Synthetic Biology and Bioenergy: Developments in India

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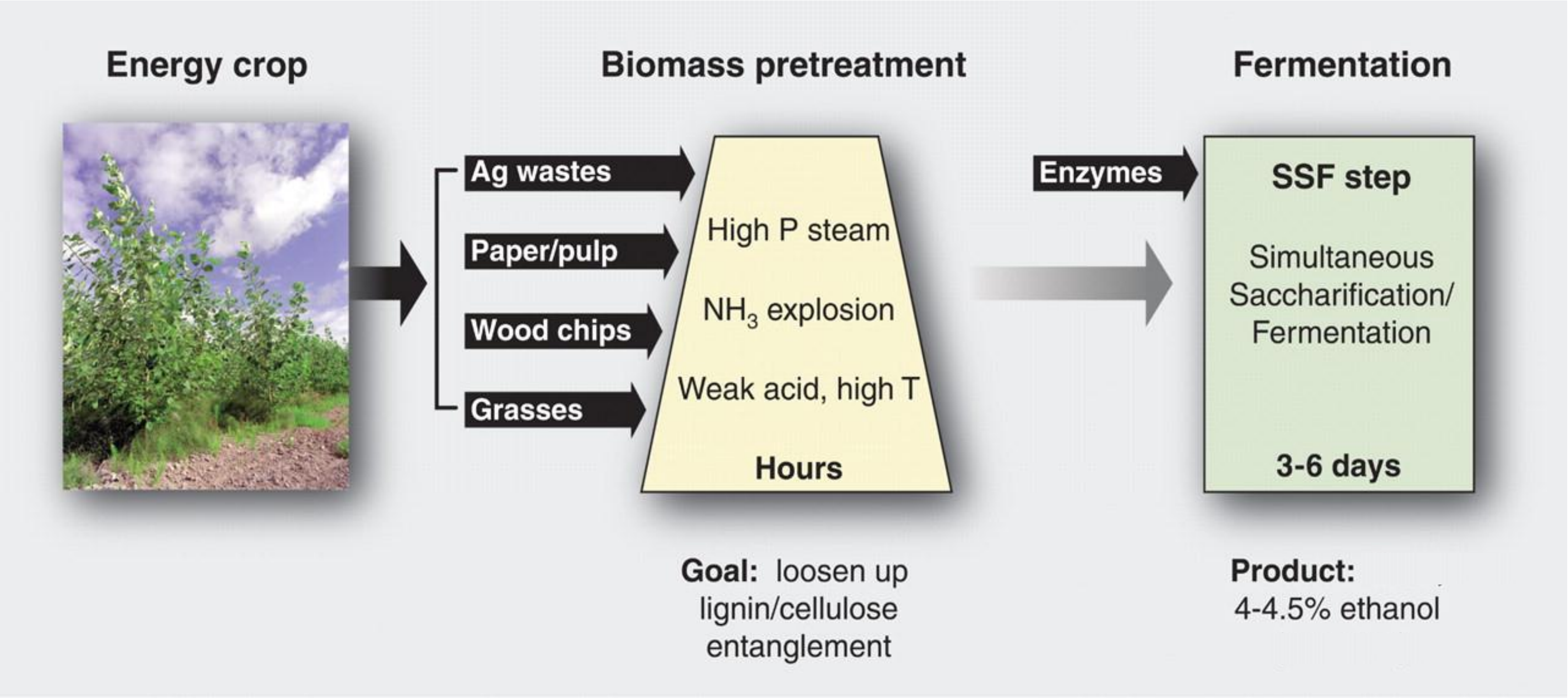
International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi

Biofuel from lignocellulosic biomass



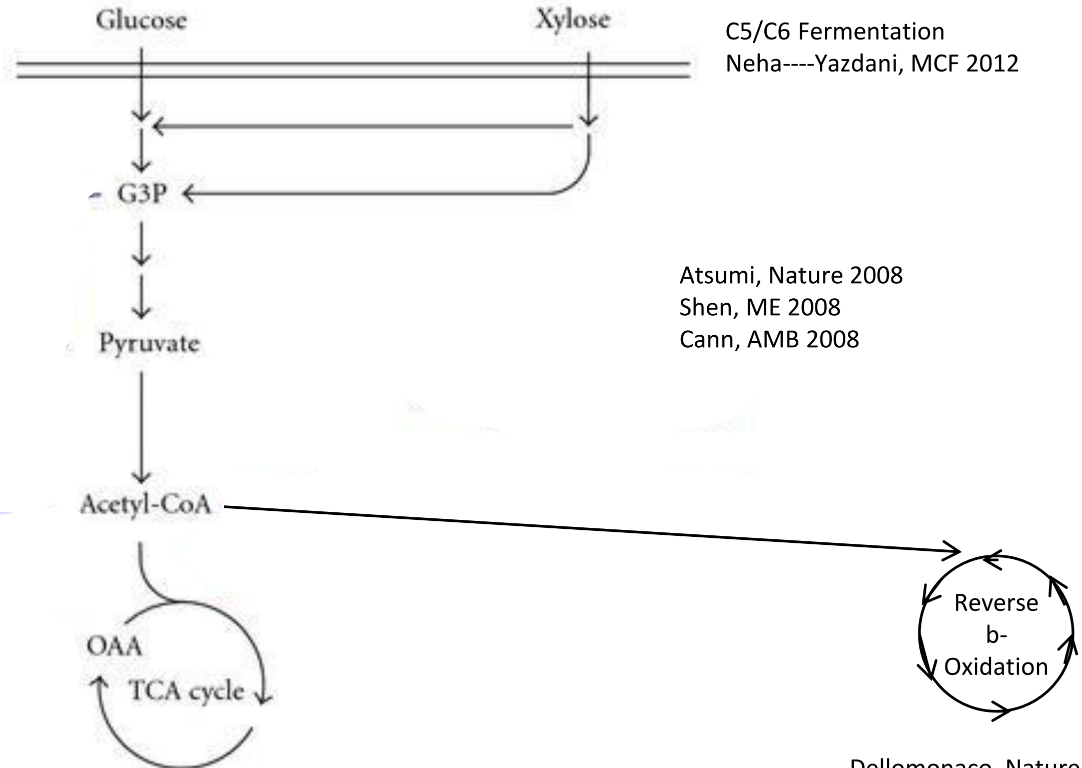
Stephanopoulos (2007) Science, 315:801-804.

