Producción de etanol carburante a partir de rastrojo de maíz con bacterias etanologénicas

Fuel ethanol production from corn stover with ethanologenic bacteria

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SMIBIO

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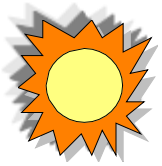
Generation Ethanol (Agro-Fuels) and (Agro-) Chemicals from Lignocellulose



The Sun

Artificial
CO_2 cycle

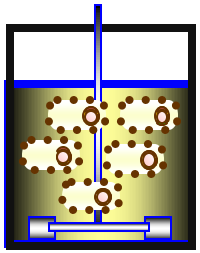
CO_2

Chemicals
Ethanol (Fuel)

Lignocellulose - Biomass
Agricultural Residues
Bagasses and Stovers
Energy Crops

Sugars

Hydrolysis

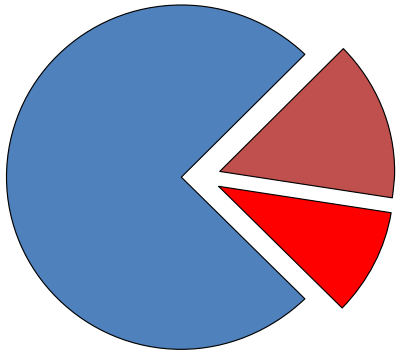


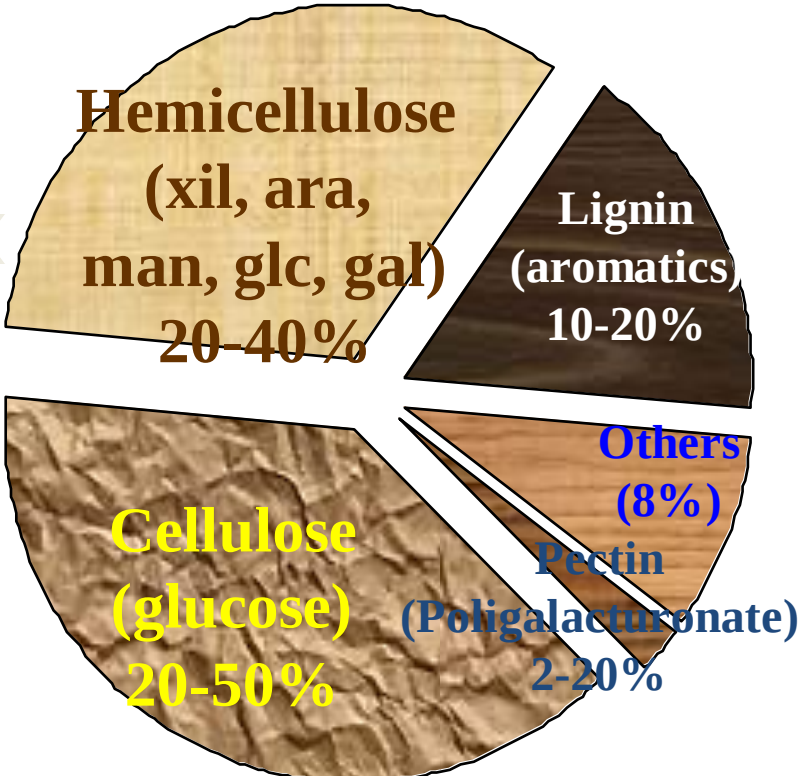
Fermentation

Purpose: Design microorganism and process to transform ALL the SUGARS contained into **lignocellulose (cellulose: glucose & hemicellulose: pentoses, hexoses, disaccharides) to ethanol (or other chemicals)**

Carreón Rodríguez et al., 2009

Lignocellulose





Hemicellulose:
Thermochemical Hydrolysis
Low or high pH, T > 100°C

Cellulose:
Enzymatic Hydrolysis
pH 4.5-5, 50°C or higher

Pentoses + Hexoses
(Xil + Ara) + (Gluc+Man+Gal)
+ Acetate + Glucuronic Acid
+ Toxins

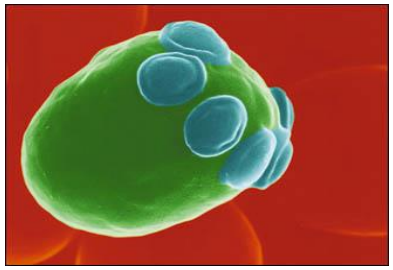
Ingram et al., 1999

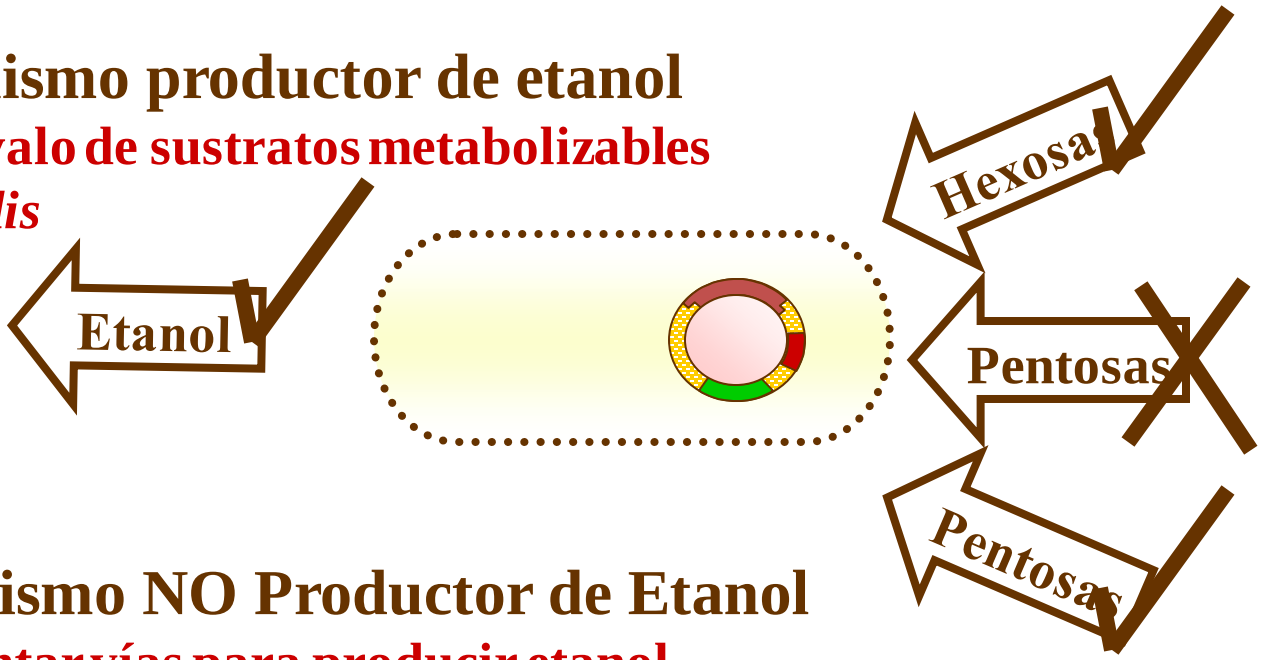
Dos principales estrategias: Pentosas → Etanol
Ingeniería de Vías Metabólicas

