Synthesis of three advanced biofuels from ionic liquid-pretreated switchgrass using *Escherichia coli*

Bokinsky et al, nov 2011 PNAS

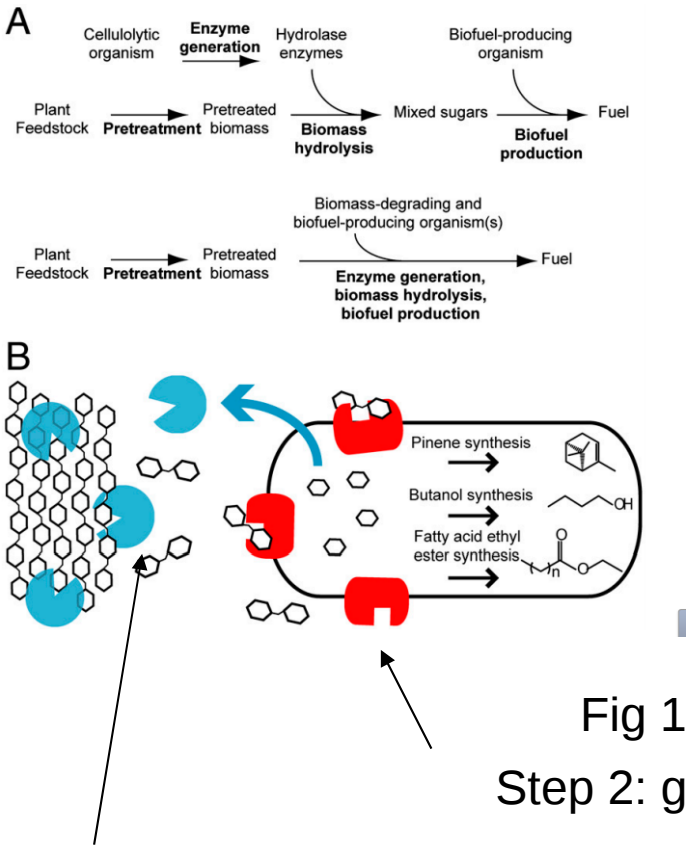
Andrew Tolonen
MSSB presentation, Genoscope
7 dec 11

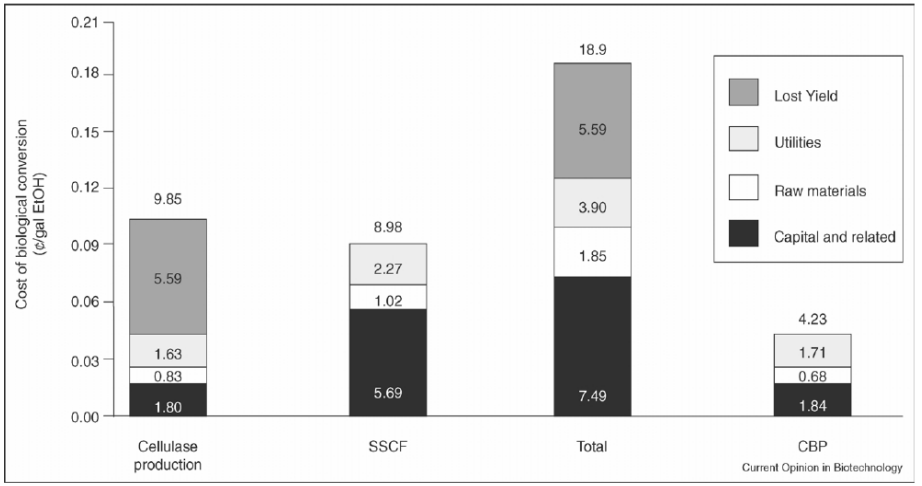
Why did I choose this paper?

- It's new (published 4 weeks ago)
- It relates to yesterday's talk (and my research)
- Shows many basic problems in facing synthetic biologists
- JBEI is doing cutting edge research in synthetic biology

jbei
Joint BioEnergy Institute

Consolidated bioprocessing for efficient conversion of biomass to fuel





Lynd et al, 2005

Fig 1

Step 2: glucosidase/xylobiosidase to cut short chains into sugars

Step 1: cellulase/hemicellulase to cut polymers into short chains

What plant feedstocks (inputs) did they use?

switchgrass: prairie grass

yard waste

eucalyptus

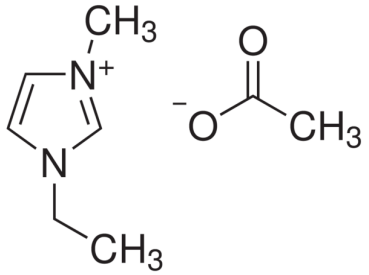
What feedstocks (inputs) did they use?