Pathway Reconstruction

Steen-----Keasling, Nature 2009
Adlakha----Yazdani, AEM 2011
Adlakha----Yazdani, AEM 2012

C5/C6 Fermentation
Neha----Yazdani, MCF 2012

Withers, AEM 2007



Atsumi, Nature 2008
Shen, ME 2008
Cann, AMB 2008

Inui, AMB 2008
Atsumi, ME 2008
Shen, AEM 2011 (Ter)

Dellomonaco, Nature 2011

Schirmer, Science 2010

Steen-----Keasling, Nature 2009

Connor&Atsumi (2010) JBB,2010:9

Development in India

Madurai Kamaraj University

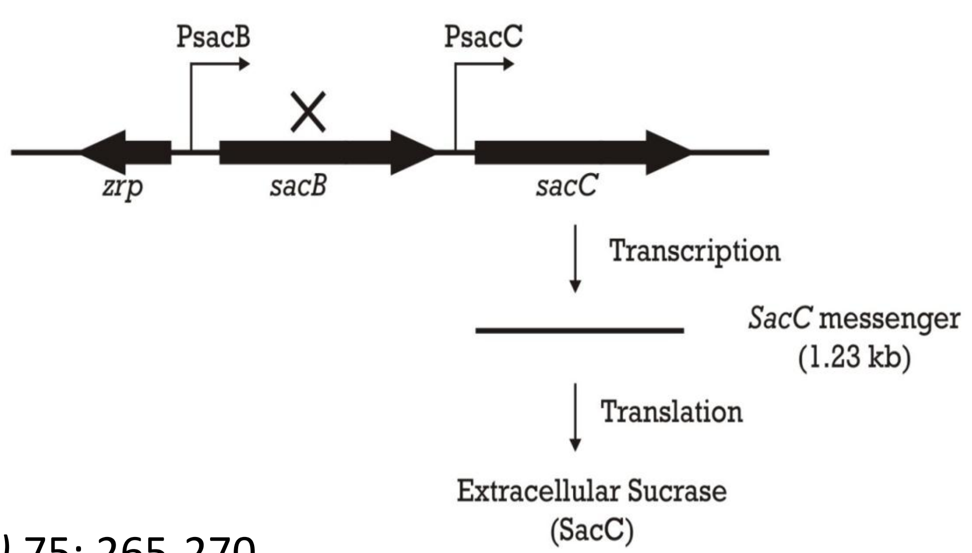
Dr. P. Gunasekaran

Levansucrase mutant of *Z. mobilis* for ethanol production

•Formation of by-products, such as levan (fructooligomer) decreases the ethanol yield.

•Therefore, *sacB* gene coding for levansucrase was deleted by homologous recombination in *Z. moblis*

Overproduction of ethanol using Δ*SacB* mutant of *Z. mobilis*



J Ferment Bioengin (1993) 75: 265-270

Madurai Kamaraj University

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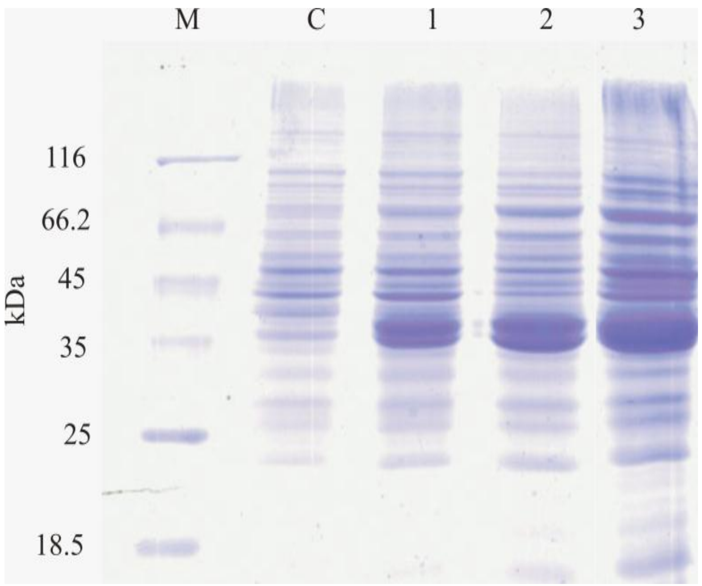
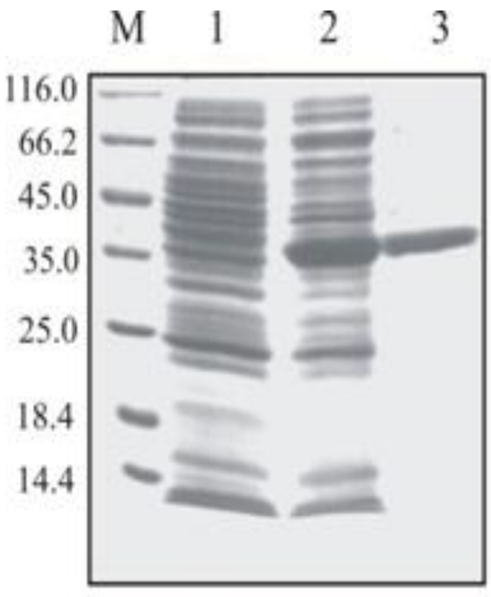


Overexpression in *E. coli*



Purification of endoglucanse

Presently working on the improvement of celA expression in Z. mobilis to improve the cellulose utilization and metagenomics for identification of new cellulolytic enzymes

Biotechnol Lett (2008) 30: 1461-1467.

Development in India

VS Bisaria, IIT Delhi

Alcoholic fermentation of xylose and mixed sugars using recombinant Saccharomyces cerevisiae engineered for xylose utilization.

Mrinal Kumar Maiti, IIT Kharagpur

Search for local isolates of oleaginous micro-organism as potential source of biodiesel production and Metabolic engineering of fatty acid biosynthesis to develop nutritionally improved Brassica seed oil

Sea6energy/Cellworks Group

To produce isobutanol from hydrolysis and fermentation of seaweeds.

DBT Initiative to set-up three Bioenergy Centre

- DBT-ICT Centre for Enenergy Biosciences, Mumbai
- DBT-IOC Centre for Advanced Bioenergy Research, Faridabad
- DBT-ICGEB Centre for Advanced Bioenergy Research, New Delhi

Development in India

– DBT-ICT Centre for Enenergy Biosciences, Mumbai

- Interested in producing butanol, isopentanol and fatty acid through synthetic biology

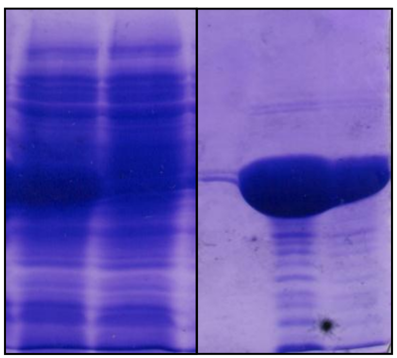
- Engineering microbes for
  - Consolidated bioprocessing
  - C5/C6 sugar fermentation
  - Hydrocarbon production