A: Microorganismo productor de etanol

Extender el intervalo de sustratos metabolizables

Zymomonas mobilis

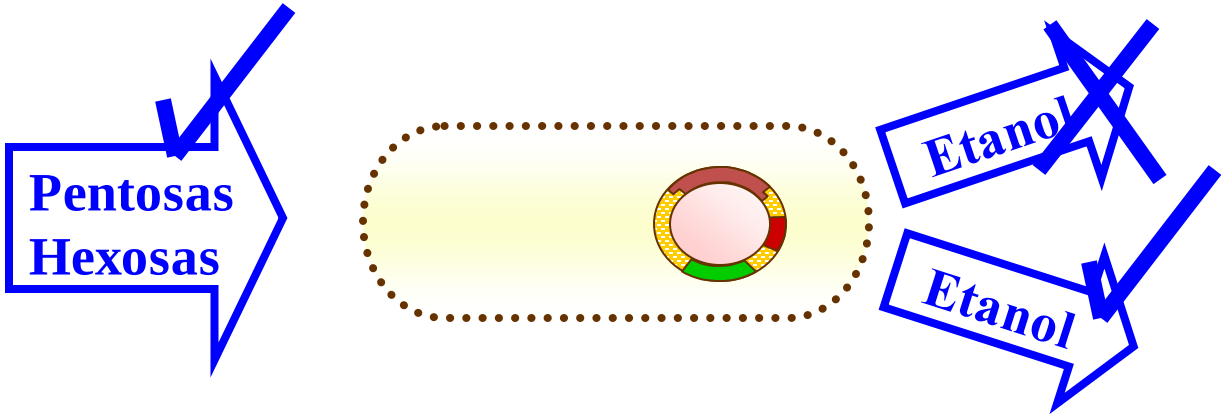




B: Microorganismo NO Productor de Etanol

Complementar vías para producir etanol

Escherichia coli



Escherichia coli Fermentative Phatways

HEXOSES (Glc, Fru, Gal, Man etc.) + PENTOSES (Xyl, Ara, Rib, Xylu, etc.)

