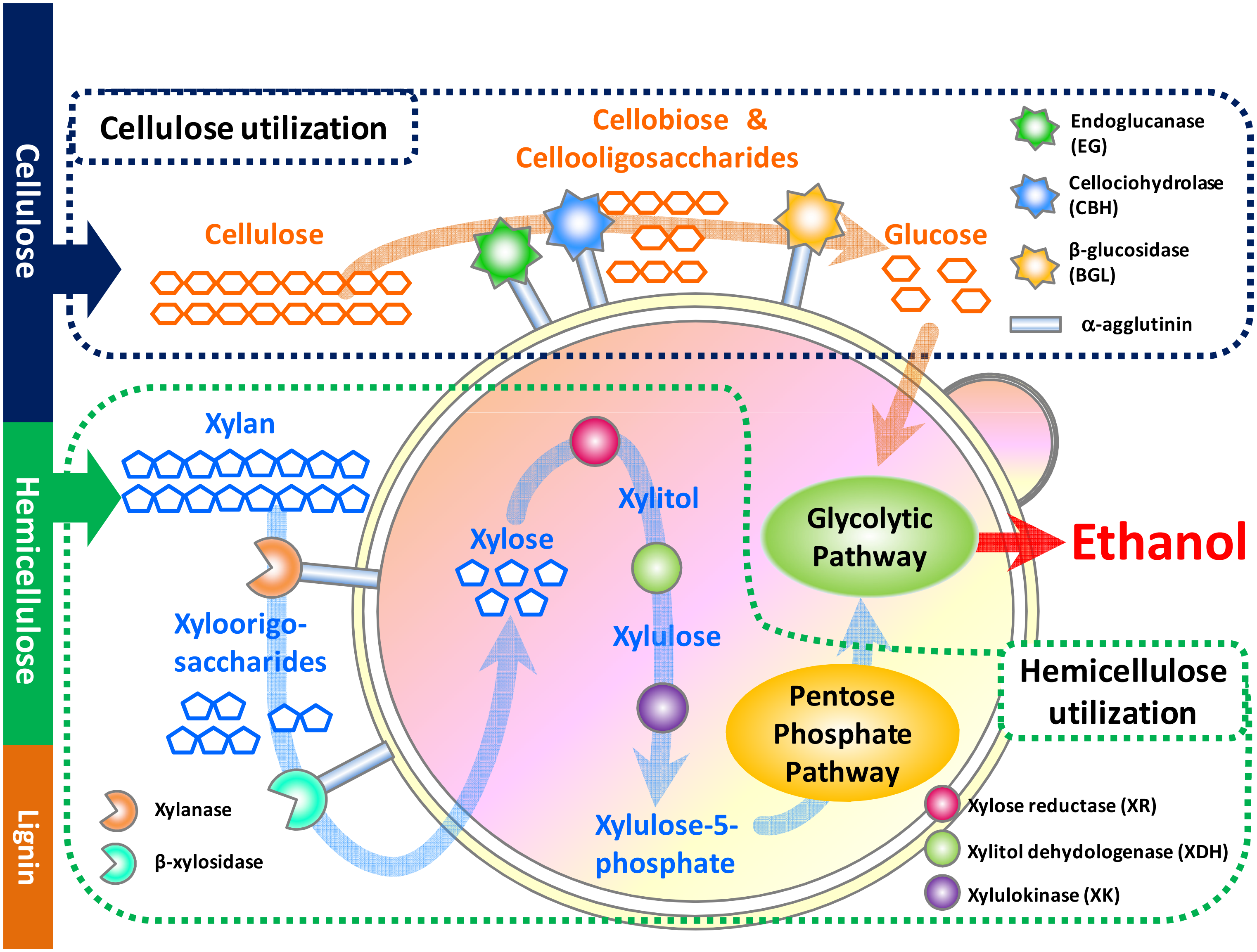

100 L
liquefaction reactor


50 L fermentation reactor


30 L fermentation reactor (x3)

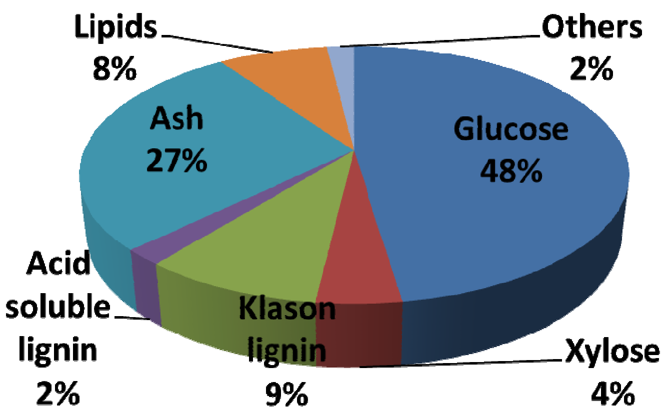

Cell recycling system

Arming Yeast for CBP

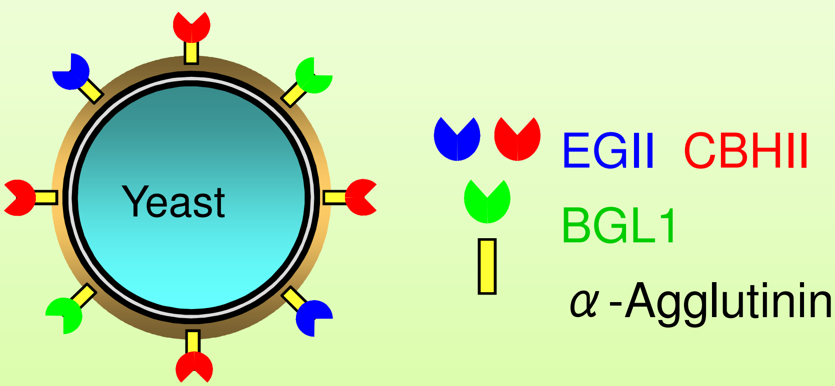


Ethanol production from lignocellulosic biomass

Composition of C6 fraction



Yeast cells co-displaying BGL1, EGII and CBHII



Rice straw

Liquid hot water treatment

C6 fraction
(Water insoluble)

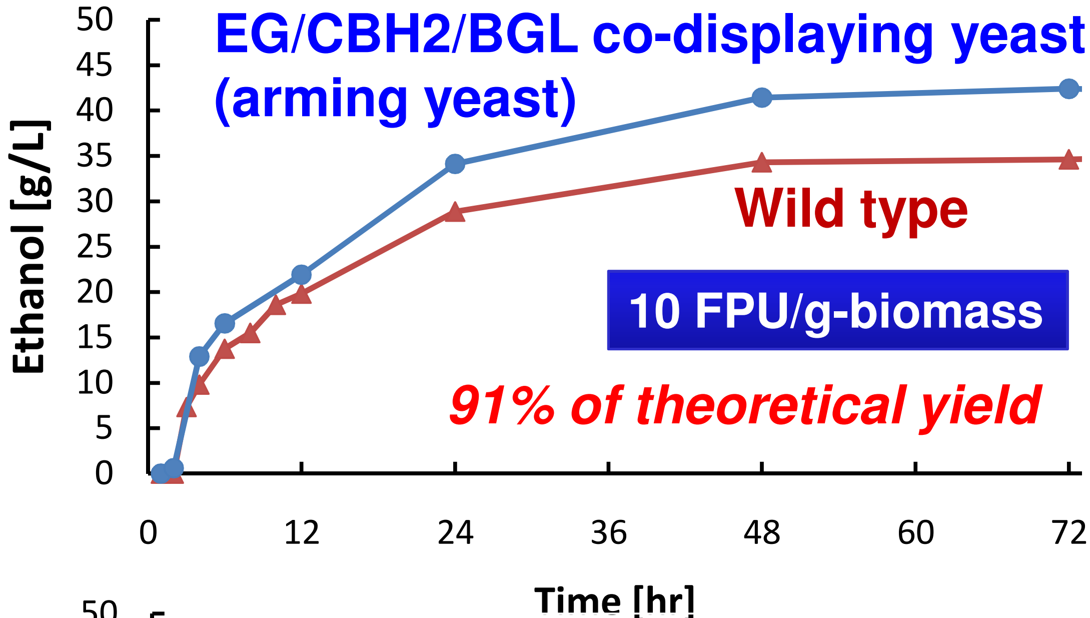
C5 fraction
(Water soluble)

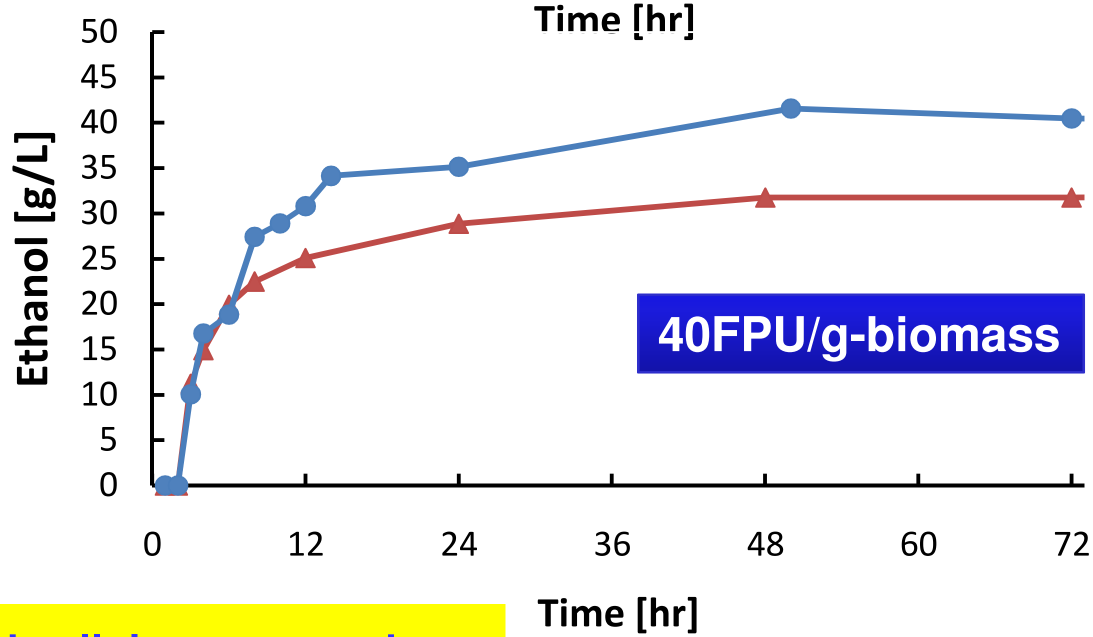
Ethanol production from high-solid biomass