switchgrass: prairie grass

yard waste

Note: the biomass was pre-treated with an 'ionic liquid'

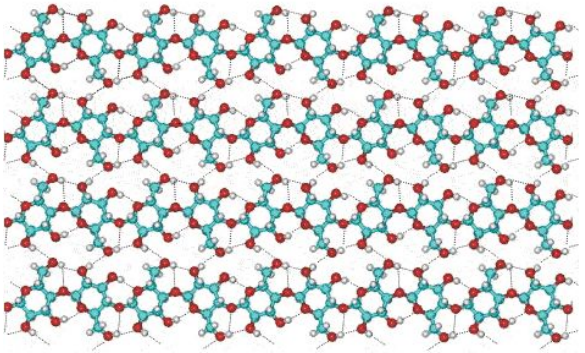
1-Ethyl-3-methylimidazolium acetate



Cost is a 'substantial barrier to commercialization' (it's expensive!)

What are environmental impacts of these liquids?

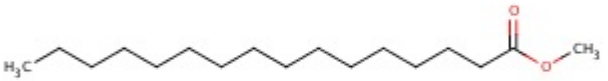
On the positive side, IL 'eliminates cellulose crystallinity'



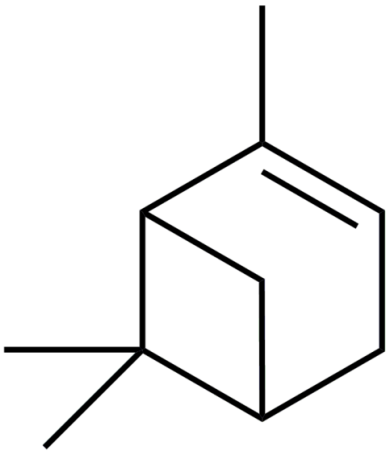
What chemicals did they produce?

Butanol (gasoline replacement)

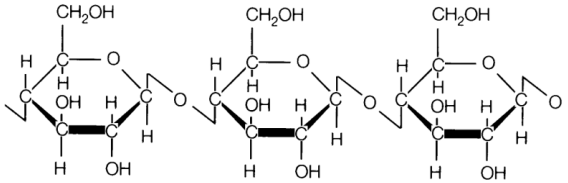
Fatty acid (biodiesel)



Pinene (jet fuel precursor)

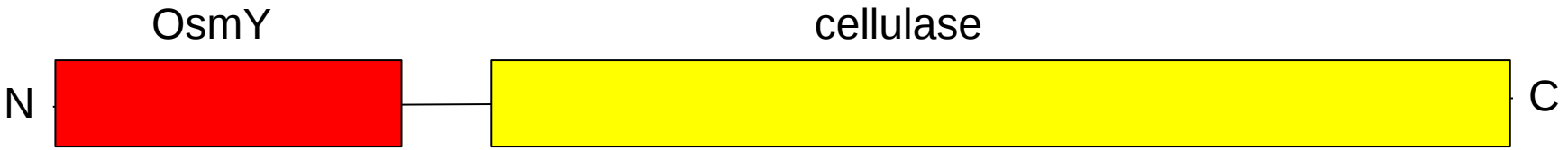


How did they make *E. coli* degrade cellulose?