Embden-Meyerhof-Parnas | Entner-Doudoroff | Pentose Patway

PYRUVATE

FRD → Succinate

LDH (Km = 7 mM) → Lactate

PFL (Km = 2 mM) → Acetyl-CoA + Formate

Acetyl-CoA → Acetate, Ethanol

Formate → CO_2, H_2

Glucose → 2 Ethanol + 2 CO_2

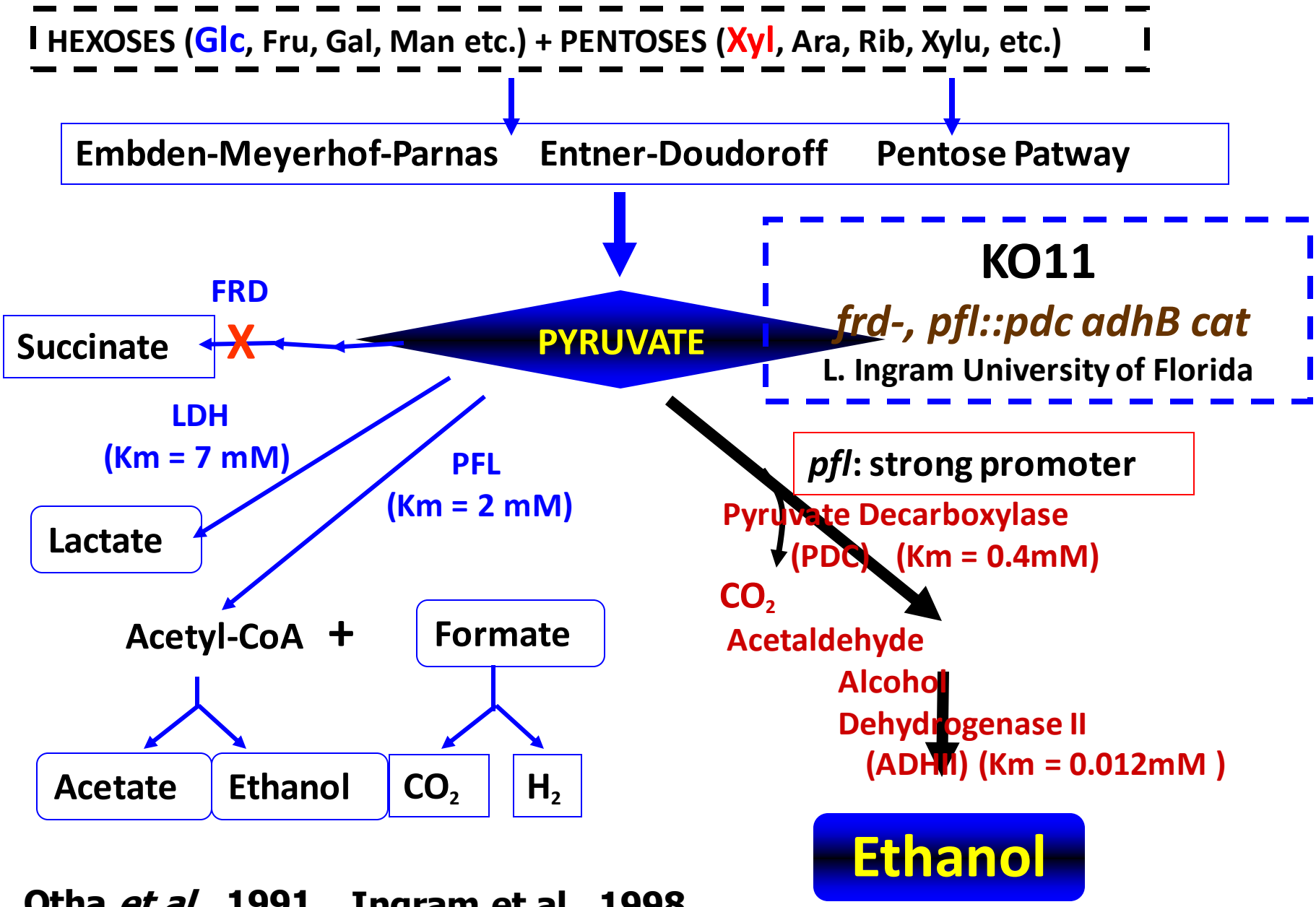
Sucrose → 4 Ethanol + 4 CO_2

Teoretical Yield: 0.51 g/g

$g_{ETHANOL} / g_{(GLC\ o\ SAC)}$

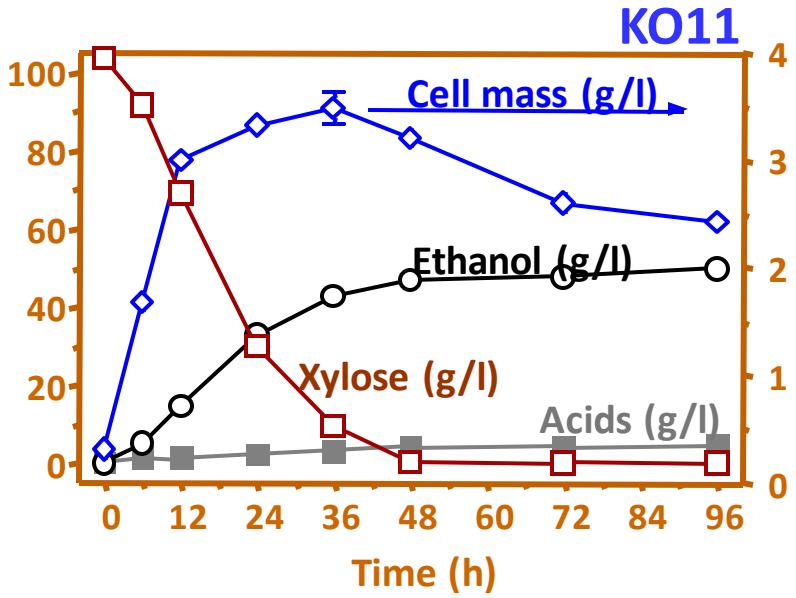
0.64 L_{EtOH}/kg

Escherichia coli Etanologénica: 1ra Generación

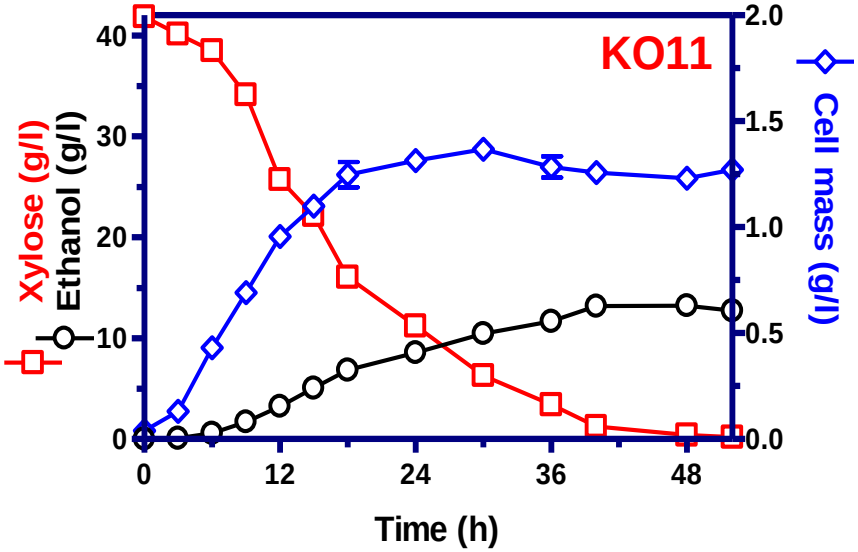


Otha *et al.*, 1991 Ingram et al., 1998

KO11: Xyl (10%) Rich Medium



Xyl (4%): Mineral Media



In comparison with rich media, in mineral media there are reductions by:

57_{Glc} & 63_{Xyl} % in cell mass formation; 25% in the specific growth rate

70_{Glc} & 60_{Xyl} % in the specific sugar consumption rate

And Q_{EtOH} is reduced to 0.42_{Glc} & 0.33_{Xyl} $g_{Et\text{-}OH}$/l h, for glucose and xylose, respectively