Liquefaction of pretreated rice straw at 50 °C for 2h

Initial biomass; 200 g/L
Cellulase; 10 -40 FPU/g-biomass

Fermentation at 35 °C

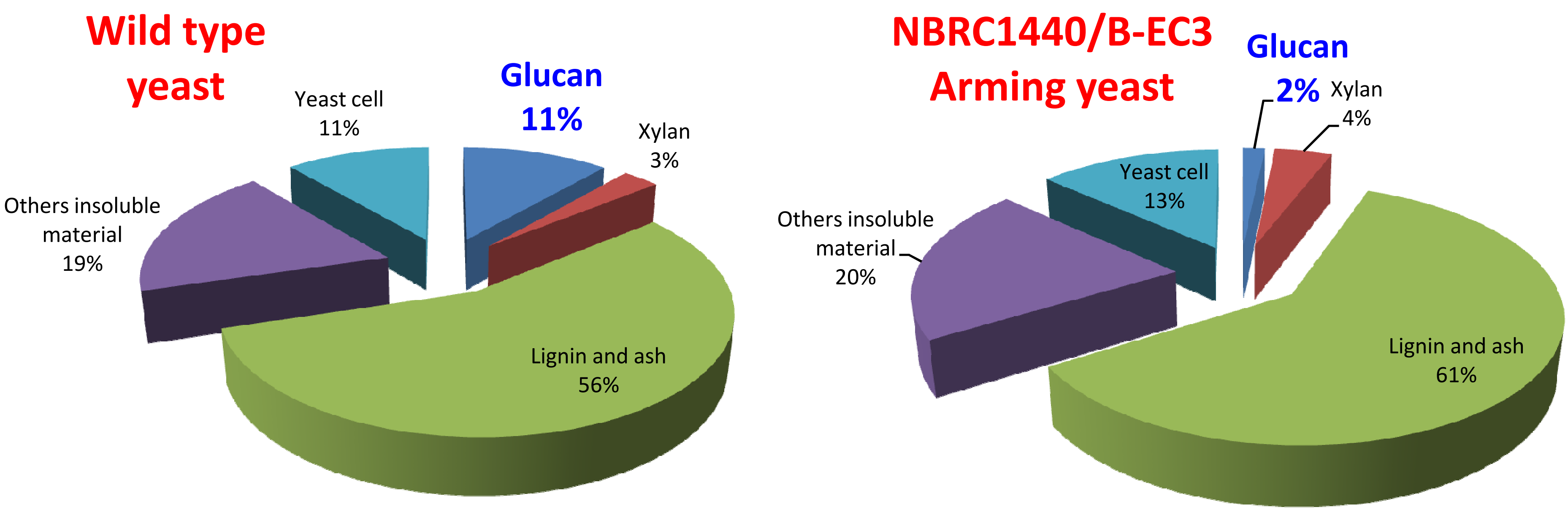




Yamada R, Poster 20 : Optimized cellulase expression

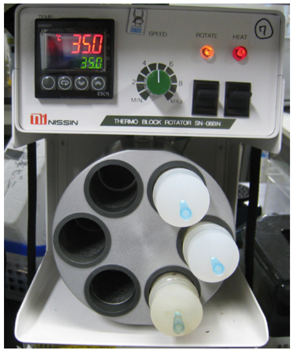
Ethanol production from high-solid biomass

Residue after high-solid fermentation



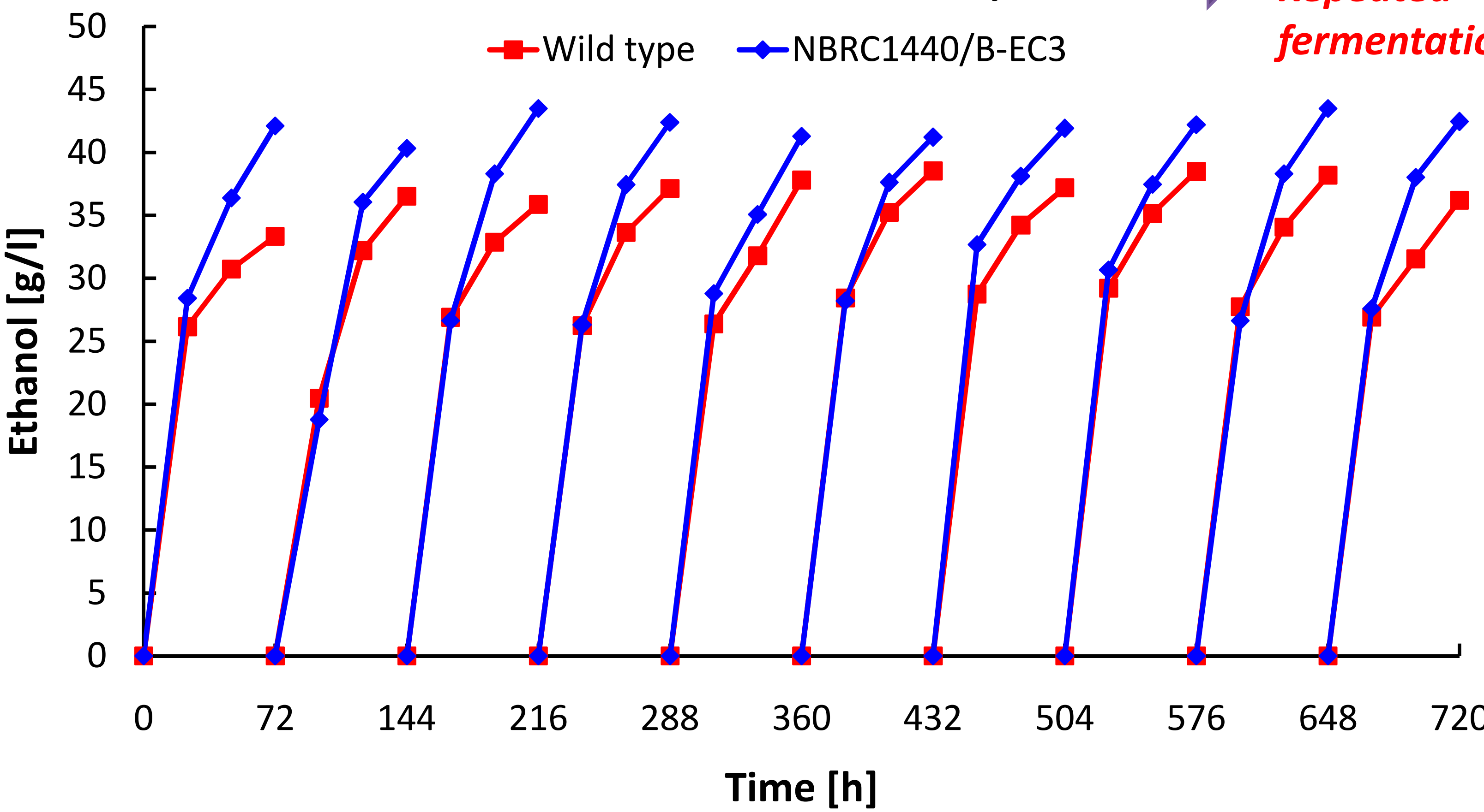
Cellulases displayed on the yeast cell surface hydrolyzed cellulose that was not hydrolyzed by commercial cellulases, leading to increased ethanol production.

Cell recycle fermentation of high-solid biomass



Initial biomass ; 200 g/L
Liquefaction; 2 h, 50ºC
Cellulase added; 10 FPU/g-biomass

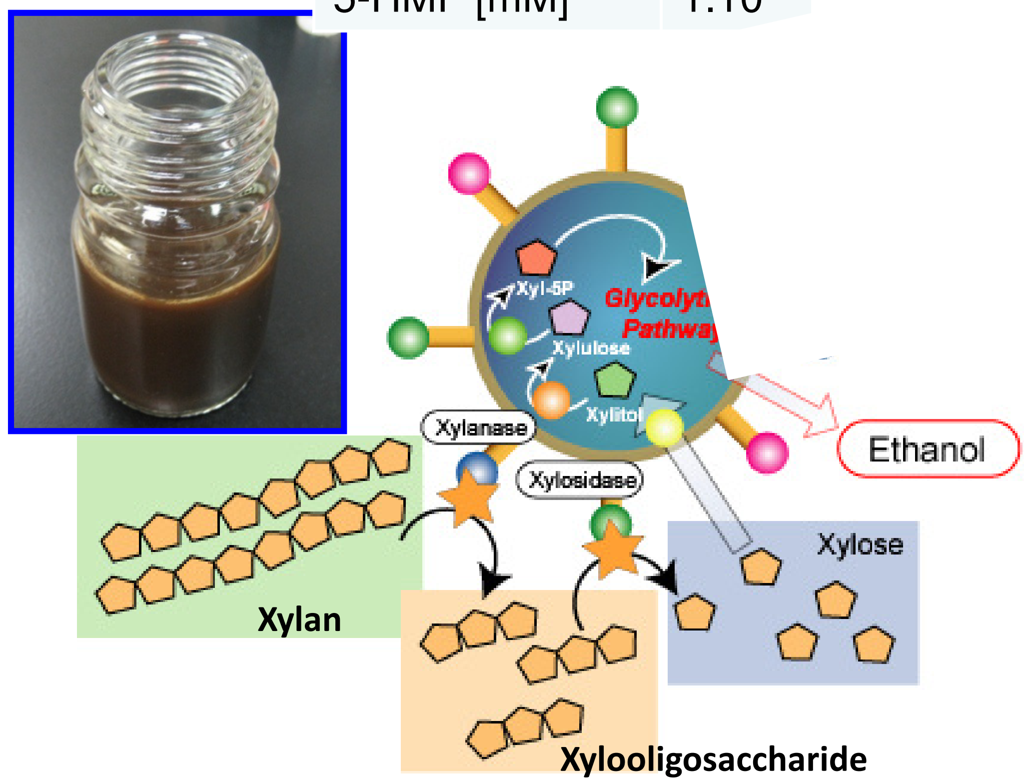
Fermentation mixture
↓ **Centrifugation at 20 x *g***
Supernatant
↓ **Centrifugation at 6,000 x *g***
pellet → ***Repeated fermentation***



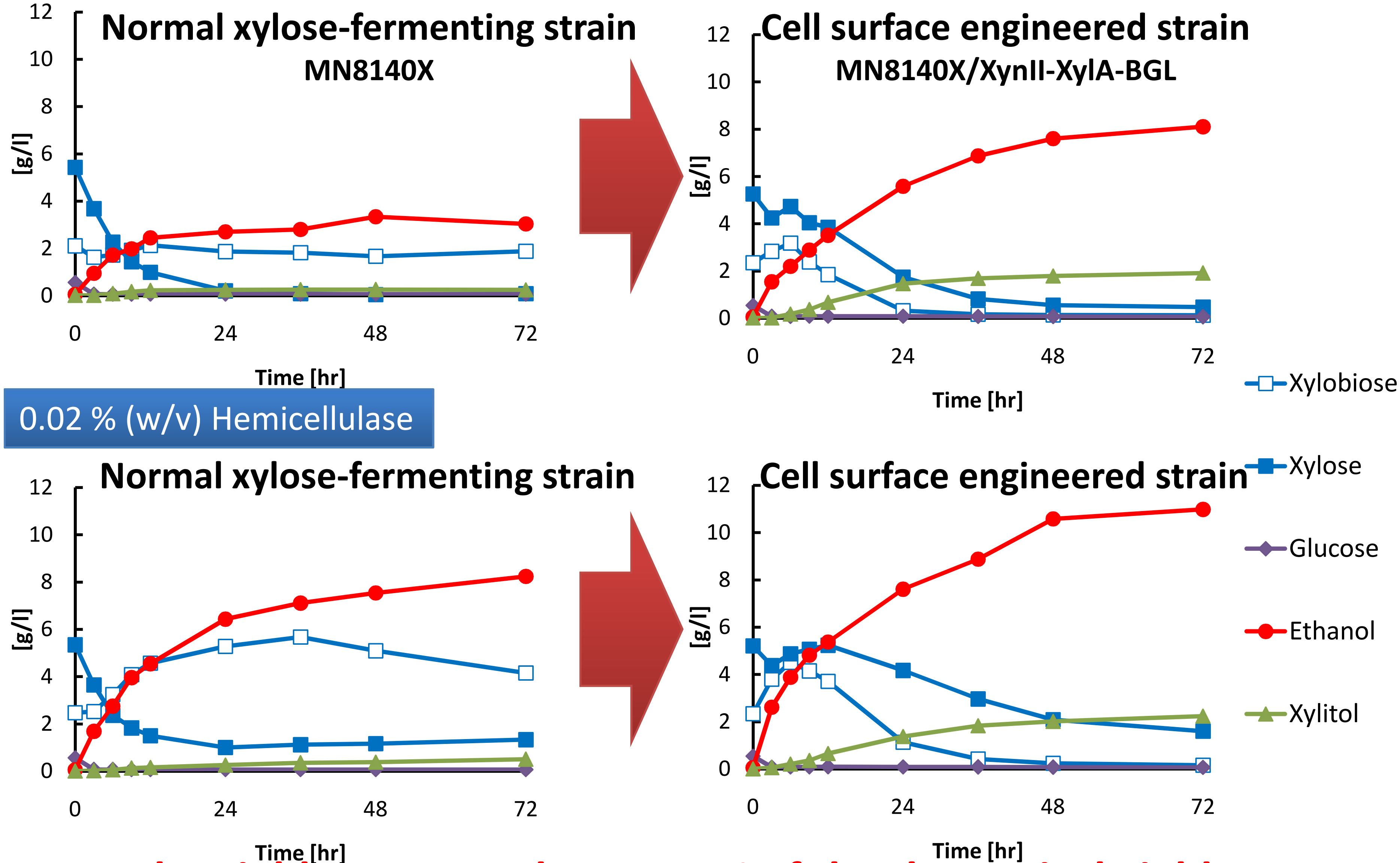
Fermentation of C5 fraction of rice straw obtained by hydrothermal pretreatment