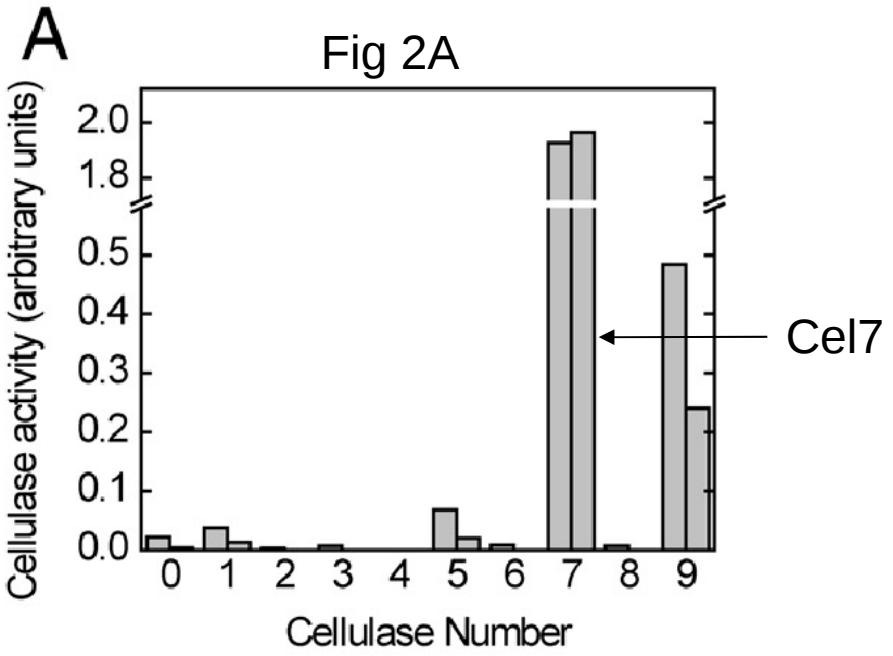
Cellulose is a polymer of glucose

Step 1: fuse cellulase genes to OsmY domain so they are secreted



How did they make *E. coli* degrade cellulose?

Step 2: find a cellulase that works in *E. coli*



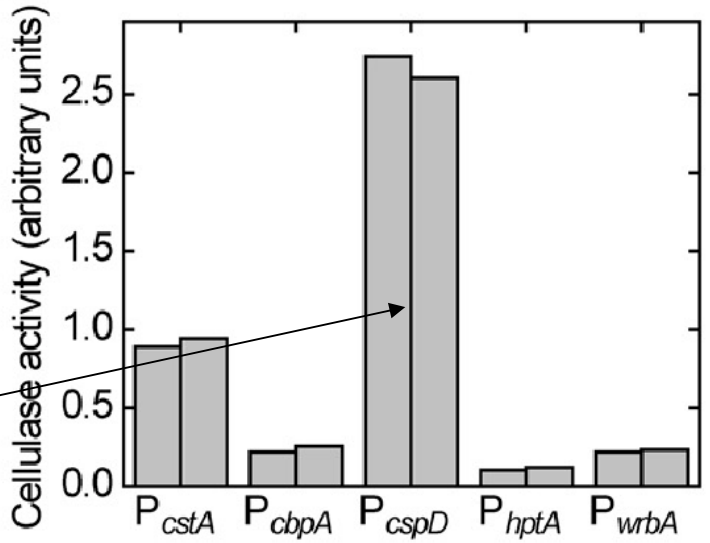
Expressed 10 cellulases and tested supernatant for cellulase activity

Question:
How would you select genes to test?
http://www.cazy.org/GH5_all.html

How did they make *E. coli* degrade cellulose?

Step 3: find a which promoter gives highest expression of *cel7*

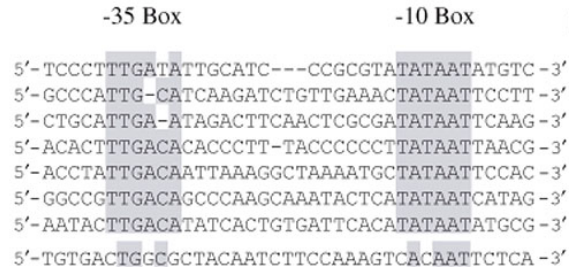
Fig 2F



Chose PcspD

CstA: peptide transporter
CbpA: chaperone
CspD: DNA replication inhibitor
CspD: DNA replication inhibitor
WrbA: flavodoxin

Questions: What is a promoter?
How would you choose promoters to test?

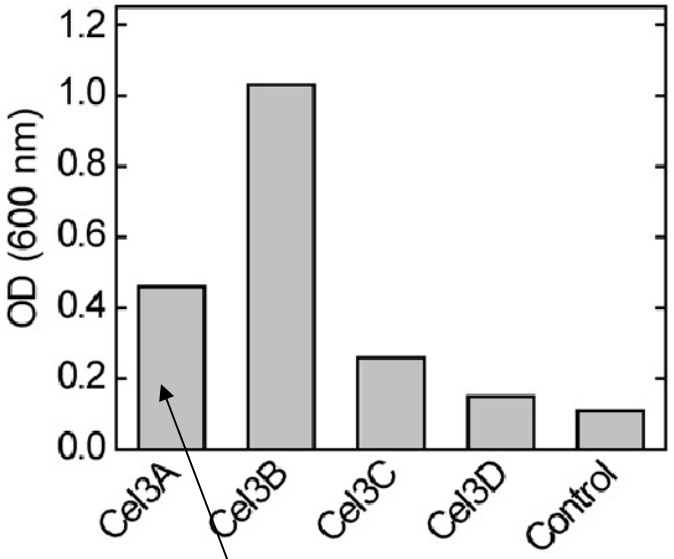


How did they make *E. coli* degrade cellulose?