37°C, 100 rpm, pH 7.0

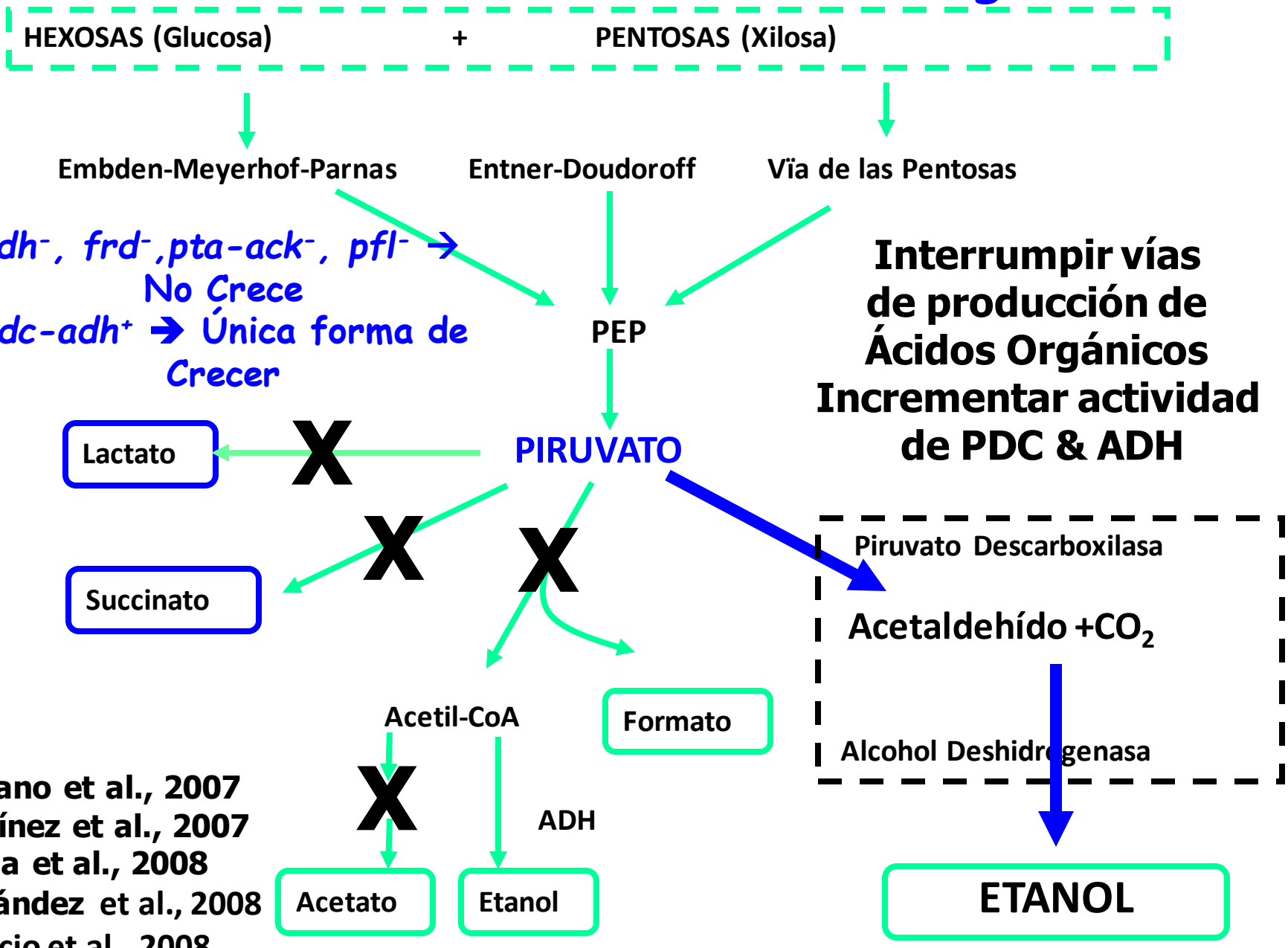
Yield >95%

Yield 60%

Tao *et al.*, 2001, Martínez et al., 1999

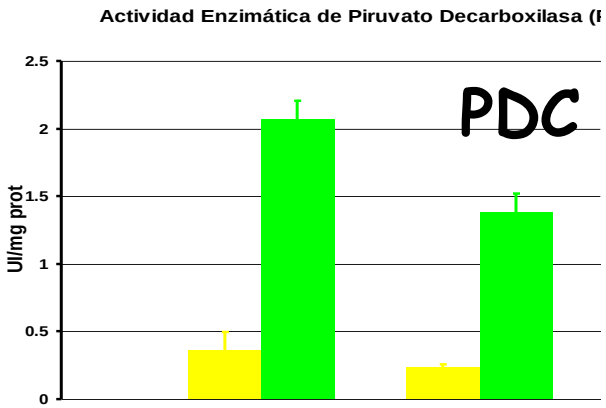
Huerta-Beristain et al., 2008

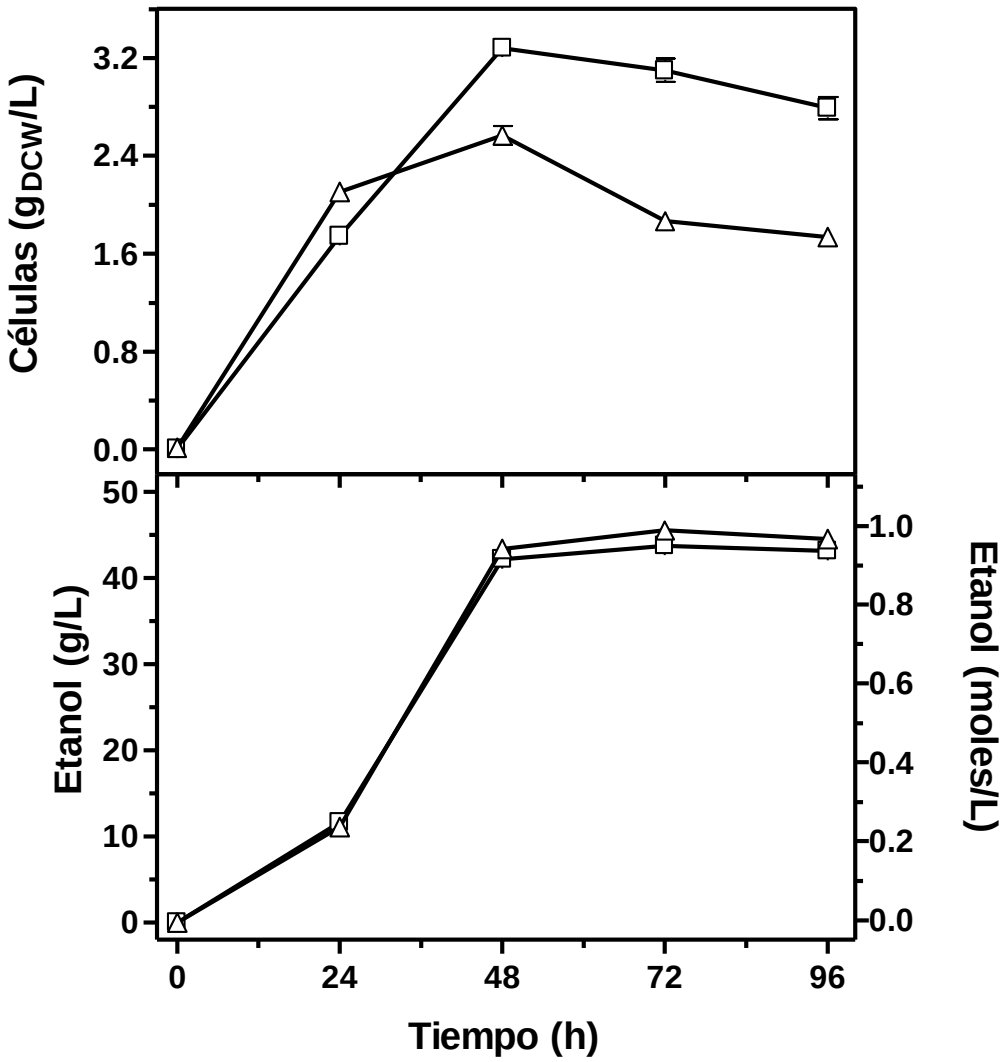
2da Generación de *E. coli* Etanologénicas



Yomano et al., 2007
Martínez et al., 2007
Utrilla et al., 2008
Fernández et al., 2008
Orencio et al., 2008