Oligosacchrides	
Xylose [g/L]	4.19
Xylobiose [g/L]	1.69
Xylotriose [g/L]	15.77
Xylotetraose [g/L]	1.10
Xylopentaose [g/L]	0.013
Xylohexaose [g/L]	0.111
Glucose [g/L]	0.036
Cellobiose [g/L]	0.002
Cellotriose [g/L]	0.019
Cellotetraose [g/L]	0
Total oligosaccharide [g/L]	9.62
Total sugar [g/L]*	38.12

Inhibitors	
Acetate [mM]	46.2
Formate [mM]	28.1
Furfural [mM]	15.77
5-HMF [mM]	1.10



Fermentation of hemicellulosic hydrolysate

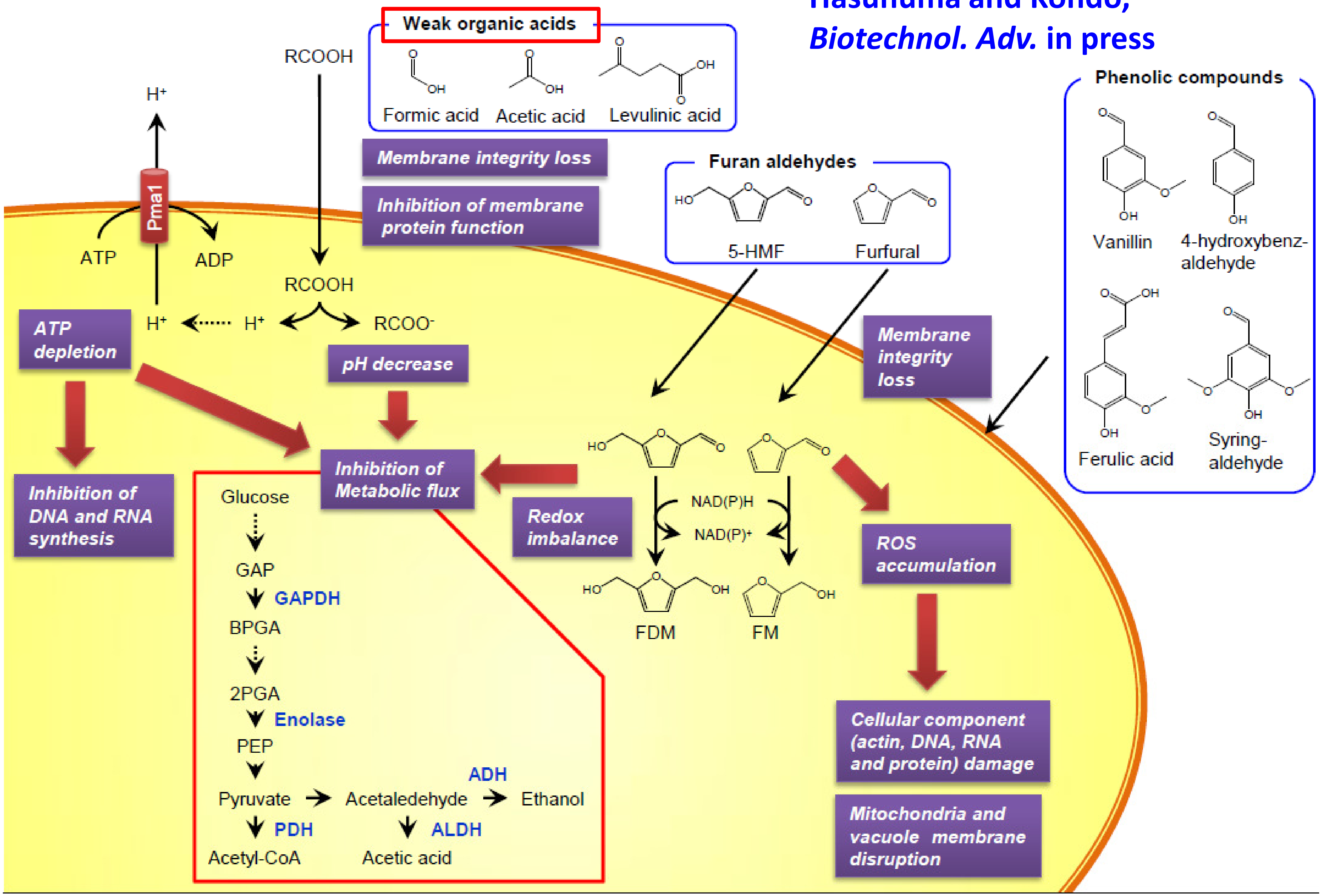


The yield corresponds to 82.1% of the theoretical yield.