Step 4: find a cellobiosidase that works in *E. coli*

B

Fig 2B



Screened 4 cellobiosidases from *Cellvibrio japonicus*. Only 4 genes?

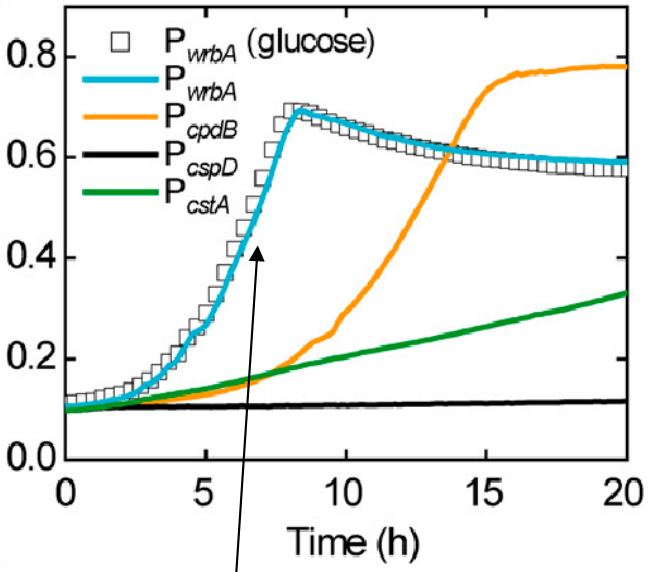
Chose Cel3A. Why?

"*E. coli* grew best on cellobiose when expressing Either *cel3A* or *cel3B*"

Step 5: find which promoter gives highest expression of cel3A

D

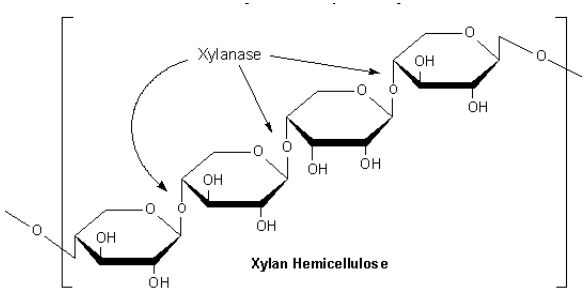
Fig 2D



chose PwrbA

How did they make *E.coli* digest hemicellulose?

hemicellulose is a xylose polymer



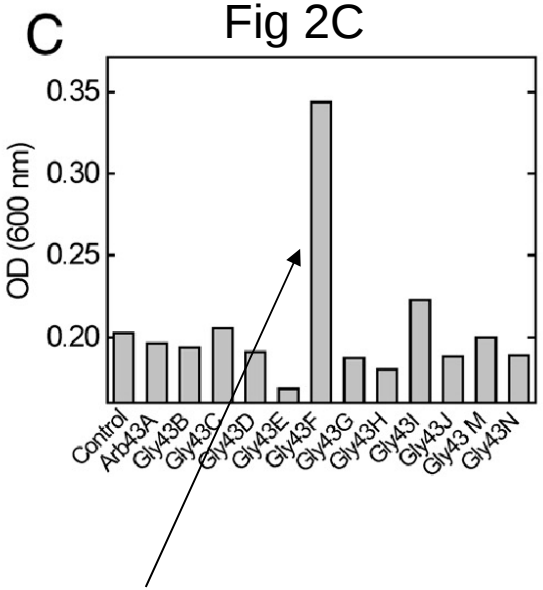
Step 1: find a hemicellulase that works in *E. coli*

This was done in another study, (Xyn10B) but I think they used the same strategy as for the cellulase

How did they make *E.coli* digest hemicellulose?

Step 2: test genes to cut short xylosaccharides by screening 12 xylobiosidases from *C. japonicus*

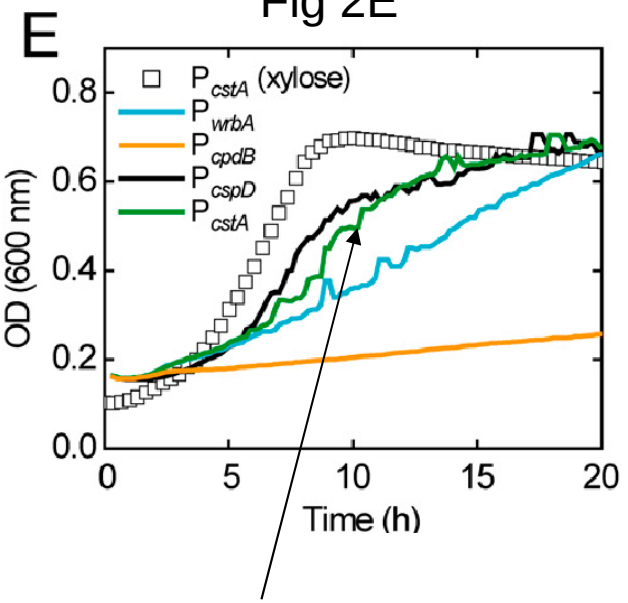
Fig 2C



How lucky that 1 worked!
But how significant is that?