- Engineering algae for
  - Growth rate improvement
  - Lipid improvement

Novel cellulolytic enzymes for CBP

Endocellulase – 41 kDa

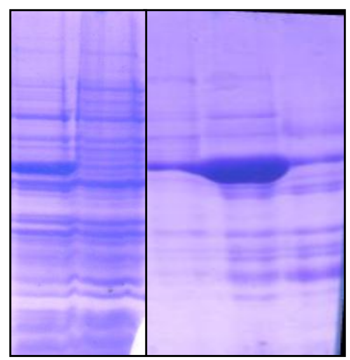
PL FT E1 E2 E3



pH optima ~ 6-7
Temp optima ~50 ^{o}C
Sp activity ~20 U/mg
GH Superfamily 5

β-glucosidase – 51 kDa

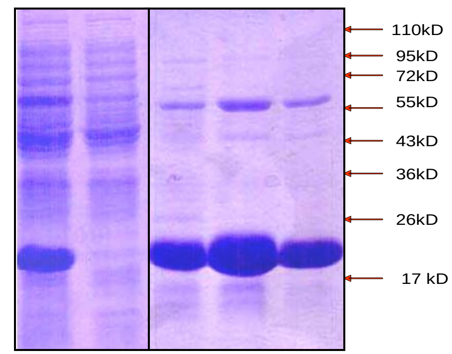
PL FT E1 E2 E3



pH optima ~ 6
Temp optima ~40 ^{o}C
Sp activity ~6U/mg
GH Superfamily 1

Xylanase – 21 kDa

PL FT E1 E2 E3

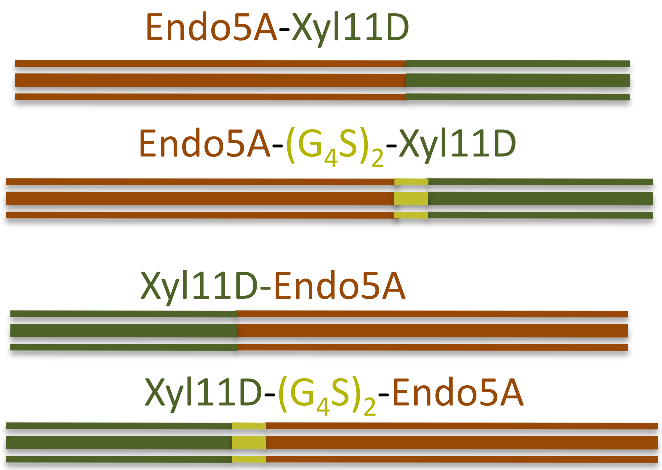


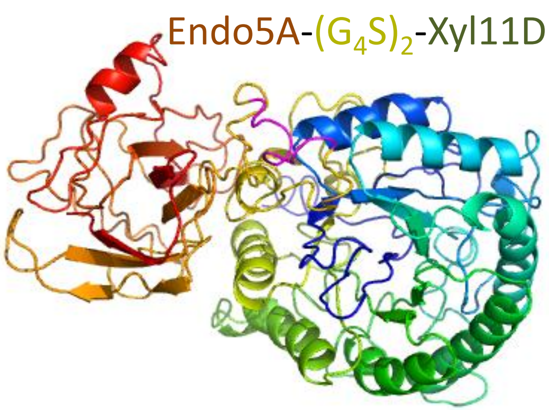
pH optima ~ 6-7
Temp optima ~50 ^{o}C
Sp activity ~17U/mg
GH Superfamily 11

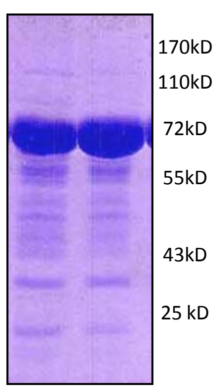
Source: *Paenibacillus* ICGEB2008 from gut of Cotton Bollworm

Design of multifunctional enzymes

Fusion protein (Endocellulase+Xylanase)

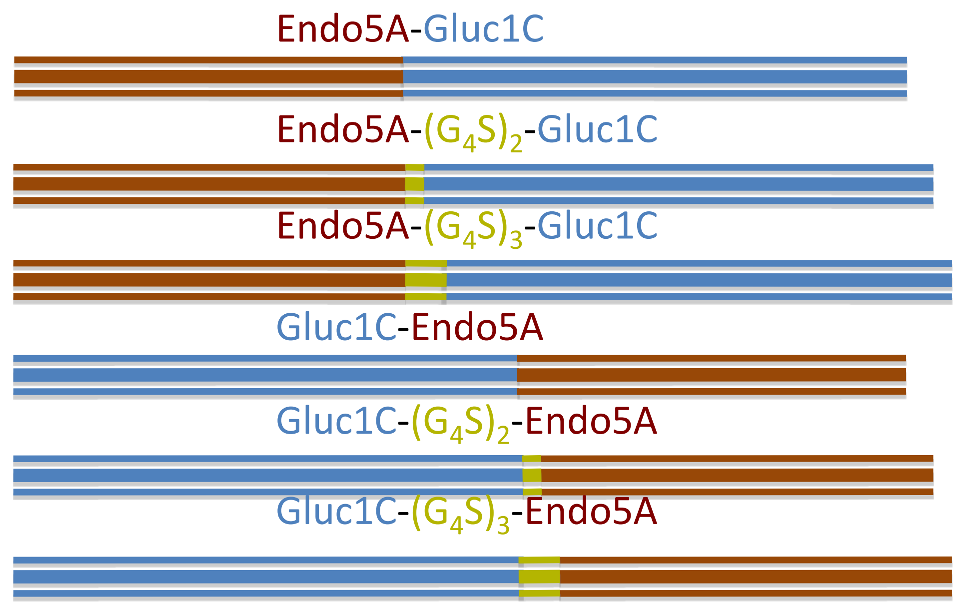


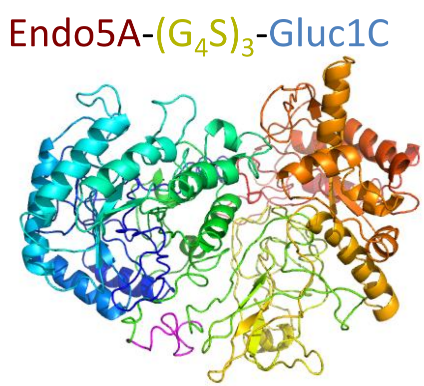


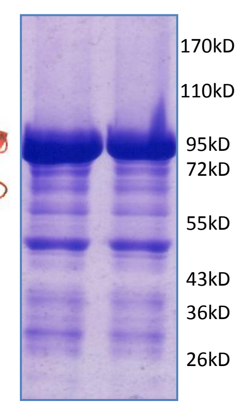


Adlakha ...Yazdani. Applied and Environmental Microbiology. July 2011

Fusion protein (Endocellulase+β-glucosidase)