Ethanol Mineral Media: Xyl-Glc



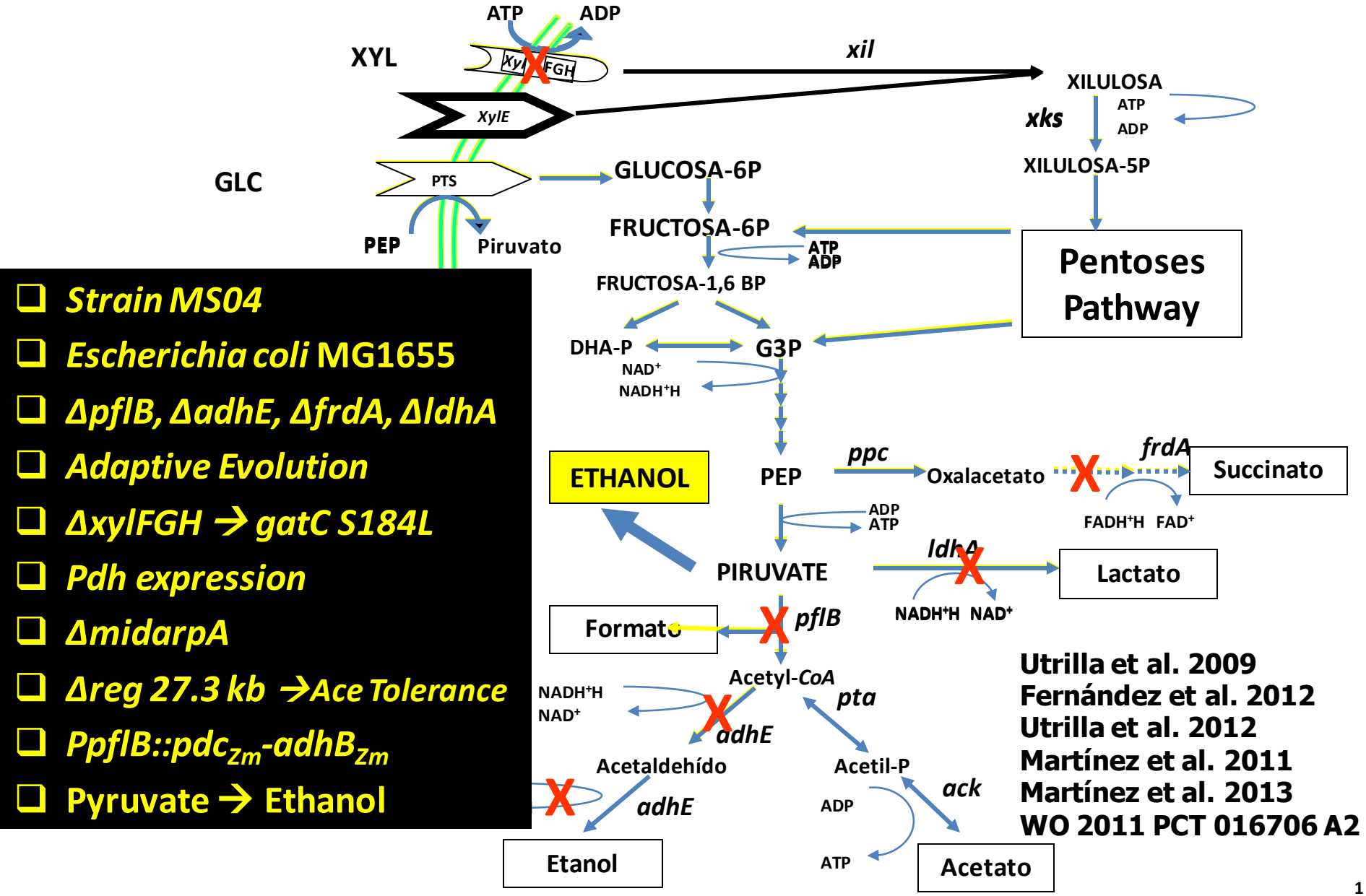


Yomano et al., 2007
Martínez et al., 2007
Utrilla et al., 2009
Fernández et al., 2011
Orencio et al., 2008

Yield >90%

Ethanologenic *E. coli* strain to use pentose-hexose mixtures

MG1655: *ΔpflB, ΔadhE, ΔfrdA, ΔxylFGH, Δldh, PpflB::pdc-adh*$_{Zm}$



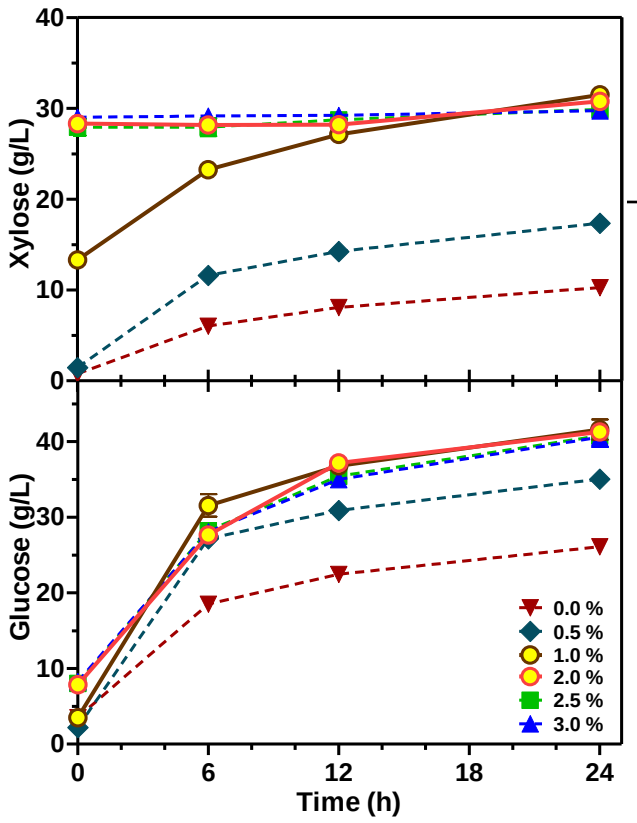
- *Strain MS04*
- *Escherichia coli* MG1655
- *ΔpflB, ΔadhE, ΔfrdA, ΔldhA*
- *Adaptive Evolution*
- *ΔxylFGH → gatC S184L*
- *Pdh expression*
- *ΔmidarpA*
- *Δreg 27.3 kb →Ace Tolerance*
- *PpflB::pdc*$_{Zm}$*-adhB*$_{Zm}$
- Pyruvate → Ethanol

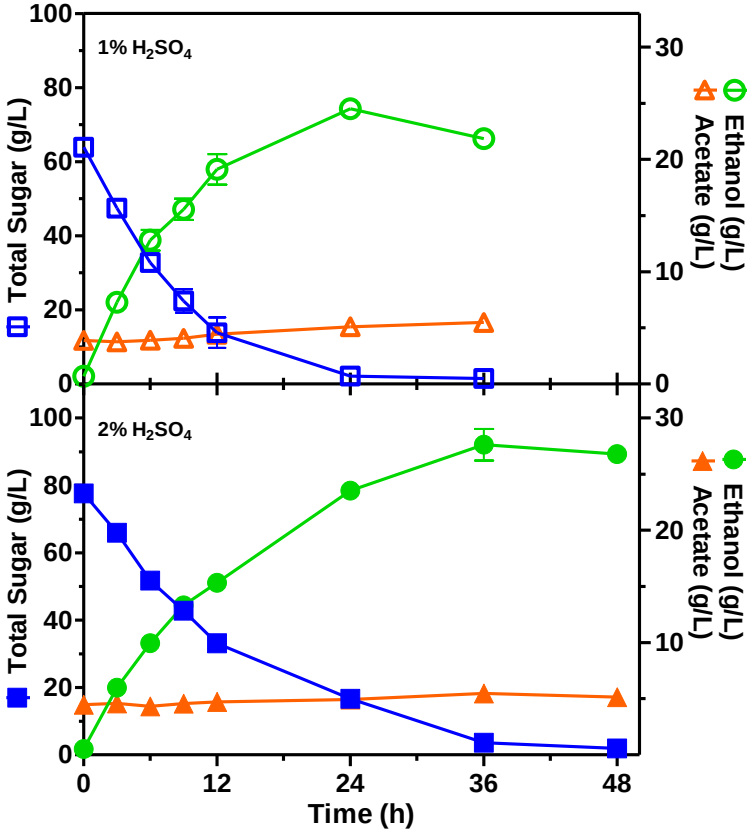
Utrilla et al. 2009
Fernández et al. 2012
Utrilla et al. 2012
Martínez et al. 2011
Martínez et al. 2013
WO 2011 PCT 016706 A2

Stover from White Corn: Sequential: Thermochemical Hydrolysis, Enzymatic Saccharification and Fermentation

Diluted Acid Pretreated Slurries
Saccharification Peg Mixer Reactor
15 UPF/gBA, 30 UCB/gBA
50 °C pH = 5.0, 60 rpm

Non-aerated Cultures with Ethanologenic *E. coli* MS04, 3.7 g/L, 0.2 L, 37°C, pH 7, 100 rpm.
No salts were added. No detox.