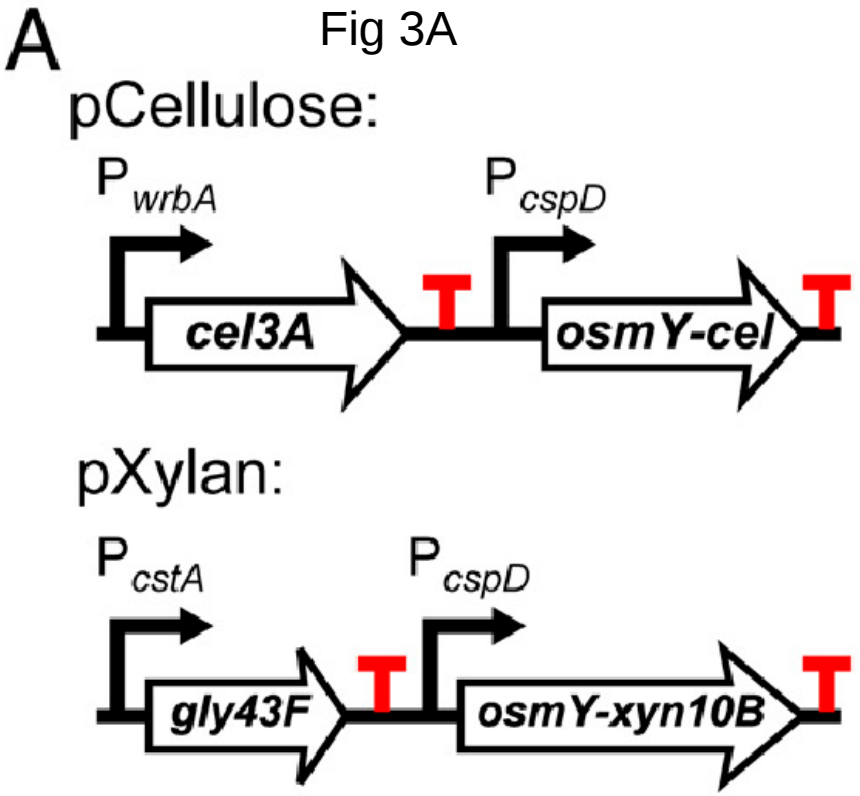
Step 3: find which promoter works best with Gly43F

Fig 2E



Chose PcstA

clone genes onto plasmid and put them in *E. coli*



Question: why do you think different promoters worked best for different genes?

