

Clostridia: Microbial Engines of Bioconversion

**Oklahoma Annual EPSCoR State Conference
April 29, 2010**

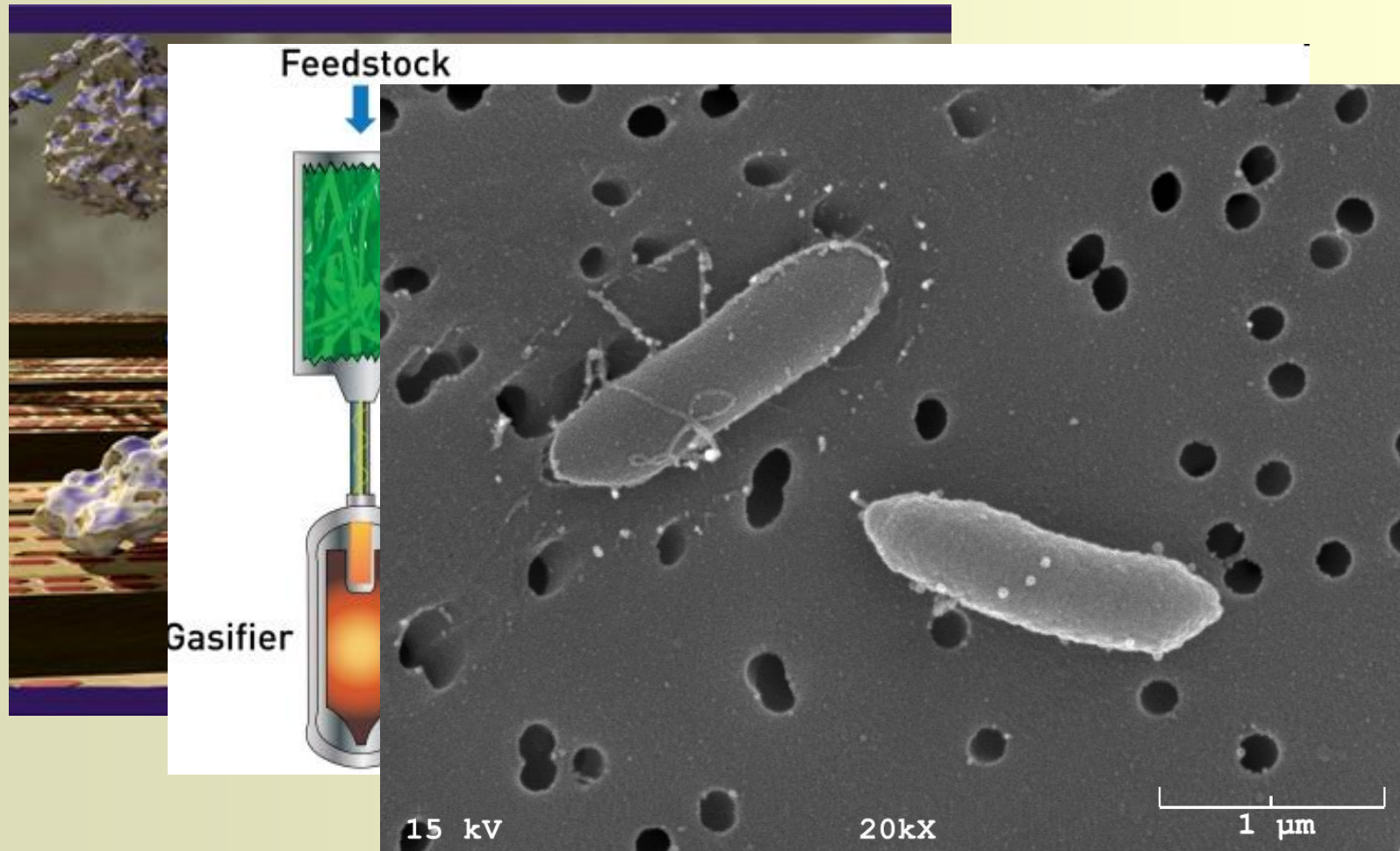
Chris Hemme and Jizhong Zhou



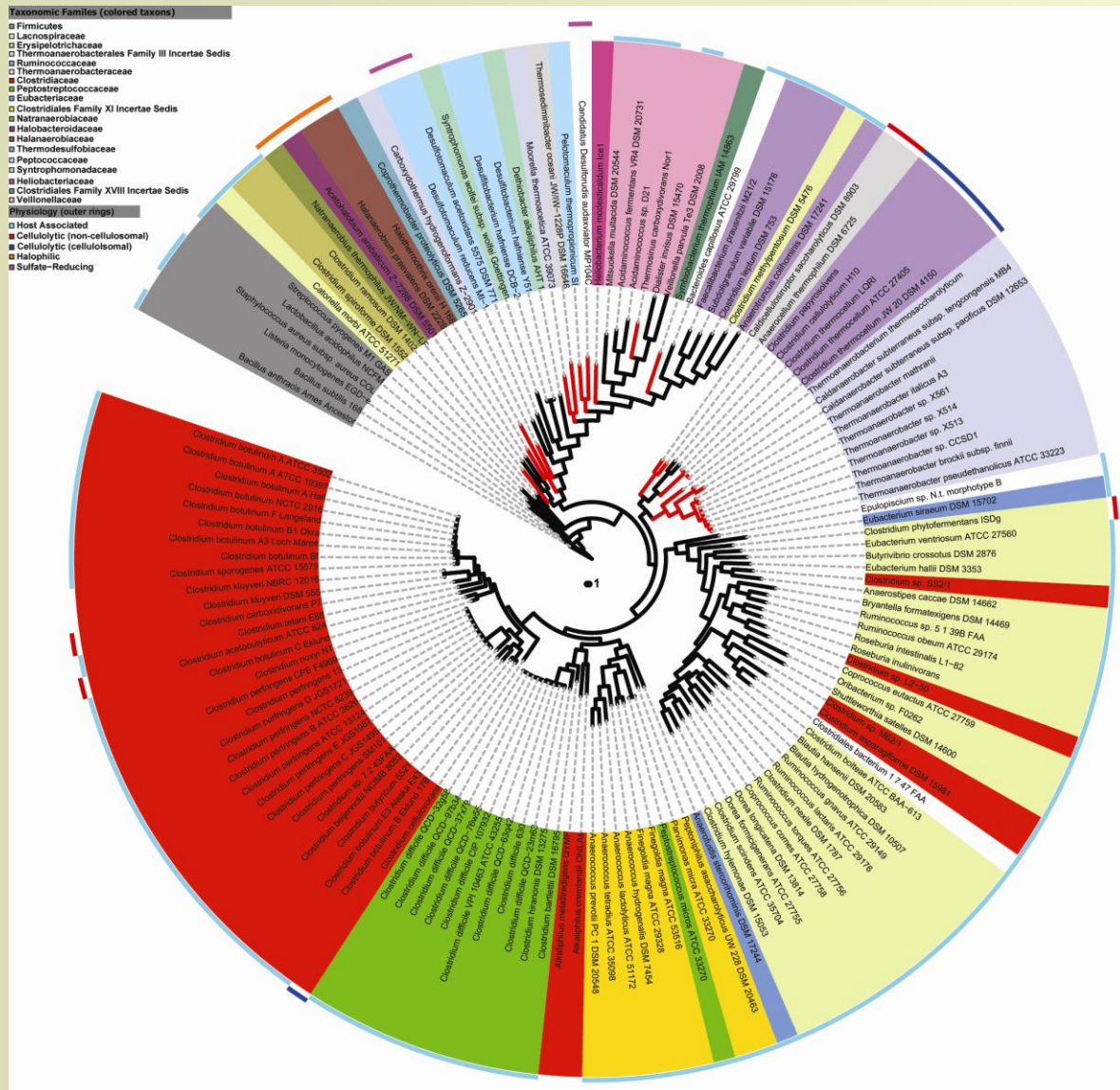