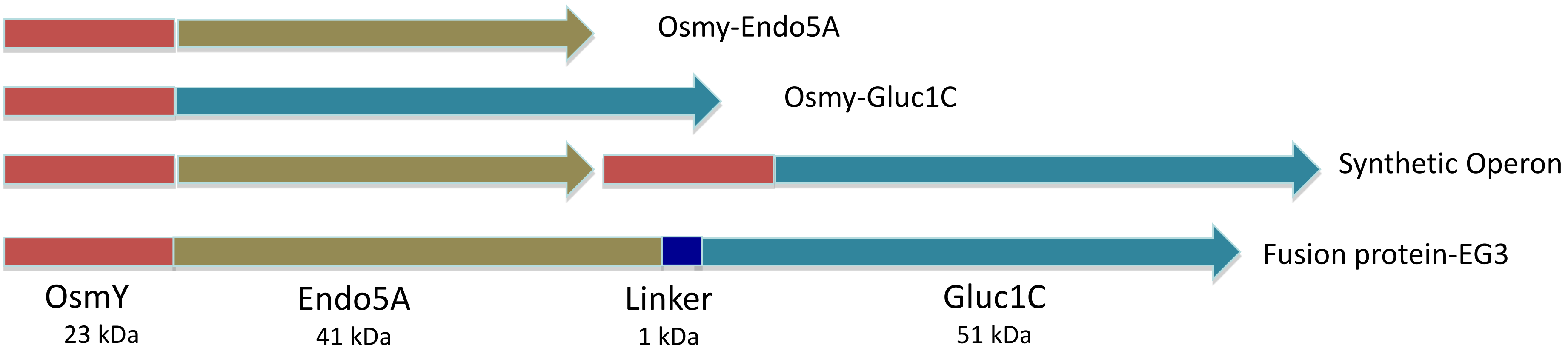


Adlakha ...Yazdani. Applied and Environmental Microbiology. Oct 2012

Kinetic parameters of recombinant enzymes

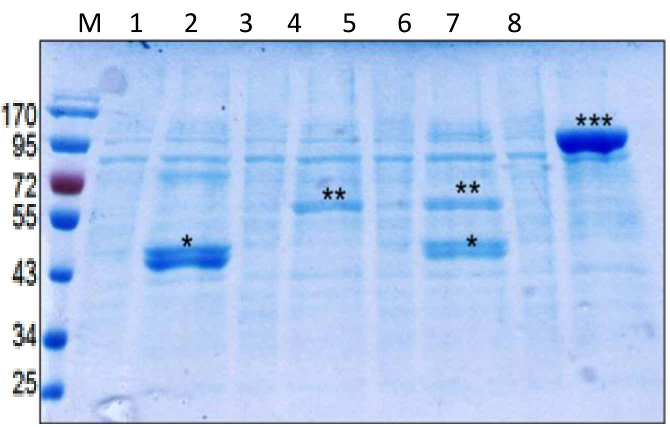
Activity and Recombinant protein	K_m (mM or mg ml^{-1})[a]	K_{cat}/K_m (mM^{-1}S^{-1} or ml.mg^{-1}S^{-1}x10^5)[b]
β-Glucosidase		
Gluc1C	4.2	3.23
EG1 (Endo5A-Gluc1C)	3.8	1.90
EG2 (Gluc1C-Endo5A)	8.1	0.93
EG3 (Endo5A-$(G_4S)_2$-Gluc1C)	6.7	0.70
EG4 (Gluc1C-$(G_4S)_2$-Endo5A)	8.4	0.62
EG5 (Endo5A-$(G_4S)_3$-Gluc1C)	2.7	6.40
EG6 (Gluc1C-$(G_4S)_3$-Endo5A)	4.3	0.89
Endoglucanase		
Endo5A	0.97	1.0
EG1 (Endo5A-Gluc1C)	1.14	1.9
EG2 (Gluc1C-Endo5A)	2.01	1.5
EG3 (Endo5A-$(G_4S)_2$-Gluc1C)	3.00	1.7
EG4 (Gluc1C-$(G_4S)_2$-Endo5A)	2.25	1.7
EG5 (Endo5A-$(G_4S)_3$-Gluc1C)	0.96	2.0
EG6 (Gluc1C-$(G_4S)_3$-Endo5A)	0.76	1.8