Sakamoto et al. (2011) *J Biotechnol*

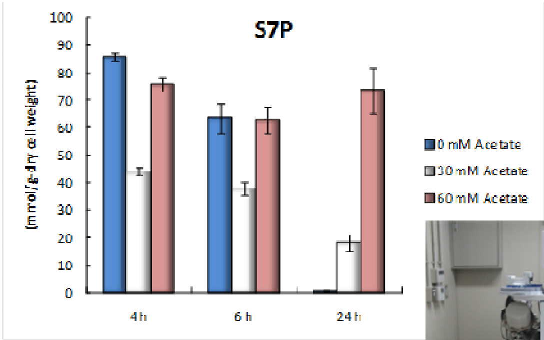
A presumed inhibition mechanism

Hasunuma and Kondo, *Biotechnol. Adv.* in press

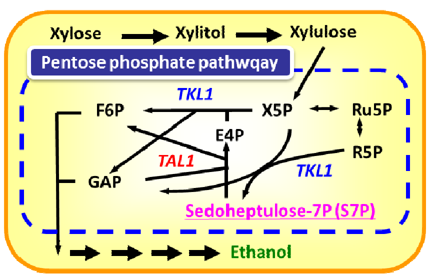


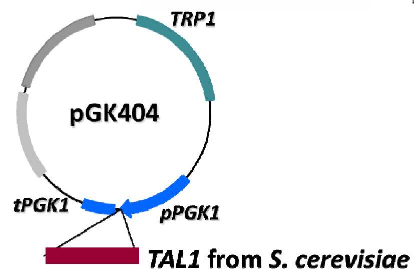
Several approaches to improve tolerance

Overexpression of TAL1 and FDH

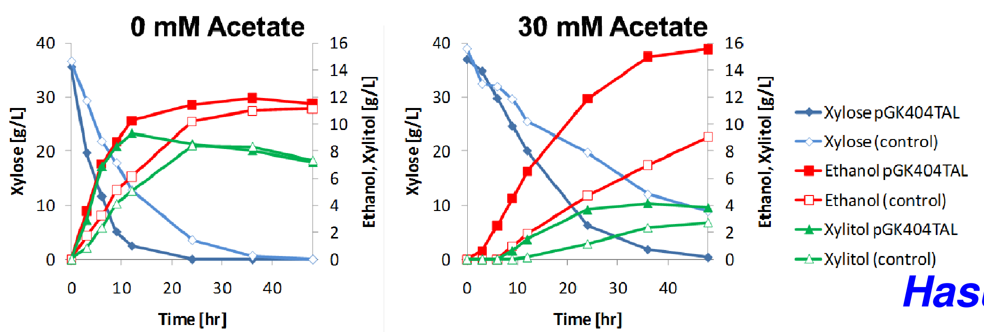


Metabolomic approach





**TAL1* encoding transaldolase (TAL)*



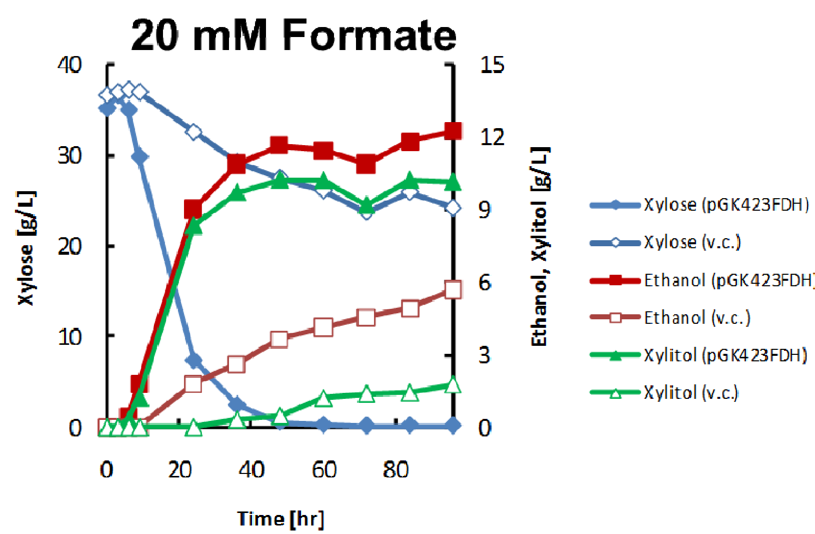
Transcriptomic approach

$HCOO^- \xrightarrow{NAD^+ \rightarrow NADH} CO_2$

FDH

Formate-responsive genes

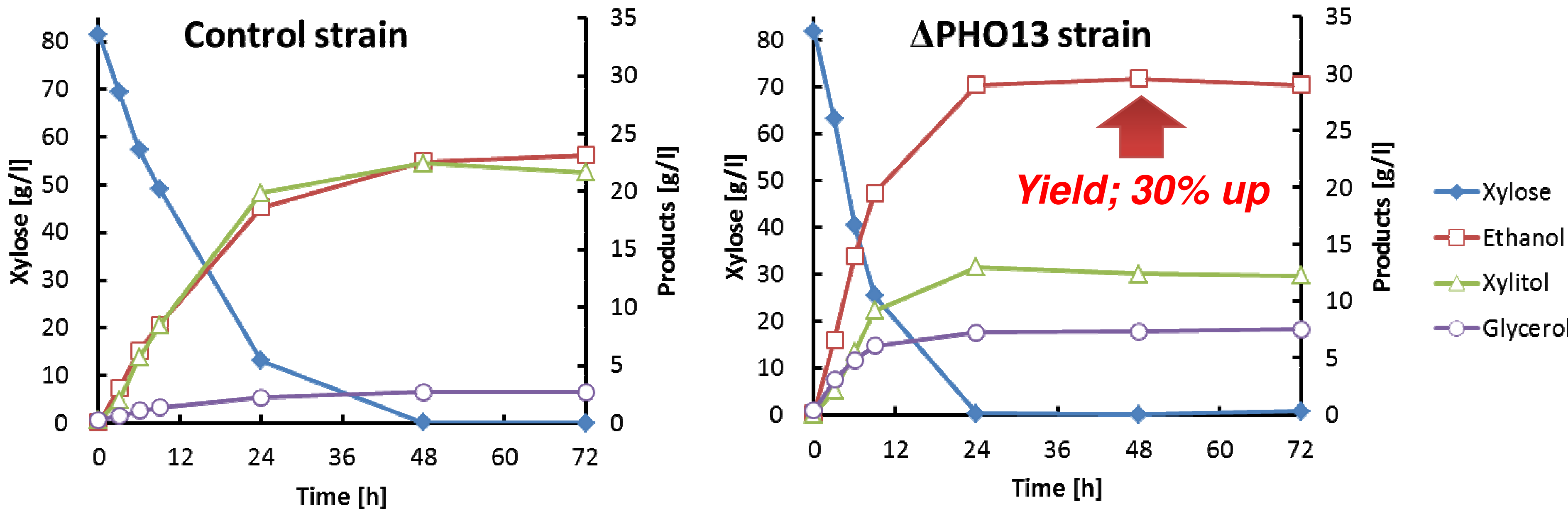
Gene	Relative mRNA expression				Description
	0 mM	5 mM	10 mM	15 mM	
FDH1	1	2.05	8.62	23.09	formate dehydrogenase (FDH)
BAT1	1	1.53	2.81	7.22	BCAA aminotransferase
ALD5	1	1.66	2.45	5.07	aldehyde dehydrogenase
HXK2	1	1.37	1.94	2.79	hexokinase
VCX1	1	1.54	2.08	2.78	Ca^{2+} ion transporter
GPD2	1	1.33	1.70	2.45	glycerol-3P dehydrogenase
HXT4	1	1.29	1.70	2.27	glucose transporter
PFK2	1	1.34	1.58	1.85	6-phosphofluctokinase



Hasunuma et al (2011) Microb Cell Fact
(2011) Appl Microbiol Biotechnol

Improvement of xylose fermentation

Effect of *PHO13* deletion on xylose fermentation



Hasunuma T, Poster12

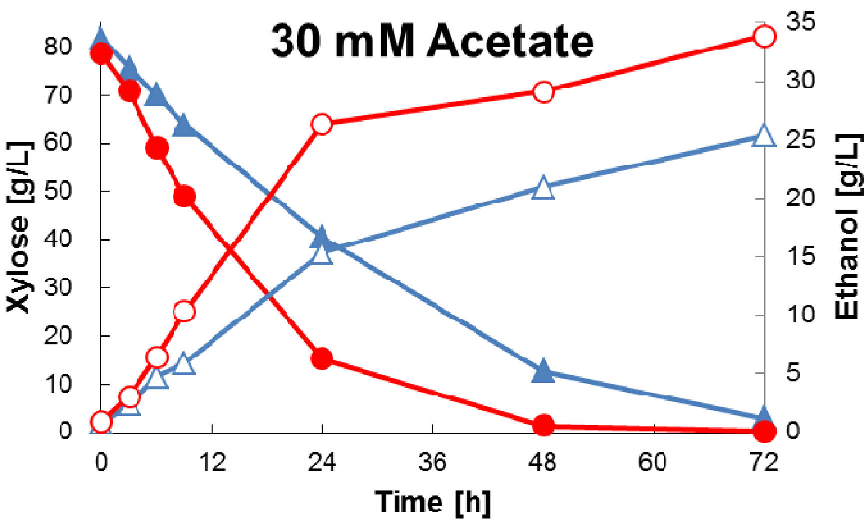
Medium; YP, 80 g/l xylose
Condition; Oxygen limited
Temp; 30^{o}C

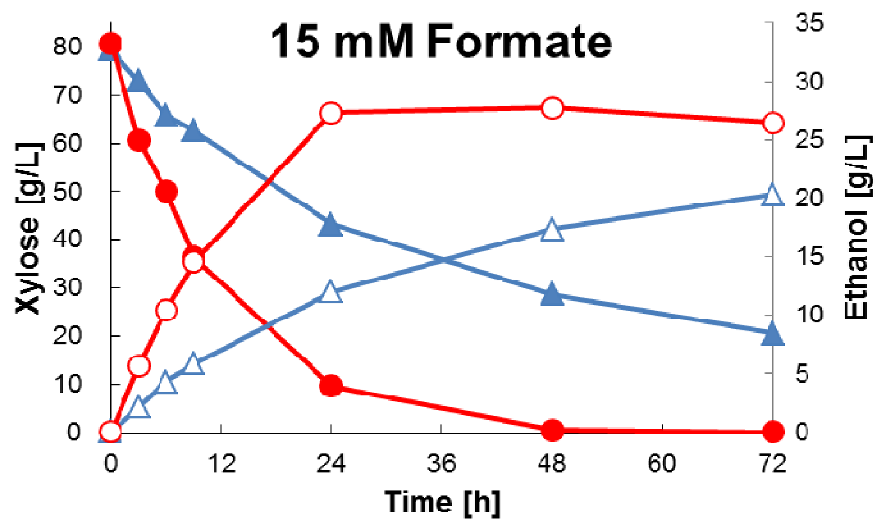
Deletion of *p*-nitrophenyl phosphatase gene, *PHO13* in xylose-fermenting strain improved ethanol production from xylose

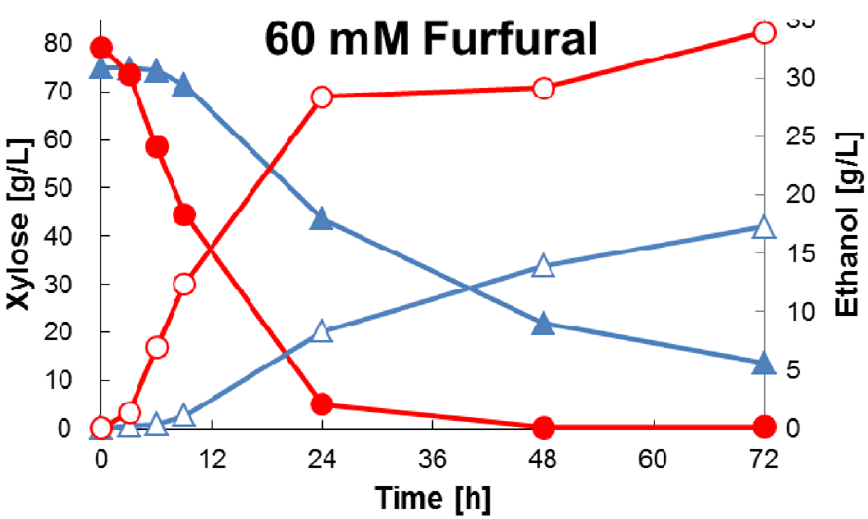
Fujitomi et al., 2012, *Bioresour Technol*

Effects of *PHO13* deletion on xylose fermentation

Improvement of xylose fermentation in the presence of inhibitors by *PHO13* deletion







***PHO13*-deleted strain**

Wild type

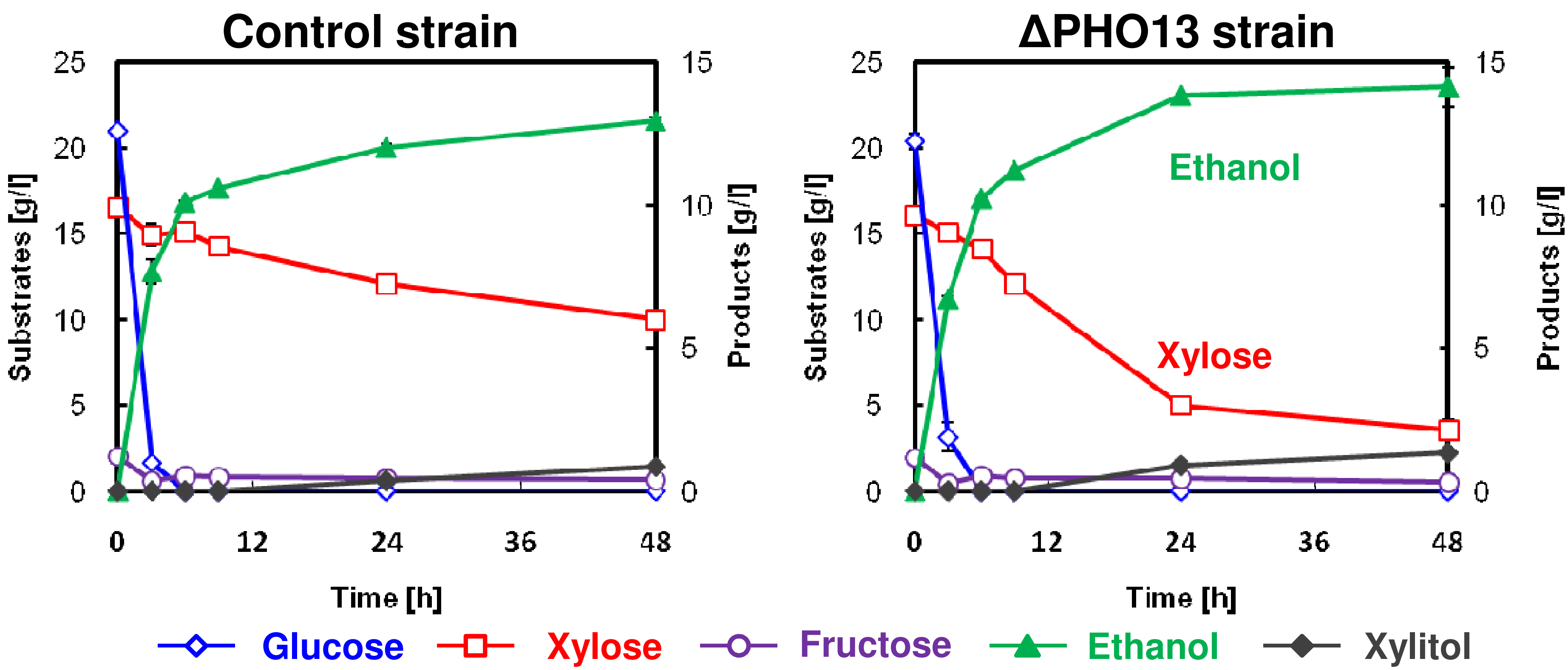
Medium; YP, 80 g/l xylose
Condition; Oxygen limited
Temp; 30ºC