strains can grow on model substrates

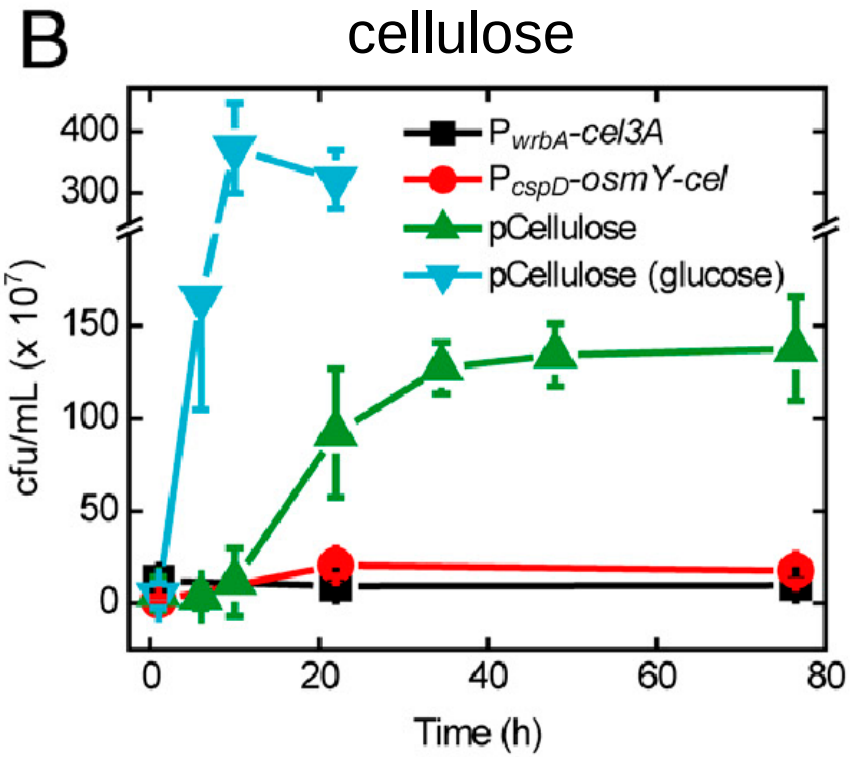


Fig 3B

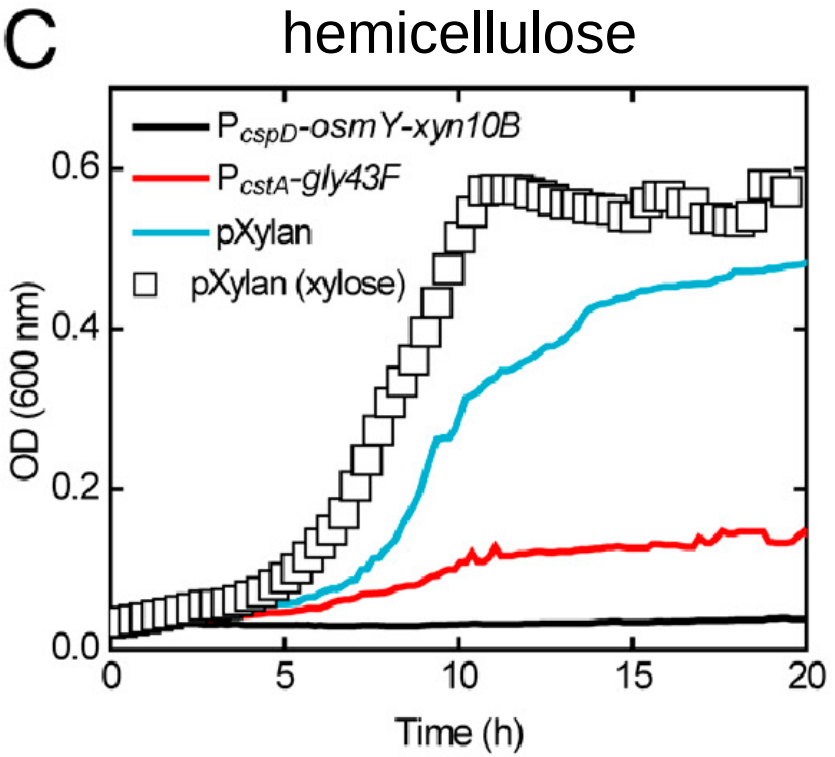
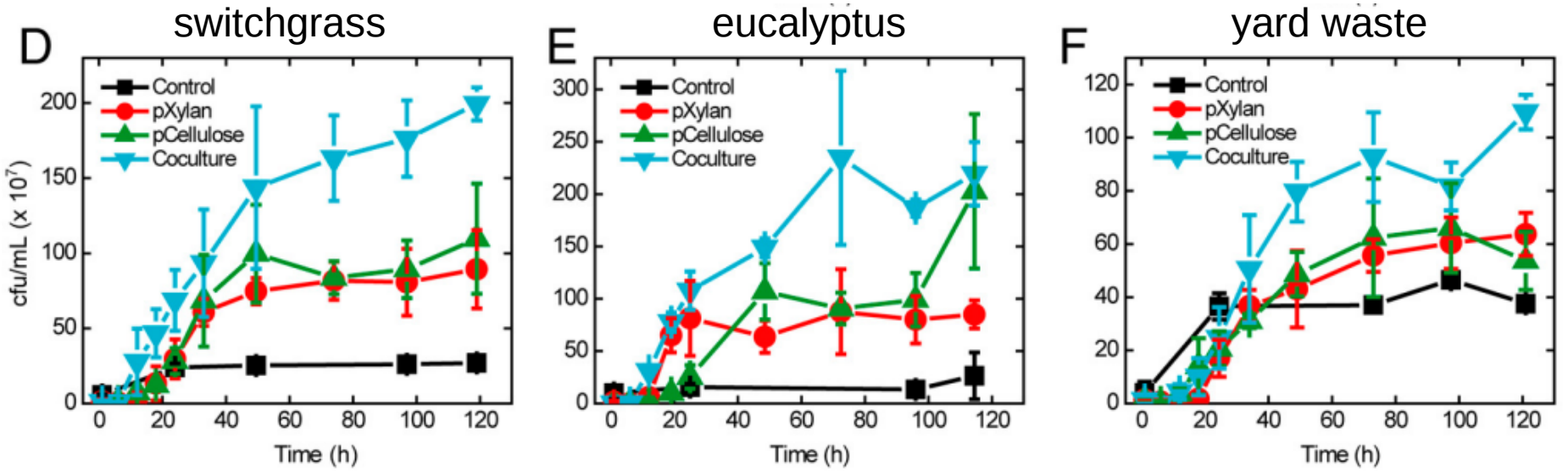