Secretion of endoglucanase and β-glucosidase in *E.coli*

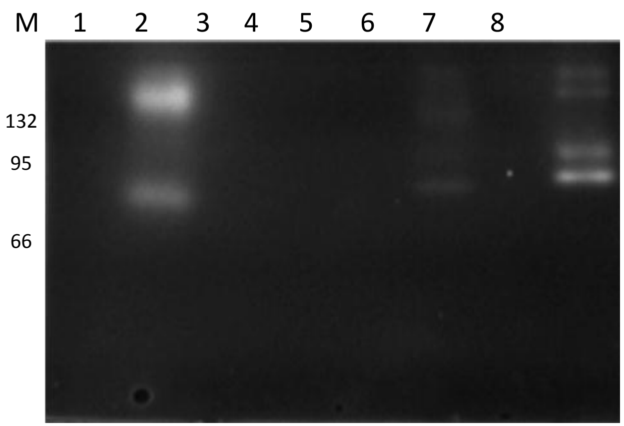


Culture Supernatant -

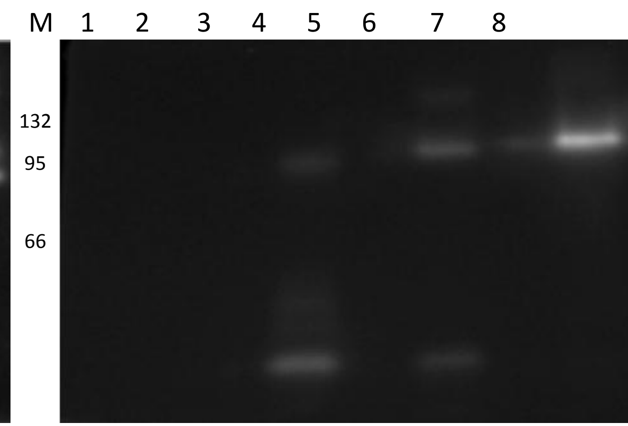
SDS-PAGE gel



Native gel – Endo activity



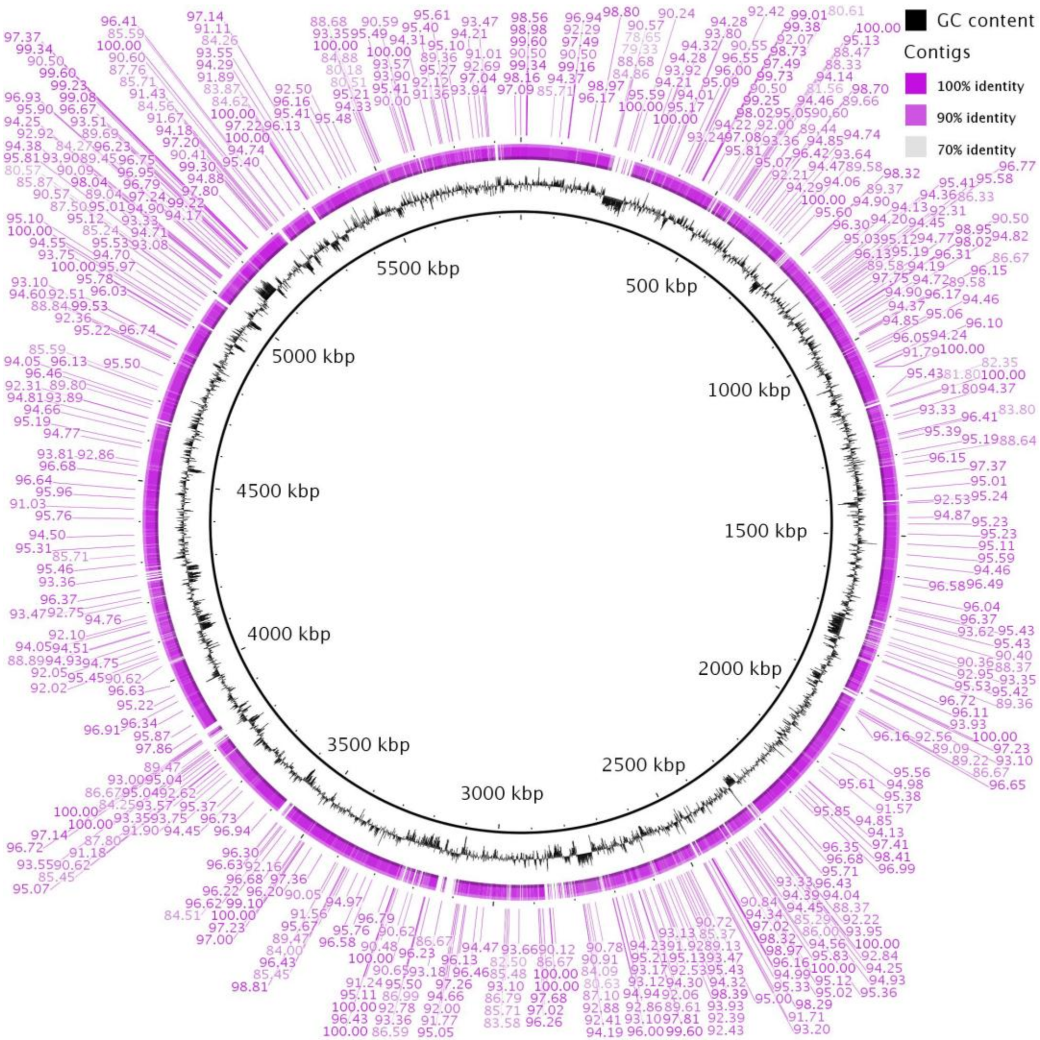
Native gel – Gluc activity

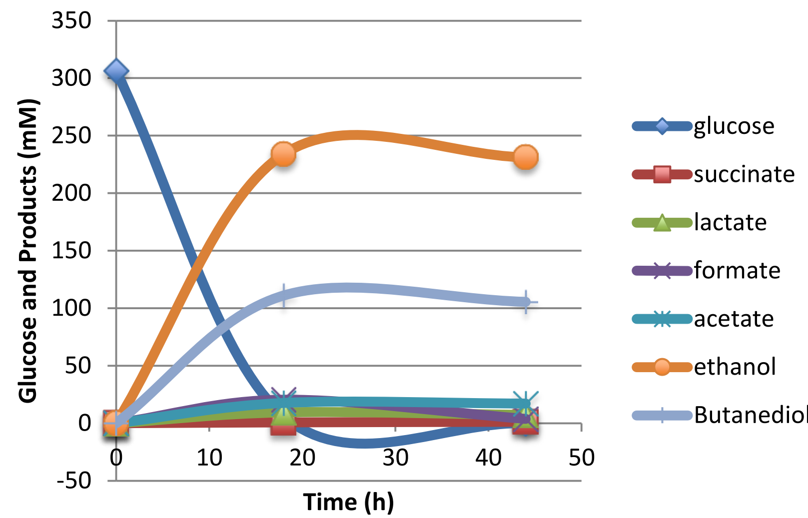


1-Unind Endo, 2-Induced Endo, 3-Unind Gluc, 4-Induced Gluc, 5-Undind SynOp, 6-Induced SynOp, 7-Unind Fusion, 8-Induced Fusion

Gupta …Yazdani. Protein Expression and Purification. Under review

Paenibacillus ICGEB2008 as a platform for fuels and chem



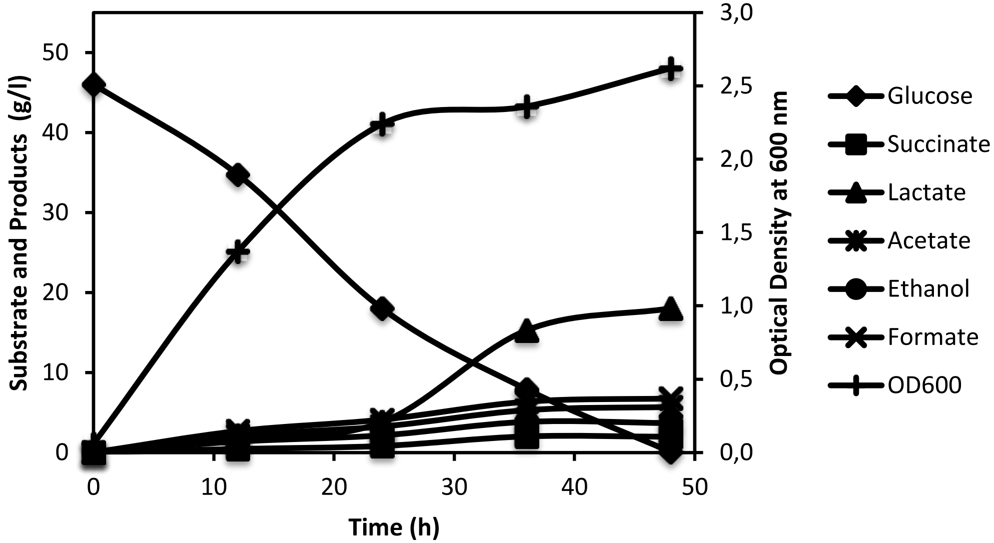


Adlakha …Yazdani. Genome Anouncement.ASM accepted

Engineering laboratory microbe for converting C5/C6 sugars to ethanol and other biofuels