Fujitomi et al., 2012, *Bioresour Technol*

Effects of *PHO13* deletion on xylose fermentation

Fermentation Inhibitors

Acetate	3.23 g/L (53.9 mM)
Formate	1.08 g/L (23.4 mM)
Furfural	1.22 g/L (12.7 mM)



Fujitomi et al., 2012, *Bioresour Technol*

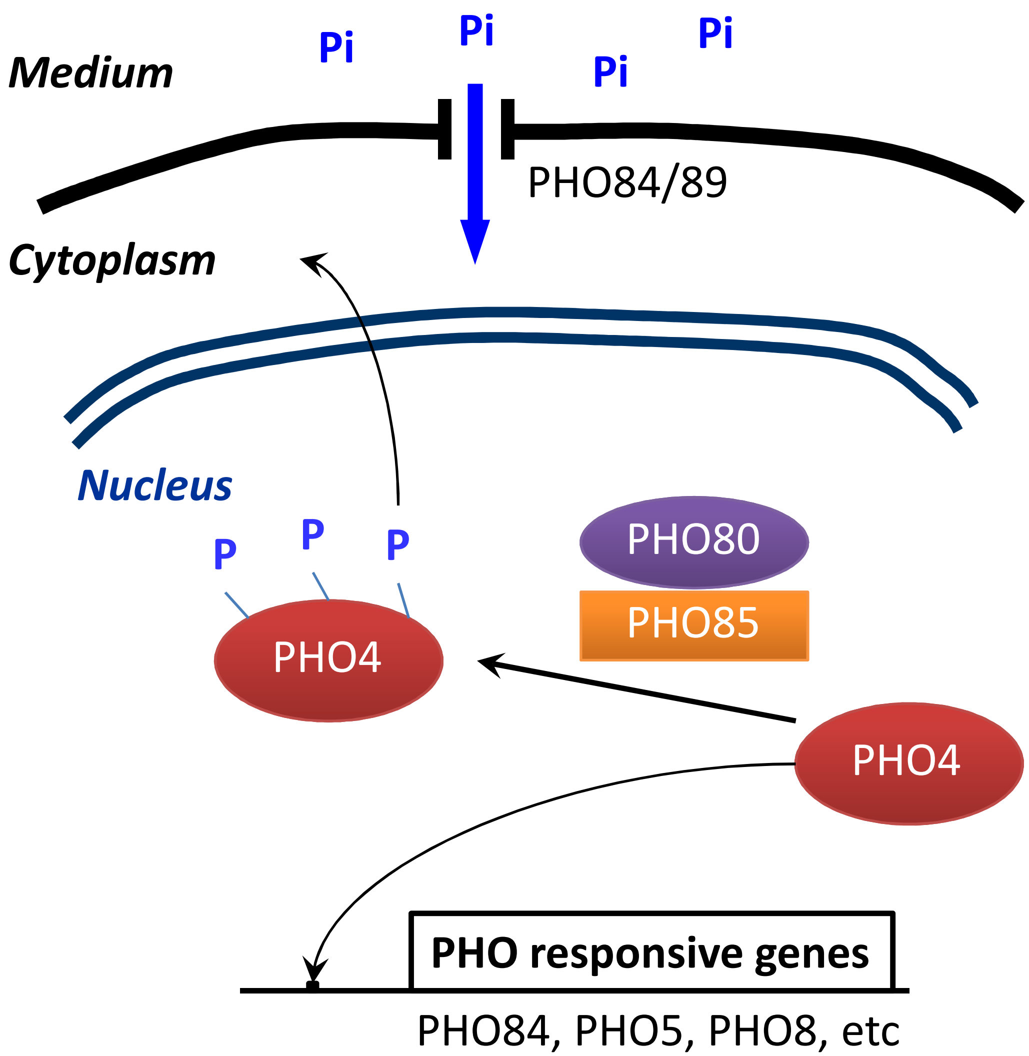
Global gene expression analysis of ΔPHO13 mutant

Genes highly up- or down-regulated by deleting the *PHO13* gene from a xylose-fermenting recombinant *S. cerevisiae* strain

Gene	Category	Annotated function	Fold change
ZWF1	Metabolic pathway (Glycolysis, PPP, Alcohol biosynthesis)	Glucose-6-phosphate dehydrogenase	4.00
SOL3		6-phosphogluconolactonase	3.67
GND1		6-phosphogluconate dehydrogenase	1.64
TKL1		Transketolase	1.57
PFK1		Alpha subunit of heterooctameric phosphofructokinase involved in glycolysis	1.55
TDH1		Glyceraldehyde-3-phosphate dehydrogenase	1.48
PDC1		Major of three pyruvate decarboxylase isozymes	2.04
ADH1		Alcohol dehydrogenase	1.94
GPD1		NAD-dependent glycerol-3-phosphate dehydrogenase	1.64
COX2	Respiratory chain	Subunit II of cytochrome c oxidase	0.33
COX3		Subunit III of cytochrome c oxidase	0.30
CYC1		Cytochrome c	0.59
QCR6		Subunit 6 of the ubiquinol cytochrome-c reductase complex	0.57
ATP1	ATP synthase	Alpha subunit of the F1 sector of mitochondrial F1F0 ATP synthase	2.24
ATP19		Subunit k of the mitochondrial F1F0 ATP synthase	2.60
ATP5		Subunit 5 of the stator stalk of mitochondrial F1F0 ATP synthase	1.64

Phosphate responsive signaling pathway

The PHO system

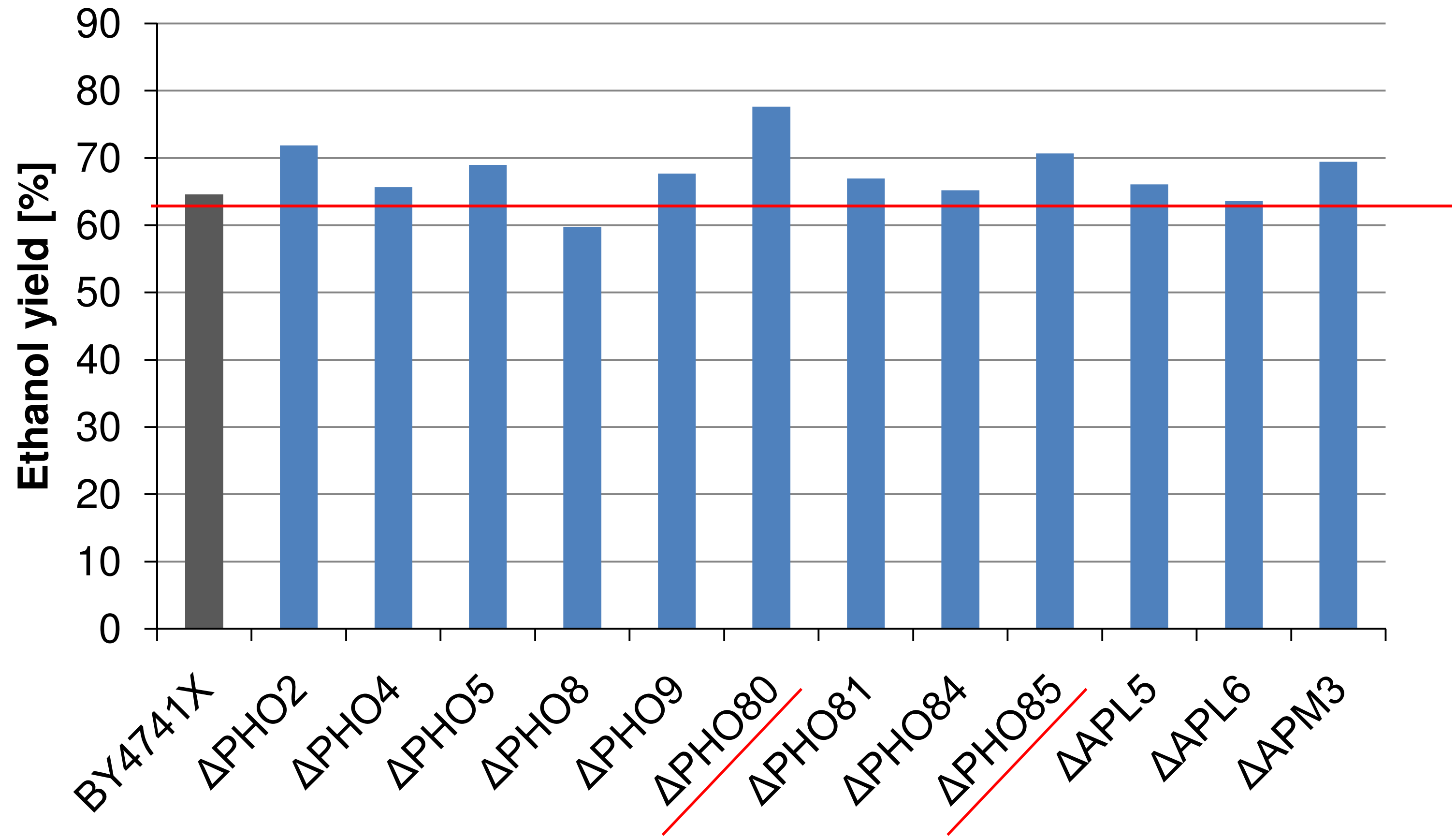


Gene	Function of gene product
Pho84	Pi-transporter
Pho89	Pi-transporter
Pho4	DNA-binding trascriptional activator
Pho80	Cyclin; inhibitor of Pho4p
Pho85	Cyclin-dependent kinase; inhibitor of Pho4p
Pho5	Repressible vacuolar alkaline phosphatase
Pho8	Repressible vacuolar alkaline phosphatase

(Ohshima et al., 1997; Lu et al., 2007)