Fig 3C

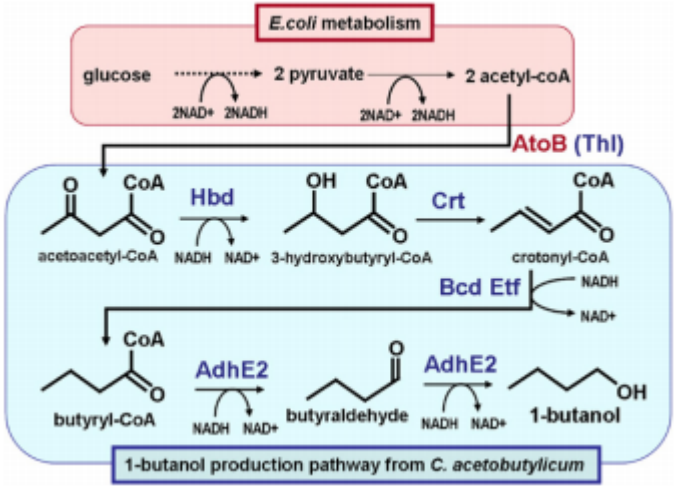
Strains can grow on biomass (sort of)



The cellulase and hemicellulases hydrolyzed 8% of sugars in switchgrass

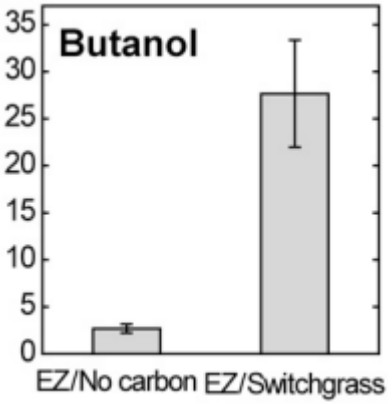
now add genes to make fuels (butanol)

Butanol=7 genes from *Clostridium acetobutylicum*



Atsumi et al, 2008

Fig 4c



now add genes to make fuels (FAEE)

Fatty acid ethyl esters=overexpress 6 genes

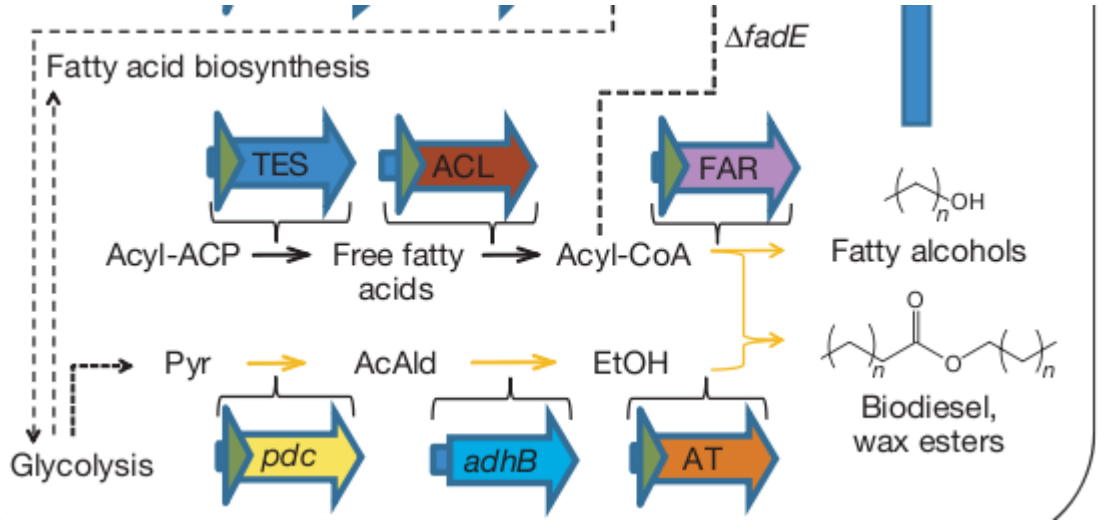
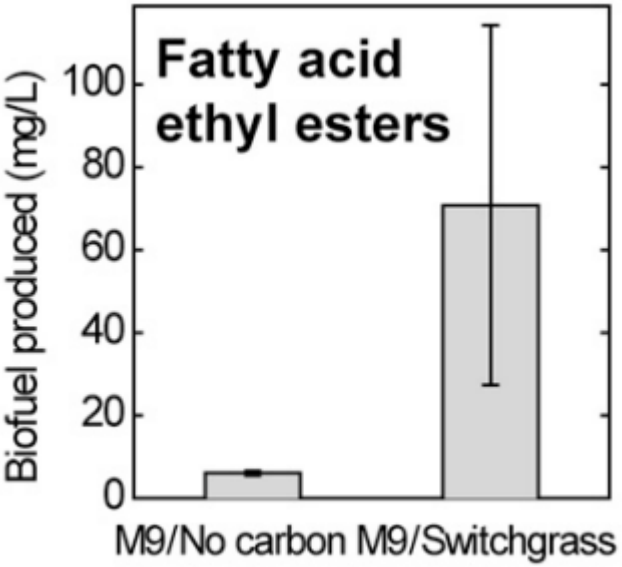