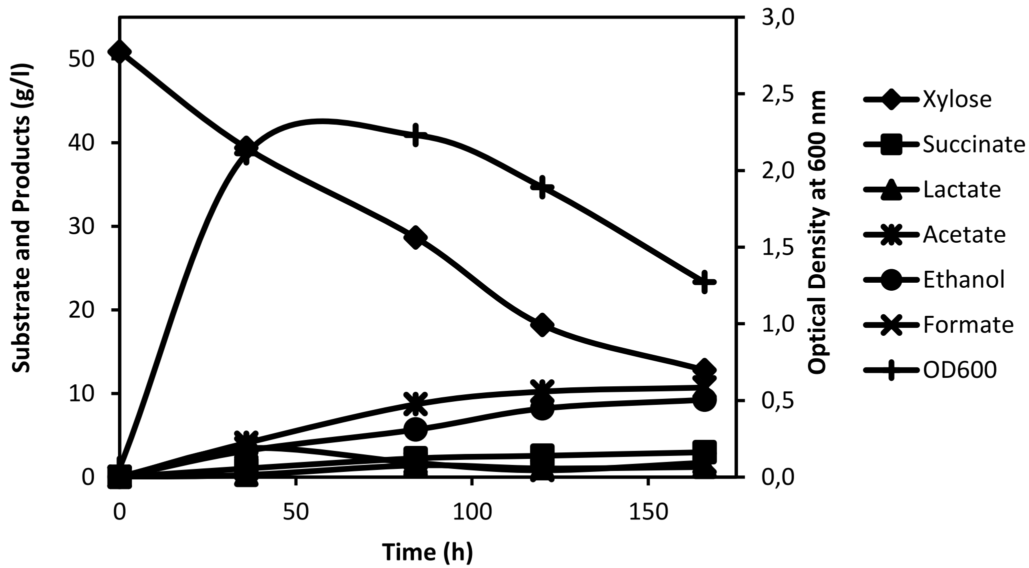
- Up to 30% pentose sugars in agricultural residues
- Traditional yeast*, Saccharomyces cerevisae,* cannot utilize pentose sugars
- *E. coli* could ferment all pentose and hexose sugars present in the lignocellulosic biomass
- It, however, produces various competing products under anaerobic condition