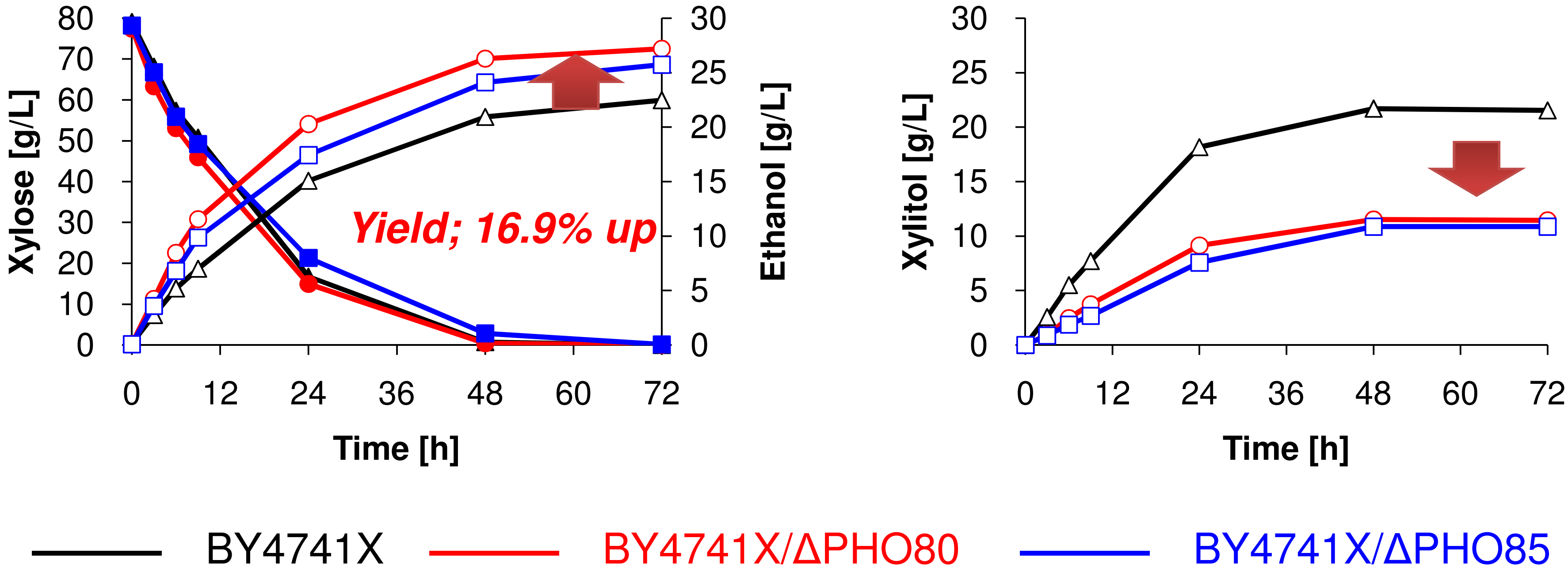
Xylose fermentation

Effect of deletion of *PHO*-related genes on ethanol production



Medium; YP, 80 g/l xylose
Condition; Oxygen limited
Temp; 30℃

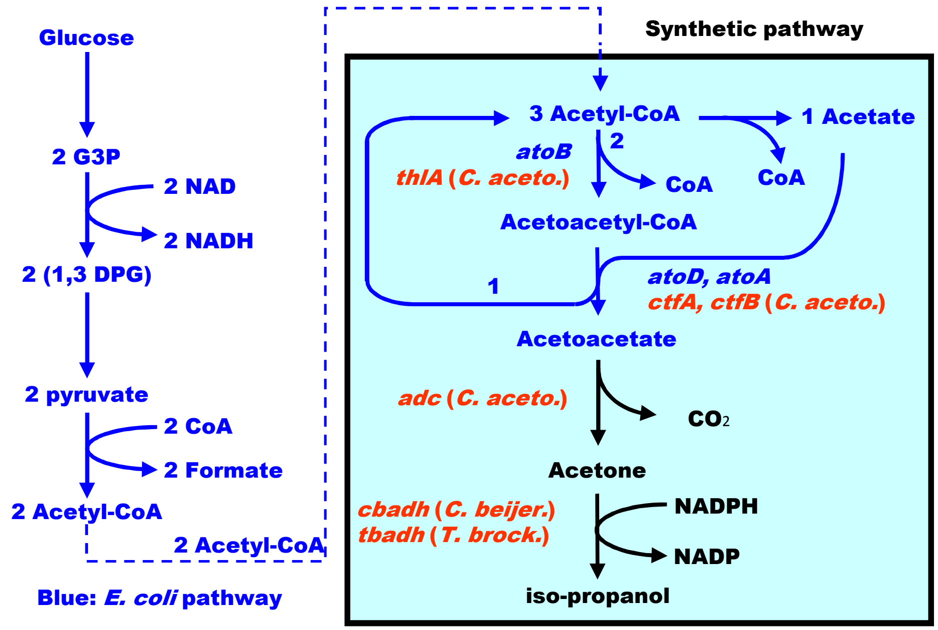
Effect of *Pho80/Pho85* deletion on xylose fermentation

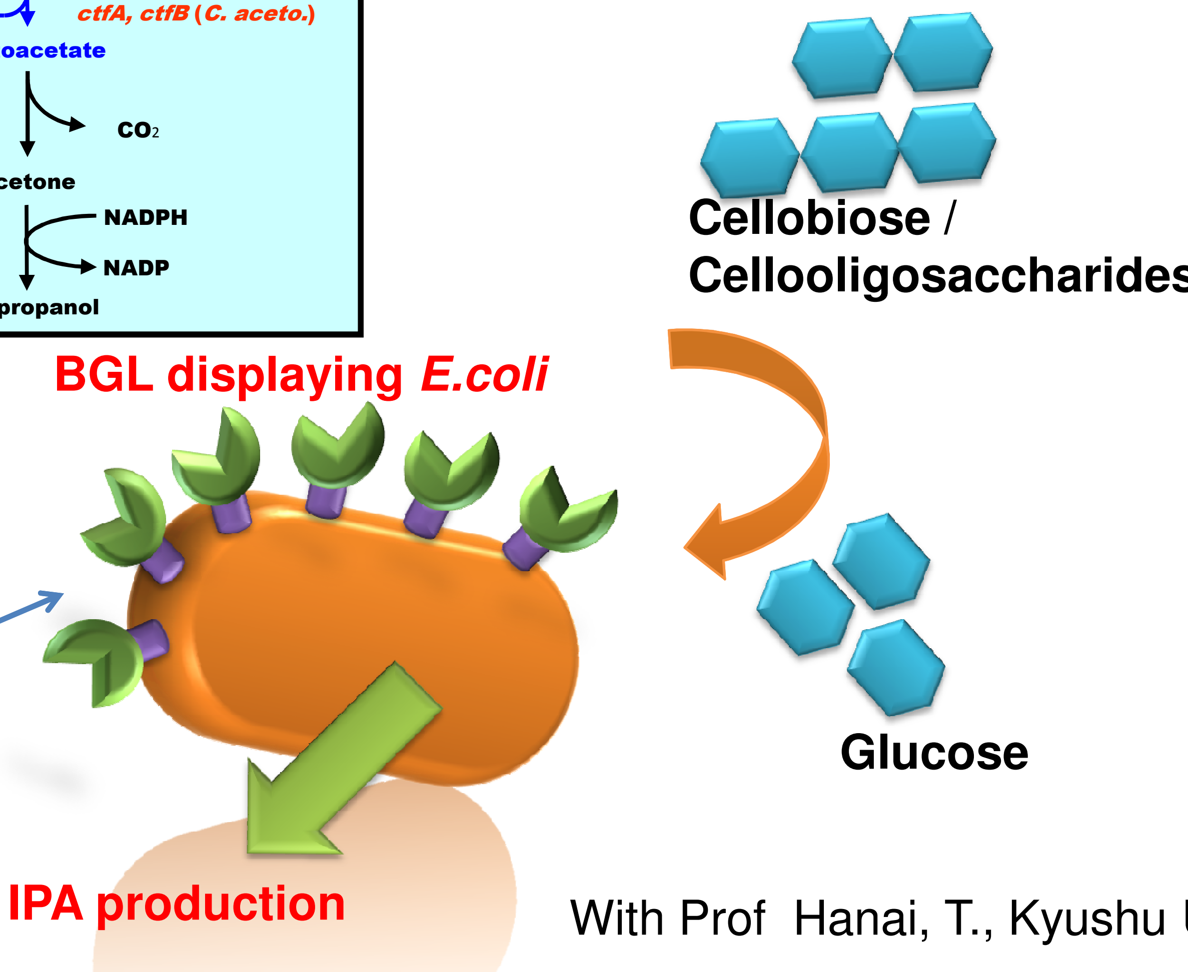


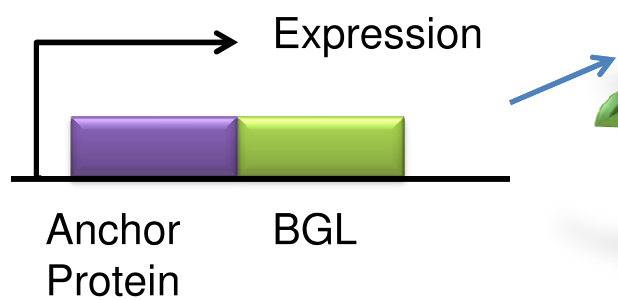
Strain	Volumetric productivity (g/L/h)	Ethanol yield (g/g)	Xylitol yield (g/g)
BY4741X	0.730	0.28	0.27
BY4741X/ΔPHO80	0.844	0.35	0.15
BY4741X/ΔPHO85	0.920	0.33	0.14

Xylose fermentation is affected by the PHO metabolism.

IPA production from cellobiose

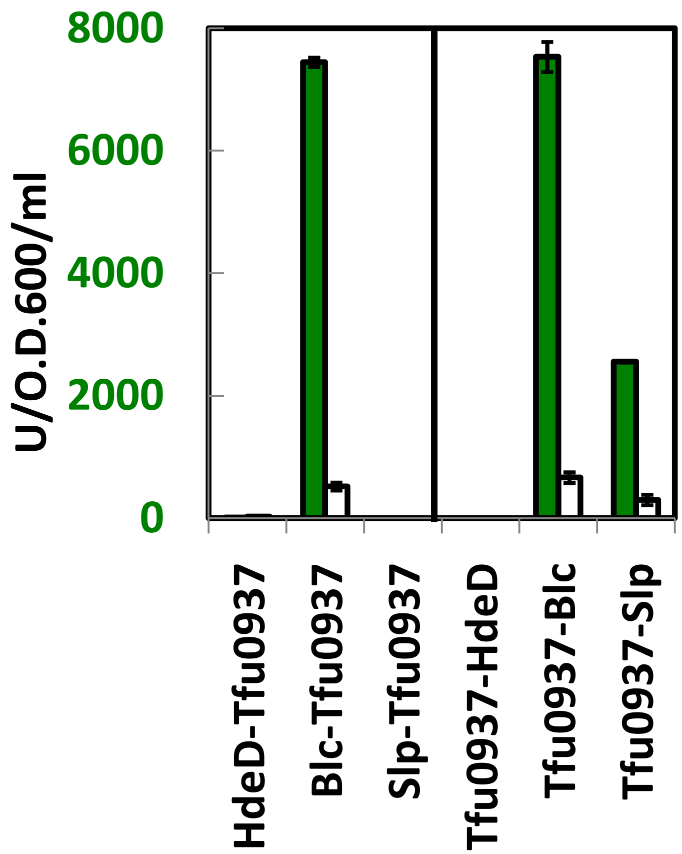




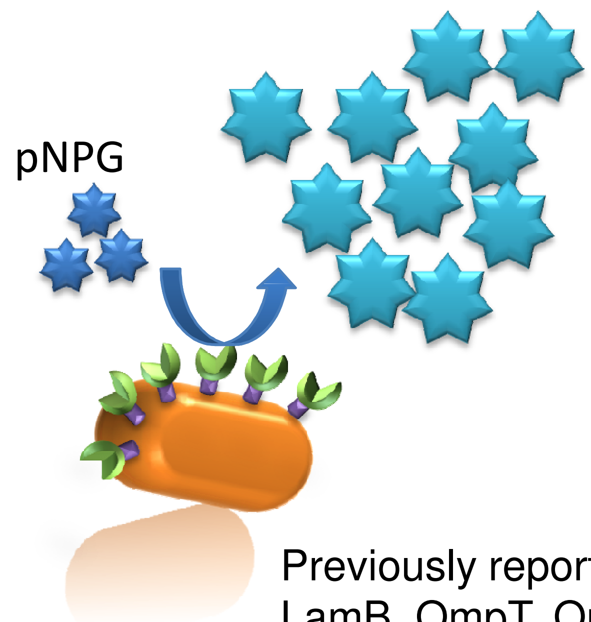


With Prof Hanai, T., Kyushu Univ

Optimization of BGL and anchor



Tfu0937 was fused to an anchor, HdeD, Blc and Slp.