Fig 4B



Steen et al, 2010

now add genes to make fuels (pinene)

Pinene=9 genes (source uknown)

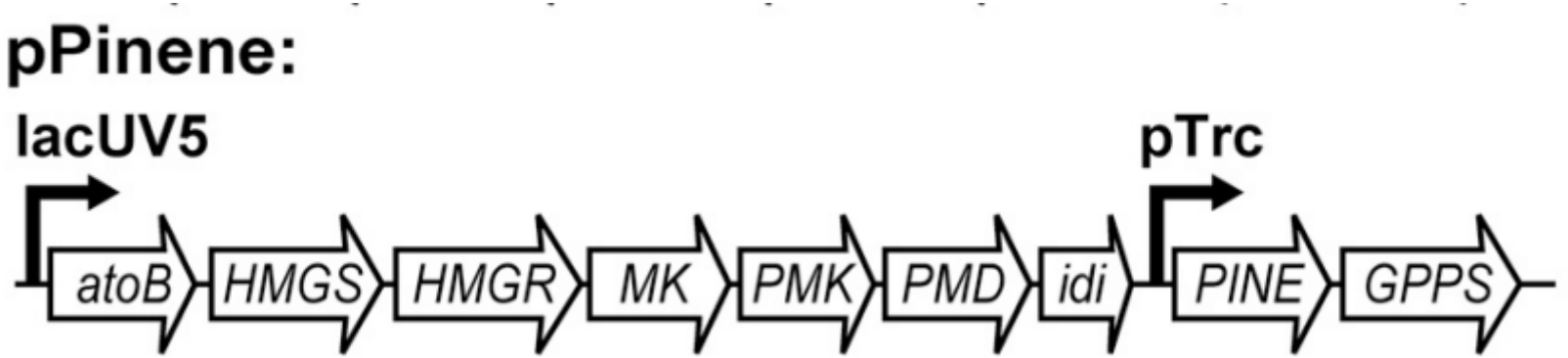
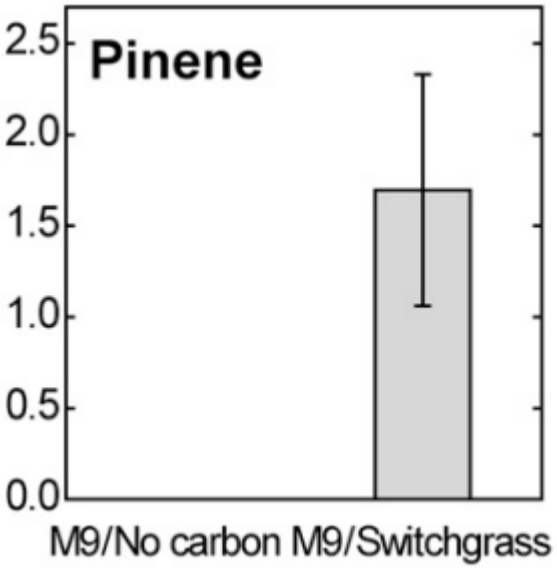


Fig 4D



Are these yields high enough to really make fuels?

butanol yield=0.028 g butanol/l of water

butanol density=0.81 g/ml

What volume of water would you need to get 1l (810g) butanol?

need 29,000 l water to get 1l butanol

What are the positives and negatives of this paper?

Positives

Made E.coli eat plants

Screen for promoters and genes that work in E. coli

Plant->fuel in single step

Pure fuels

Negatives

Side products?

Are other mutations needed? Transporters?

More mutations?

Make intermediates at higher conc?

Only 8% of plant degraded