End products of *E. coli* under fermentative condition



Max Ethanol Productivity = 0.14 g/l/h

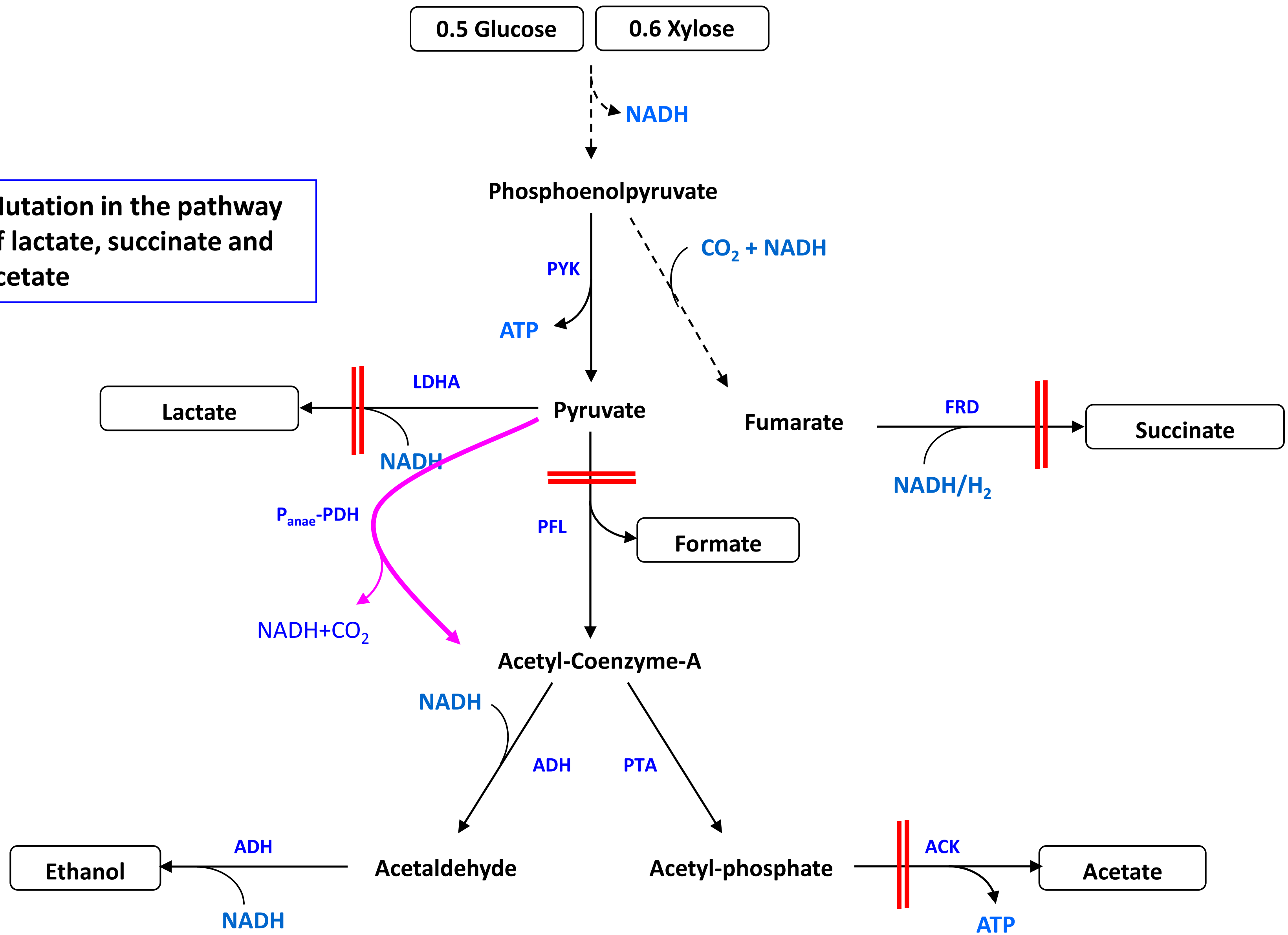
Ethanol Yield = 0.08 g/g glucose



Max Ethanol Productivity = 0.09 g/l/h

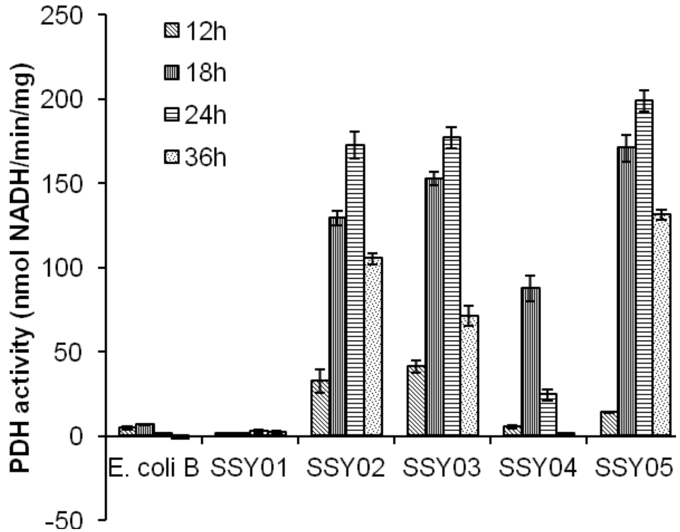
Ethanol Yield = 0.24 g/g glucose

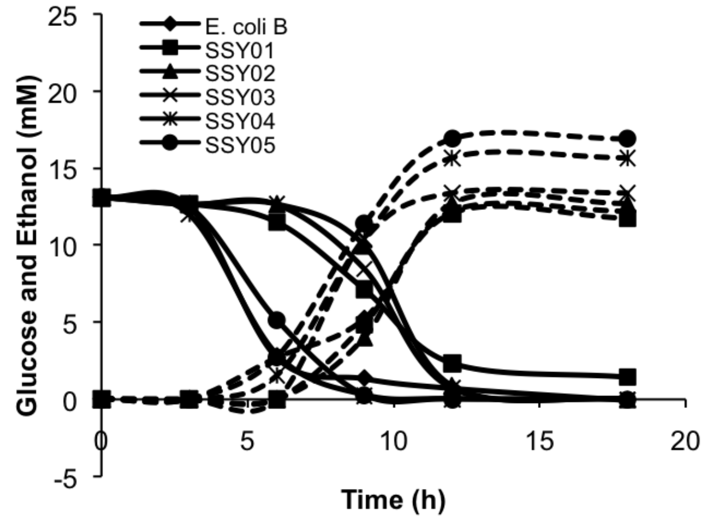
Availability of reducing equivalence for reduced products



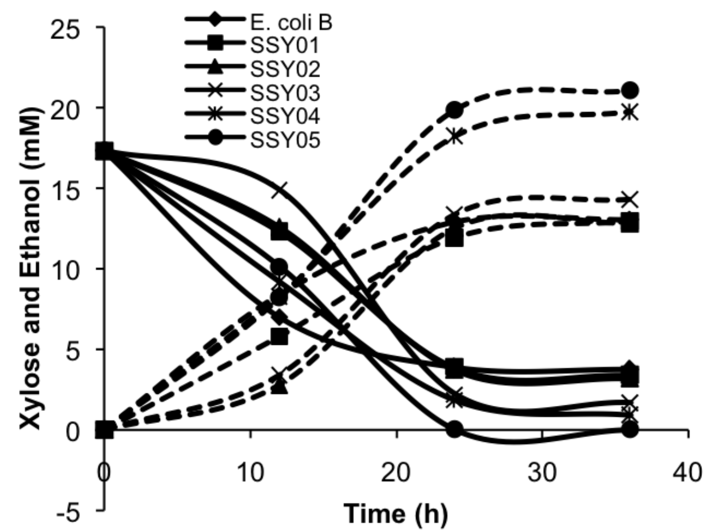
Mutation in the pathway of lactate, succinate and acetate

Promoter engineered strains demonstrate higher ethanol





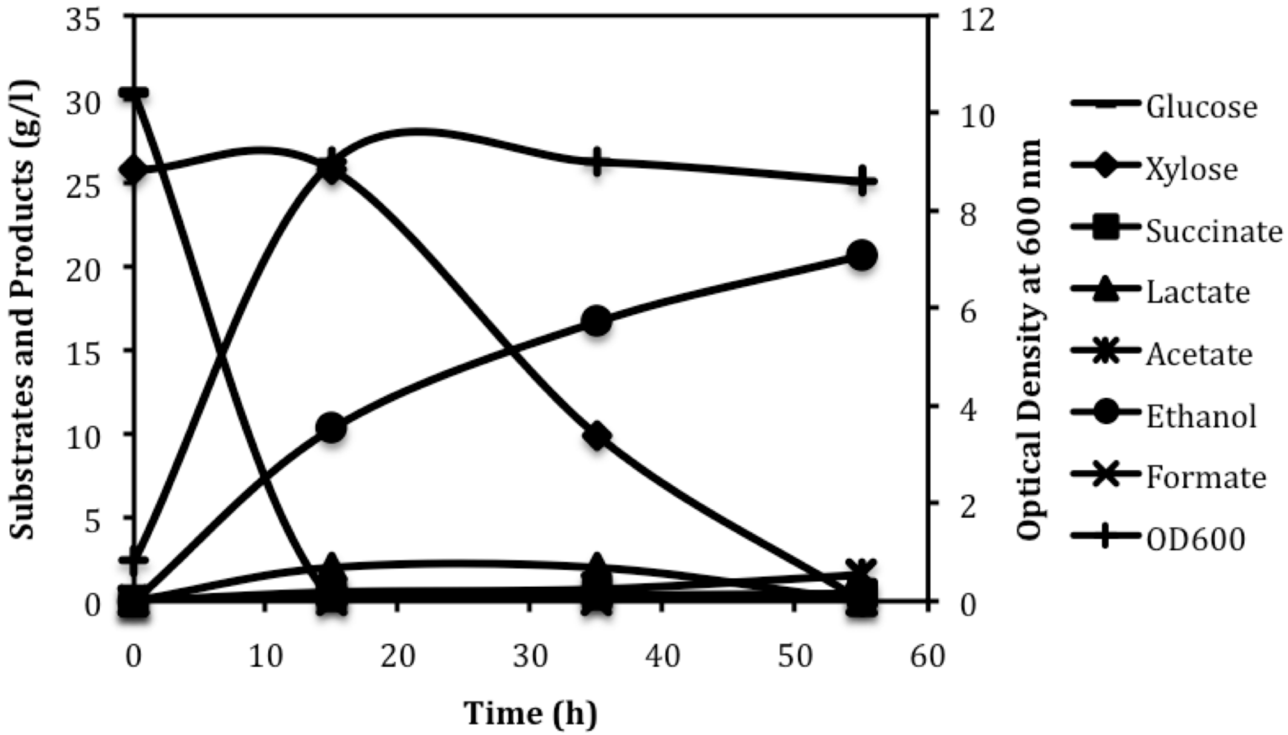
SSY01 – P_{ldhA}PDH,
SSY02 –P_{frdA}PDH,
SSY03 – P_{pflB}PDH,
SSY04 – P_{adhE}PDH,
SSY05 – P_{gapA}PDH.