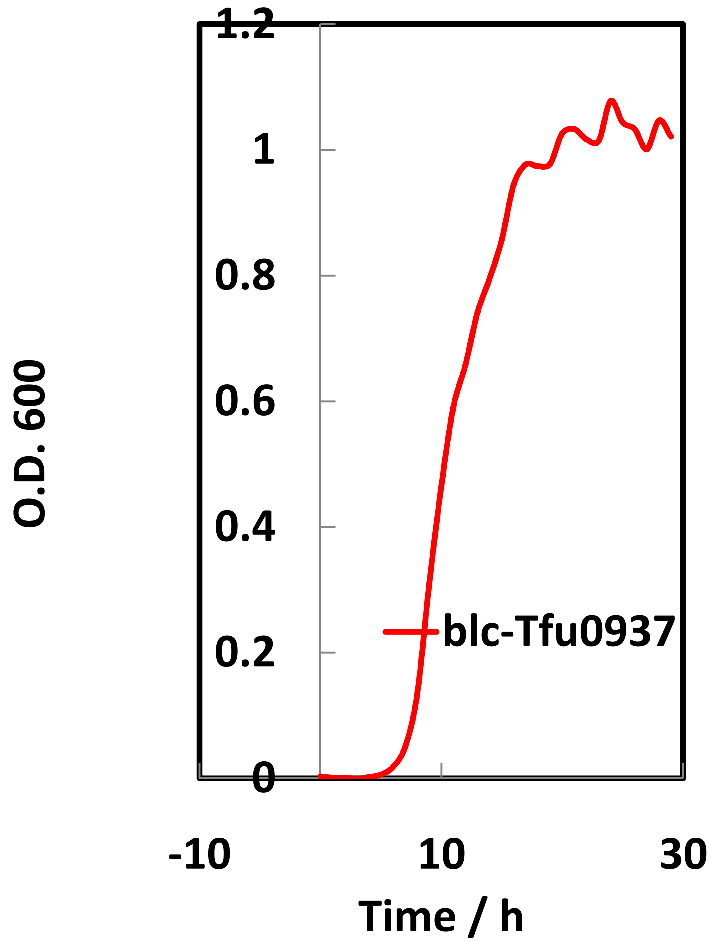


Previously reported anchor such as LamB, OmpT, OmpL did not work for activ BGL display

BGL activity of Blc-Tfu0937 were improved up to 70-fold higher compared to PgsA-BglA.

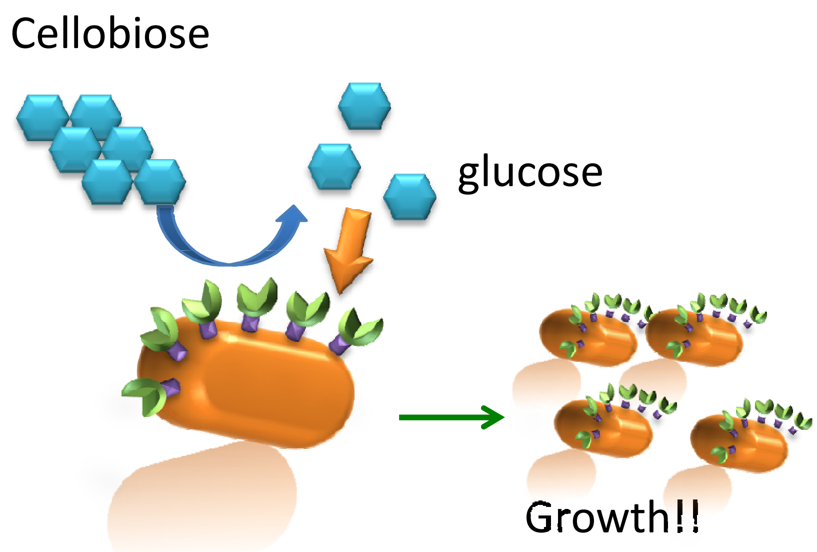
Tanaka et al (2012) Appl Environ Microbiol

Growth on 0.2% cellobiose using BGL-displaying *E.coli*



0.2% cellobiose as the sole carbon source

M9 minimum medium, Initial O.D.600 = 0.01



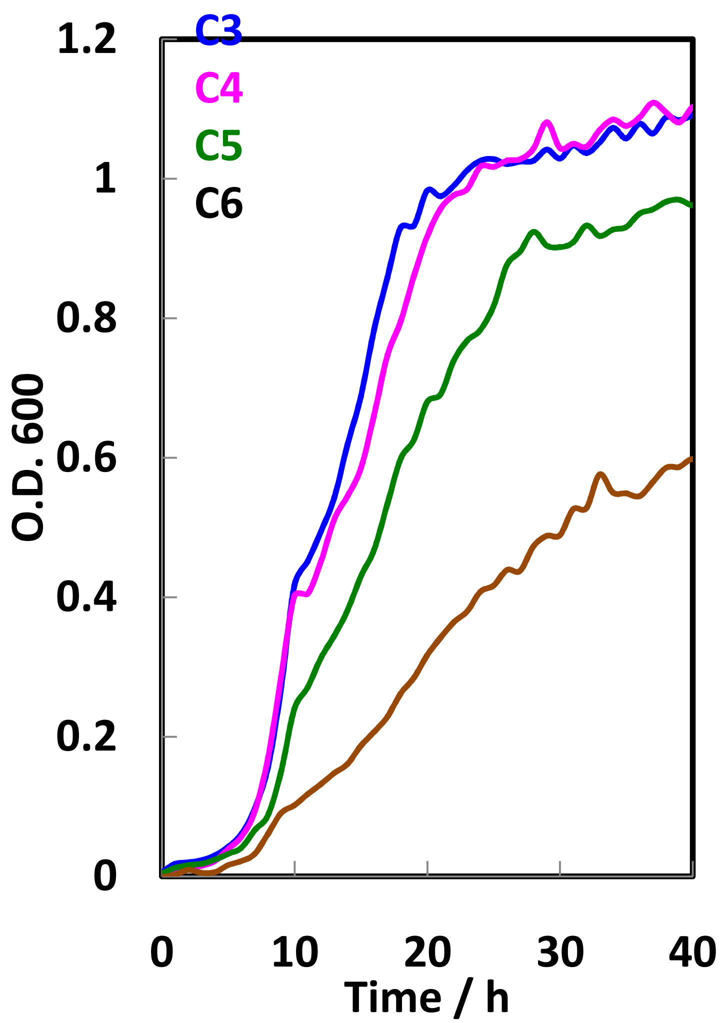
Growth on 0.2% cellobiose

Blc-Tfu0937 was growth on 0.2% cellobiose

O.D.600=1.05 after 20h cultivation, almost same levels of glucose

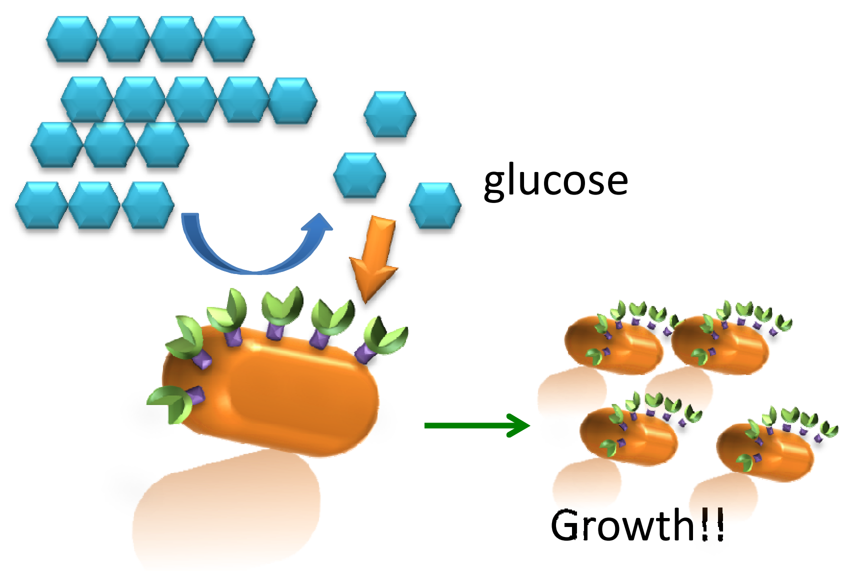
Tanaka et al (2012) Appl Environ Microbiol

Growth on 0.2% cellooligosaccharide



- Cellotriose (C3), Cellotetraose(C4), Cellopentaose(C5), Cellohexaose(C6) were used as carbon source, respectively