Most of the Xyl & Glc are released for pretreated S-WT above 1% SA in 12 h

All sugars are fermented to ethanol by ethanologenic *E. coli* MS04 in 36 h

Corn and Agaves

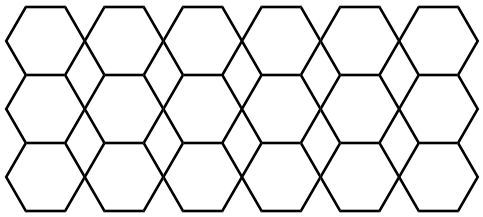
% DM (w/w)	Corn Stover	Agave Bagasse
Glucan	29.9 (1.2)	38.6 (2.5)
Xylan	19.6 (0.8)	13.6 (1.1)
Arabinan	3.4 (0.2)	3.4 (0.1)
Acetyl	4.9 (0.4)	4.2 (0.9)
Lignin	21.7 (0.6)	16.0 (1.3)
Extractives	6.8 (0.2)	12.9 (0.7)
Ashes	10.9 (0.7)	5.5 (0.4)
Other	2.8 (2.05)	4.1 (0.5)

275 L_{EtOH}/Ton Dry Corn Stover
Bajio 2012: 3,000,000 Tons CS →
825 x 10^6 Liters

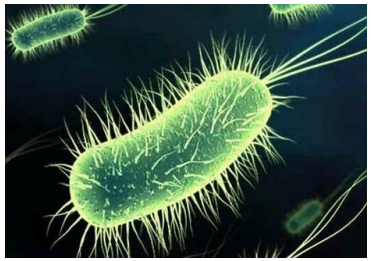
330 L_{EtOH}/Ton Dry Agave Bagasse
Tequila 2008: 220,000 Tons AB →
72 x 10^6 Liters

Consolidated Bioprocessing

Hydrolases: cellulases and β-glucosidases



Glucose



EtOH

Pentoses: xylose and arabinose

Hexoses: glucose, manose and galactose

van Zyl et al. (2007) Adv Biochem Eng Biotechnol.
Lynd et al. (2005) Curr Opin Biotechnol.

***E. coli* MS04:**

Homo-Ethanologenic strain

Hexoses Pentoses

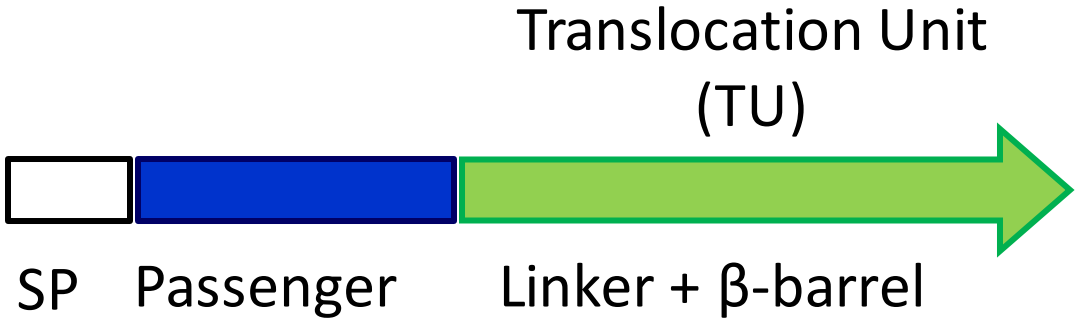
Acetate tolerant

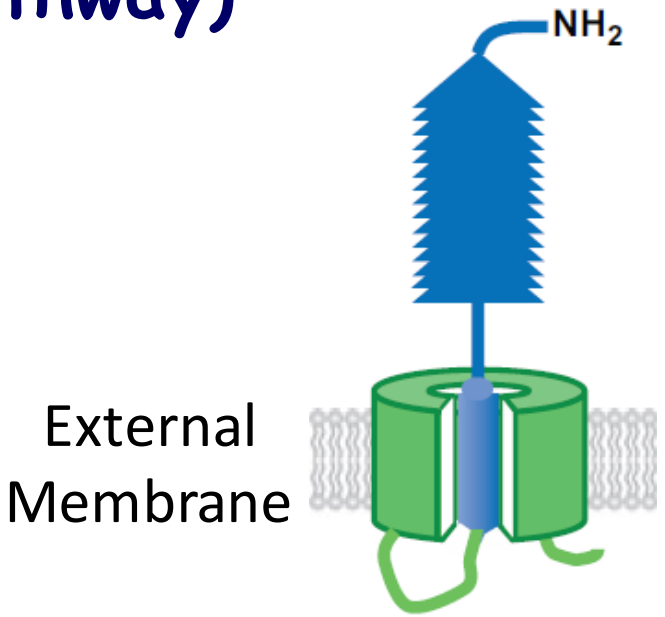
Purpose:

MS04 derivative strain with the ability to secrete a β-glucosidases to produce ethanol from cellobiose →Cellulose

At relatively high Temperature

Type Va Secretion System (Autotransporter pathway)





Dautin and Bernstein (2007) Annu Rev Microbiol

Autotransporter	Source
AIDA-I of diarrheagenic *E. coli* has been used for the secretion of many heterologous proteins.	Maurer et al. (1997) J Bacteriol. Jose and Meyer (2007) Microbiol Mol Biol Rev.

Beta-glucosidase to be secreted in *E. coli*

Parameter	*T. fusca* BglC[b]	*A. Niger* BG_S[c]
Temperature (°C)	50	65
pH	7	3.5-4
MW (kDa)	53	200
Vmax (mmol min^{-1} mg^{-1})[a]	28	68
Km (mM)[a]	0.35	2.7

[a] Parameters obtained at 25°C.
[b] Spiridonov and Wilson (2001) Curr Microbiol.
[c] Seidle et al. (2004) Protein J.