Munjal …Yazdani. Microbial Cell Factories. 2012,11:145

Fermentation of Glucose and Xylose mixture by SSY10

SSY10 - P_{gapA}PDH Δ*ldhA* Δ*frdA* Δ*ack* Δ*pflB* (pZSack)



Ethanol Productivity = 0.7 g/l/h

Ethanol Yield = 0.43 g/g glucose

Munjal …Yazdani. Microbial Cell Factories. 2012,11:145

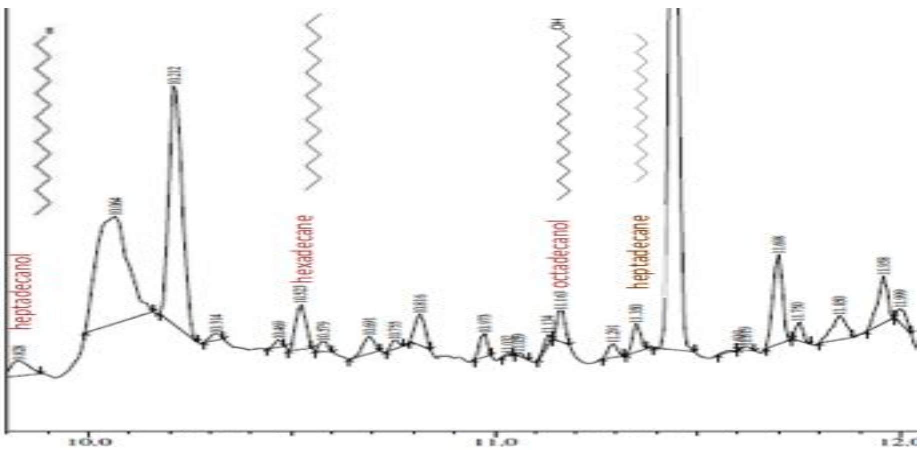
Engineering microbe for production of higher value advance biofuel molecules

- Ethanol is hygroscopic, corrosive, less energy dense, difficult to transport through pipeline, need to be blended up to 15%
- Butanol and alkane/alkane is closer to fossil fuel in terms of their properties

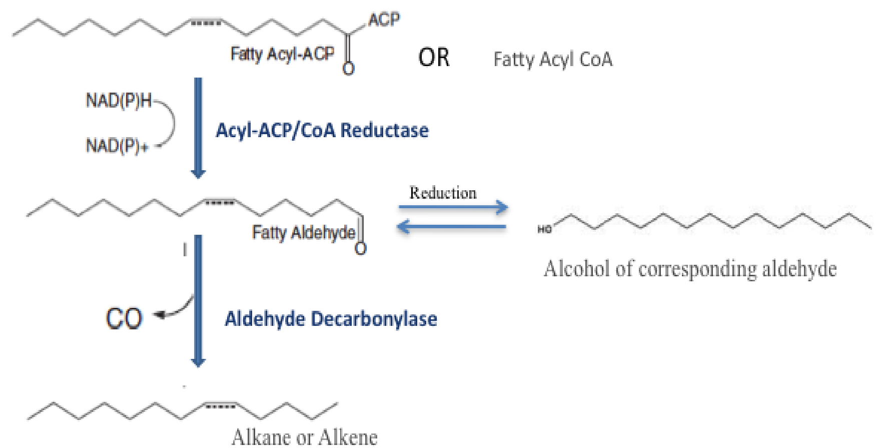
Engineering bacteria for production of hydrocarbon

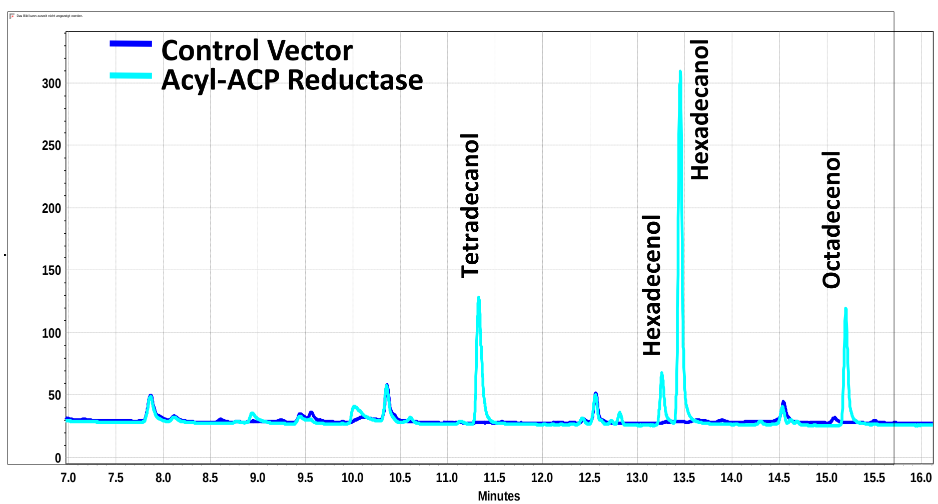
- Engineering *E. coli* for alkane production

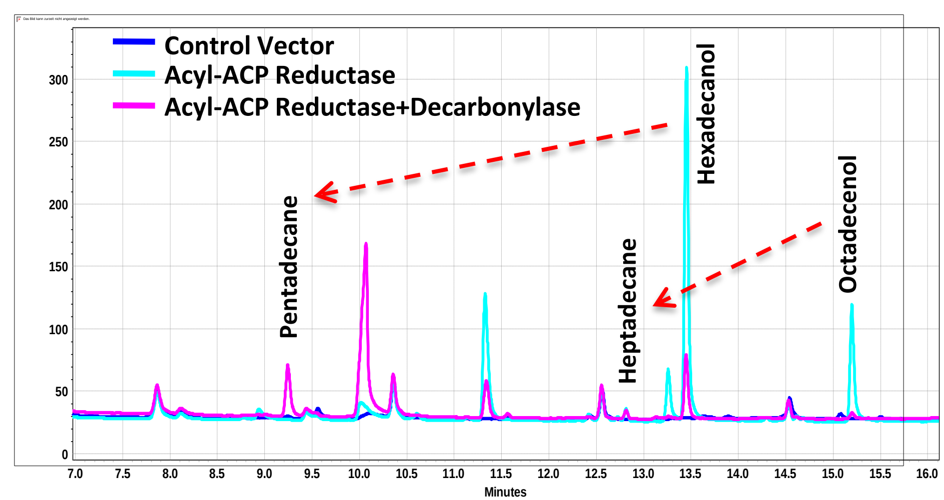
Cyanobacterial extract



Pathway







Knowledge gap and future direction

- High risk, high capital area, less institutes involved
- Integrated knowledge of hard core molecular biology, basic biology, bioengineering and systems biology needed, which is lacking in individual entity.
- There is a need for scientists having expertise in these fields to join hand, especially metabolic engineers and system biologists.
- There is a need for the funding agencies to recognize the potential of these areas and should fund high risk projects.

Acknowledgment

- ICGEB Team
  - Nidhi, Graduate Student
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  - Zia, Graduate Student
  - Alok, Research Associate
  - Zeenat, JRF
  - Vamsi, JRF

DBT-ICGEB Centre for Advanced Bioenergy Research

- Collaborators
  - Insect Resistance Group, ICGEB
  - DBT-ICT Centre for Energy Bioscience, Mumbai
  - DBT-IOC Centre for Advanced Bioenergy Research, Faridabad

- Funding
  - DBT, Govt of India
  - DST, Govt of India

Thank You