Cellooligosaccharides

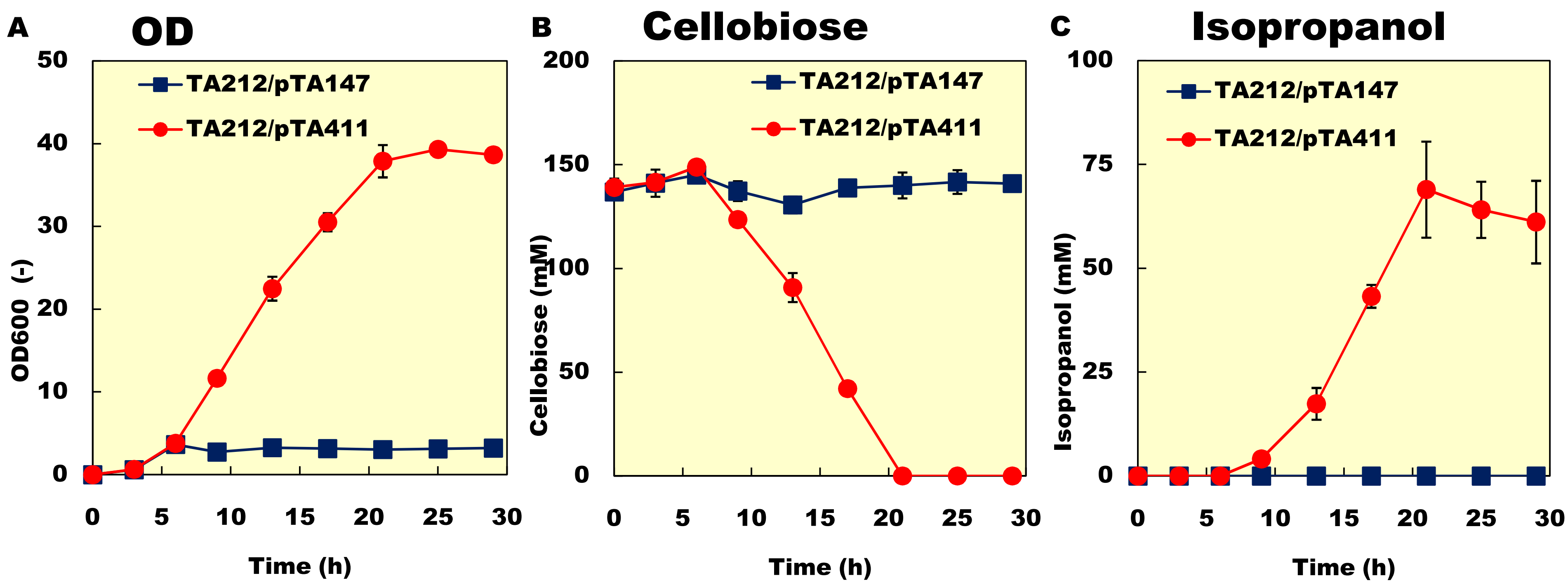


Successful growth on cellooligosaccharides

Tanaka et al (2012) Appl Environ Microbiol

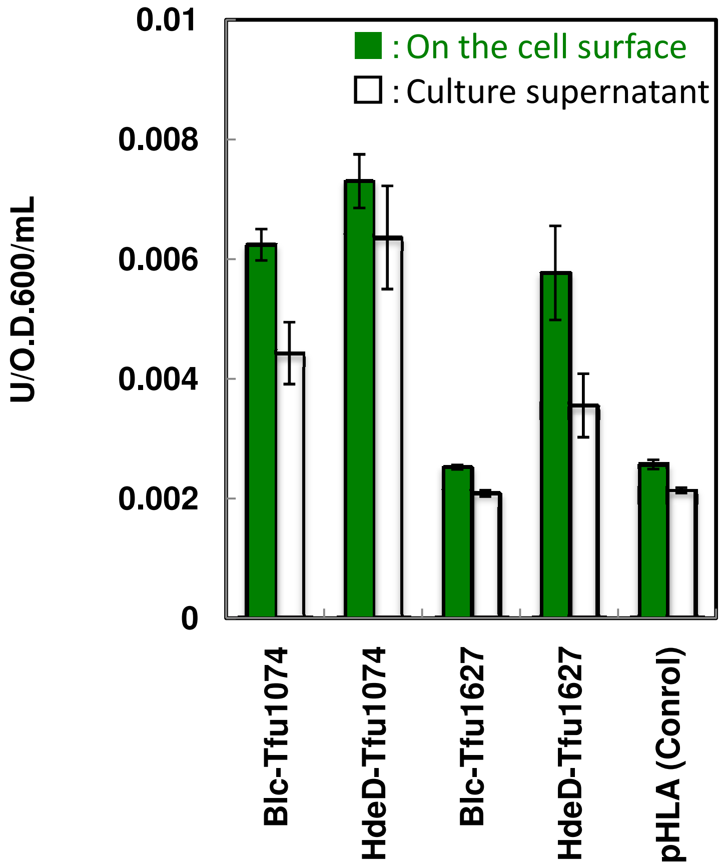
Direct IPA production from cellobiose Using BGL-displaying engineered *E.coli*

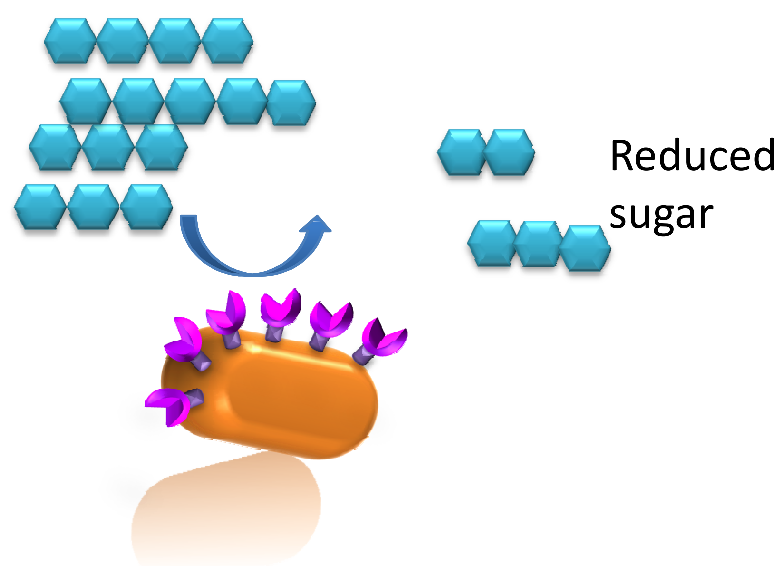
5wt% vol cellobiose in SD8 medium



Successful IPA production from cellobiose (69 mM = 4.1 g/L)

EG activity displayed on the *E.coli*

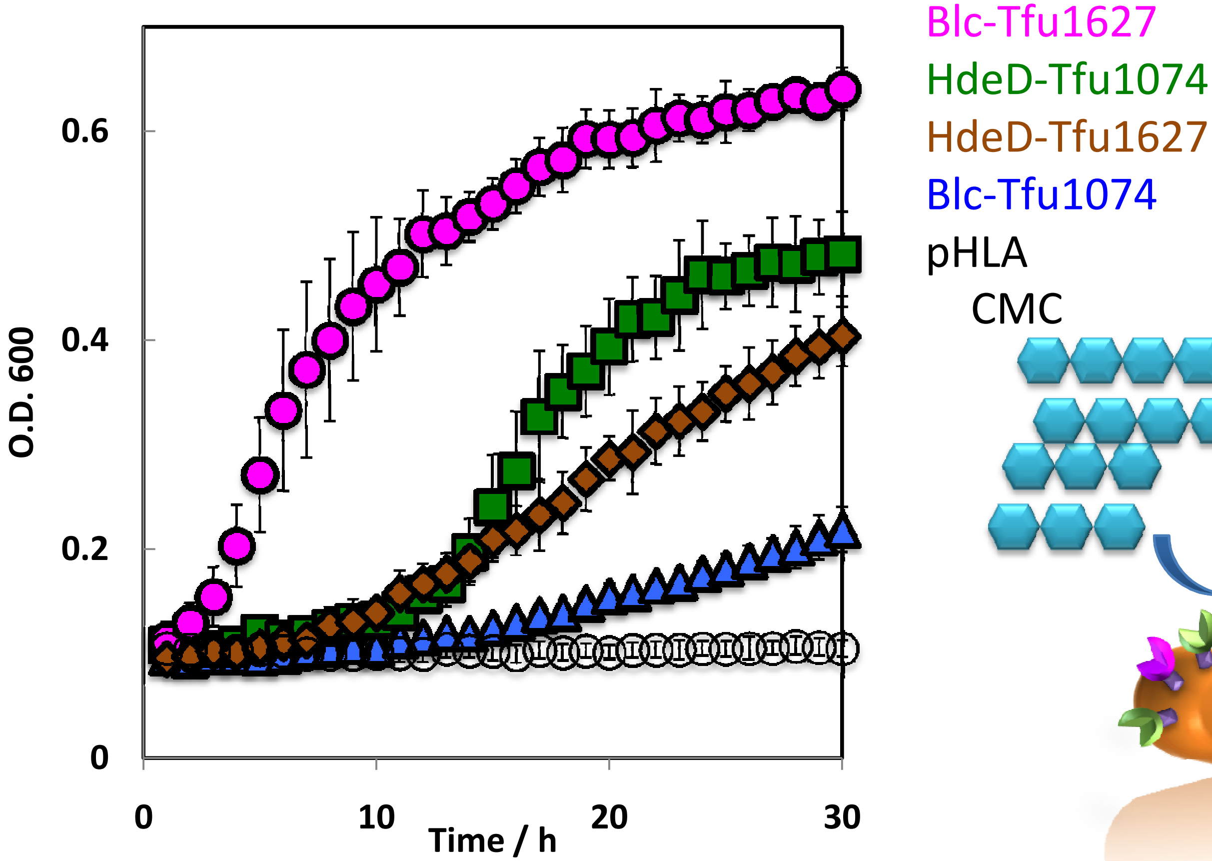


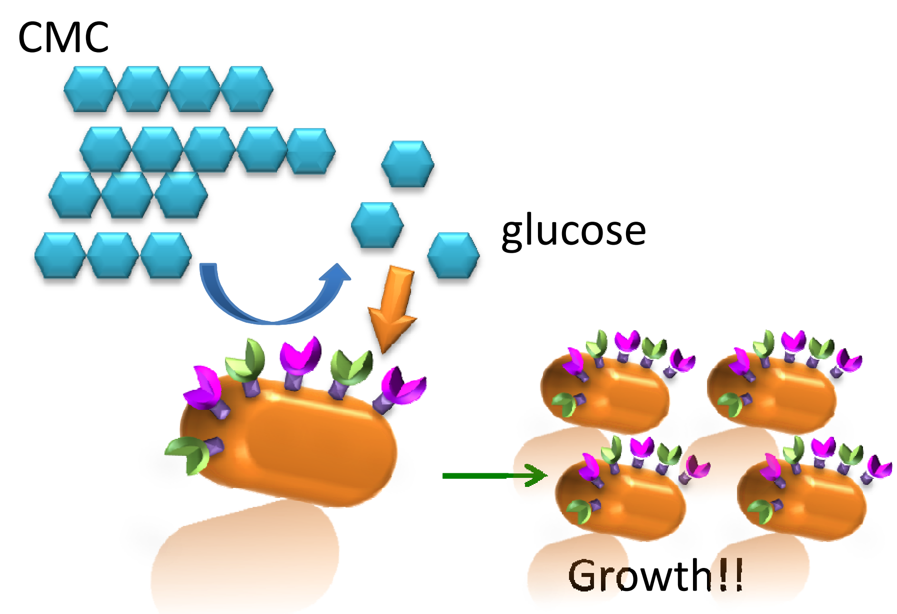


EG displayed on the cell surface has CMC degradation activity

Submitted

Direct growth on CMC both BGL and EG displaying *E. coli* in the minimum medium





EG displayed on the cell surface has CMC degradation activity

Submitted

Thank you very much for your attention

NEDO 技術開発機構

文部科学省 MINISTRY OF EDUCATION, CULTURE, SPORTS, SCIENCE AND TECHNOLOGY-JAPAN

JST 独立行政法人 科学技術振興機構 Japan Science and Technology Agency

Matsuda F, Poster28 Effect of metabolic inhibitors

Strategy for construction of super microbial cells

Metabolome analysis

UPLC/Q-TOF MS

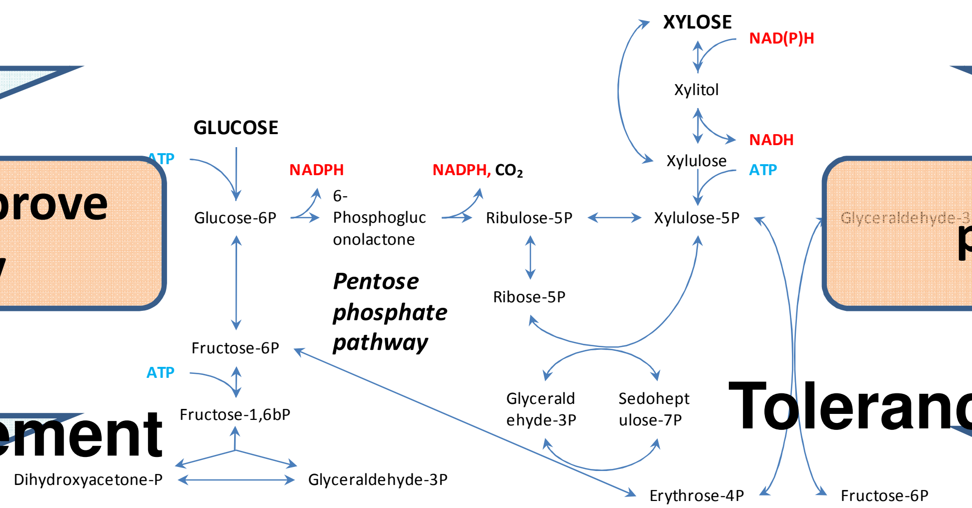
GC/TOF MS

CE/TOF MS

Transcriptome analysis

Key genes to improve productivity

Flux improvement



Mechanisms of pH, temperature tolerance

Tolerance improvement

Super microbial cells for cell factories