MS04/pAIDABglCRHis

Cell surface display of the ß-glucosidase BglC from *Thermobifida fusca* on the surface of the ethanologenic *Escherichia coli* strain MS04
For ethanol production from cellobiose

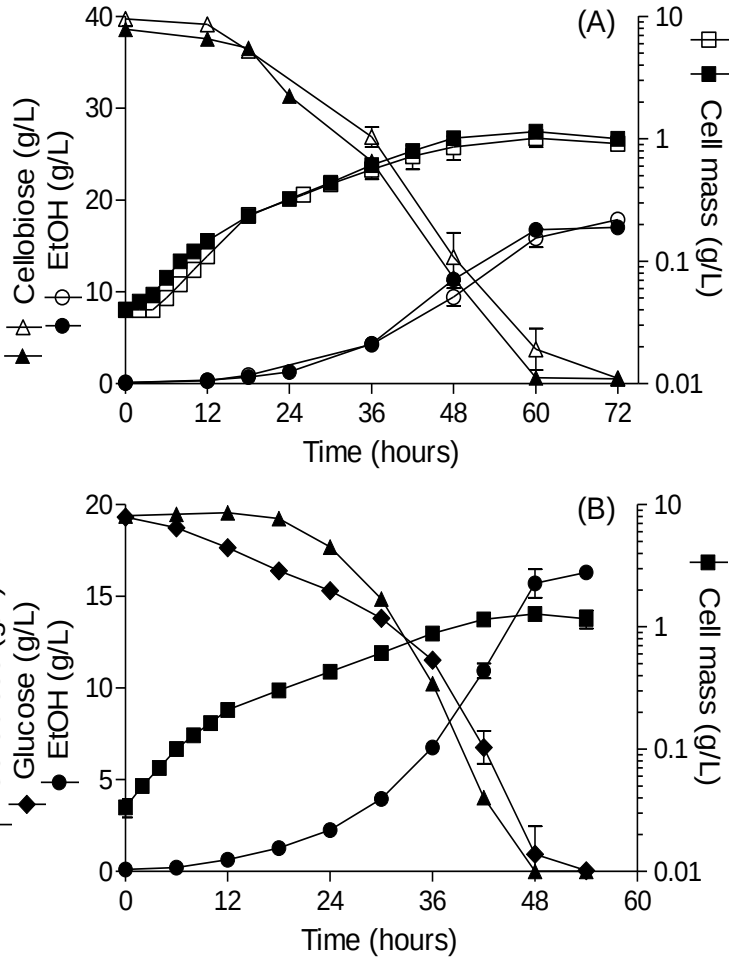
Cellobiose

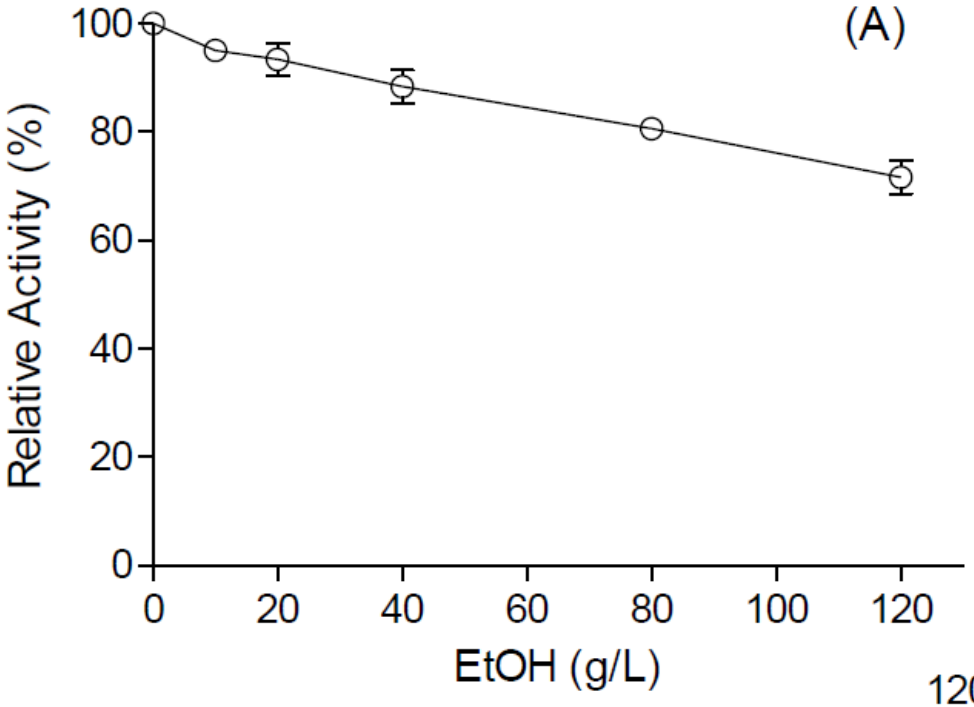
β-glucosidasa *Thermobifida fusca:* BglC

T. (ºC)	pH	MW (kDa)
50	7	53

Cellobiose – Glucose

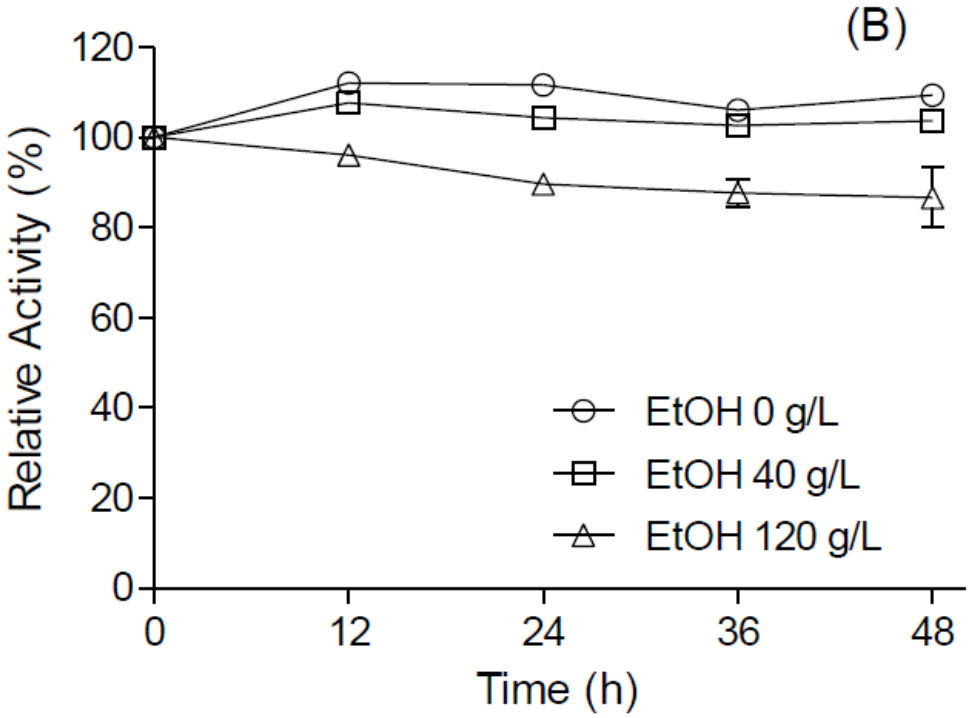
Muñoz-Gutiérrez et al., 2012





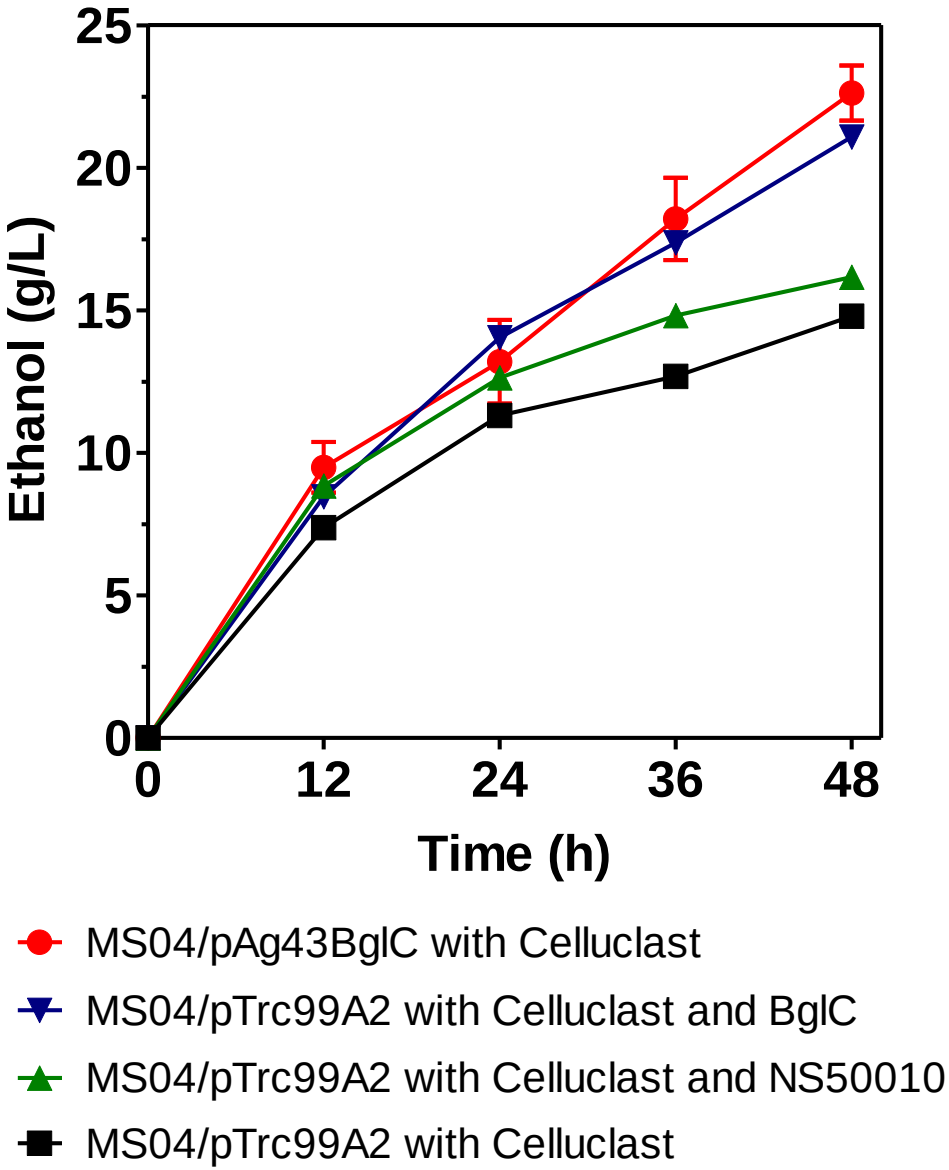
Effect of EtOH on the activity of BglC attached to MS04

Effect of EtOH on the stability of BglC attached to MS04



SSF AVICEL 45°C pH 6

- Ethanol production during the SSF process of 40 g/L Avicel by MS04 carrying plasmid pAg43BglC or pTrc99A2.
- With Exo-endo Cellulase addition



Remarks. From Cellulose. Other applications.

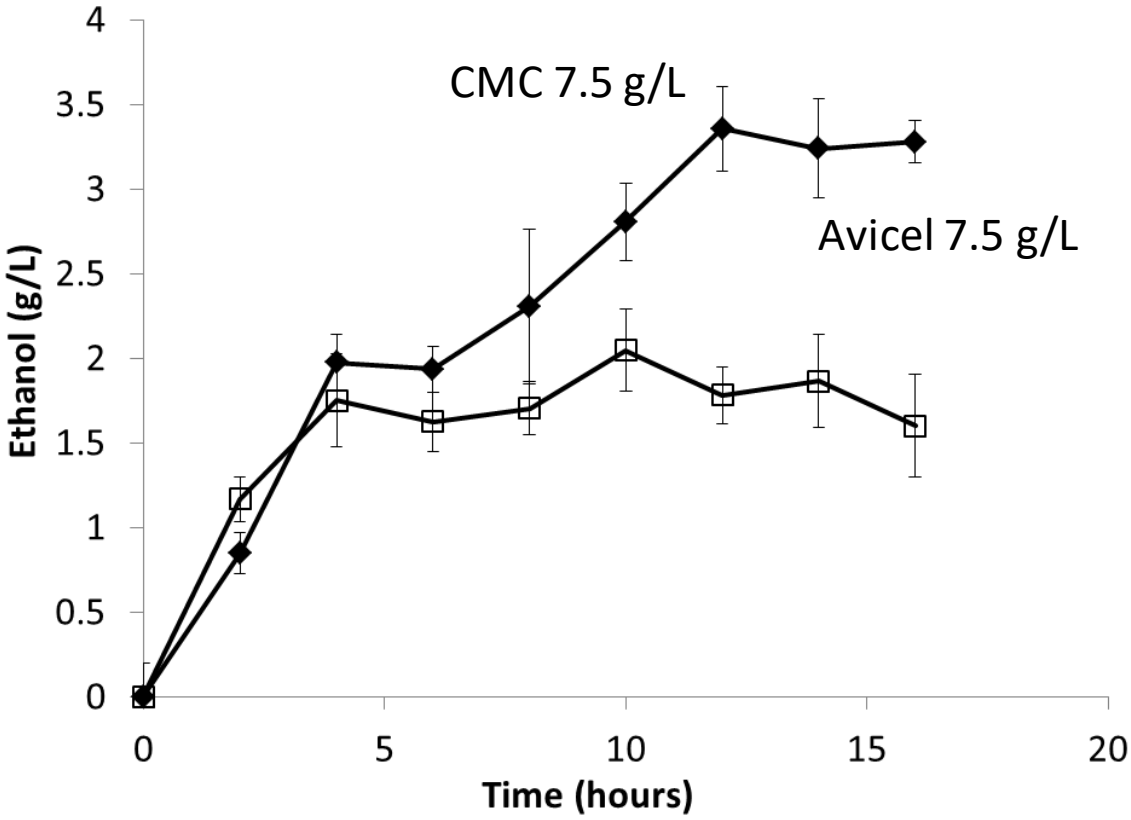
MSO4 – EndoG

Ethanol production

MS04-EndoG / MS04 – BglC

No cellulase addition. 45°C, pH 6

Loaces, I, et al., 2016



Gracias

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