INSTITUTE FOR ENVIRONMENTAL GENOMICS

UNIVERSITY OF OKLAHOMA

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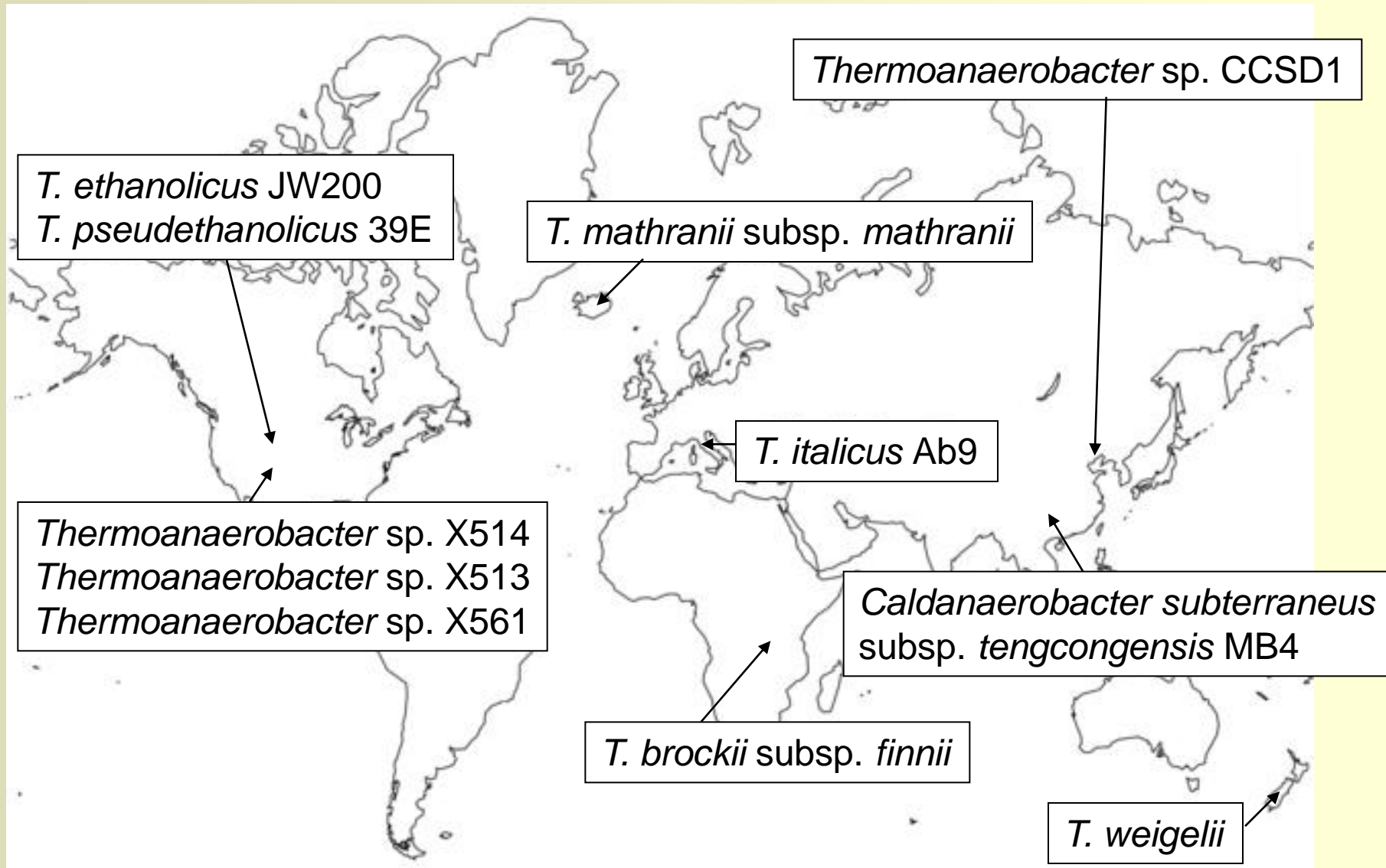
Clostridia Phylogenomics



- Taxa colored based on 16S rRNA classification
- Red lineages = thermophiles
- Suggests taxonomic relationship between thermophilic saccharophiles and cellulosome-encoding Clostridia



Thermoanaerobacter Genomics

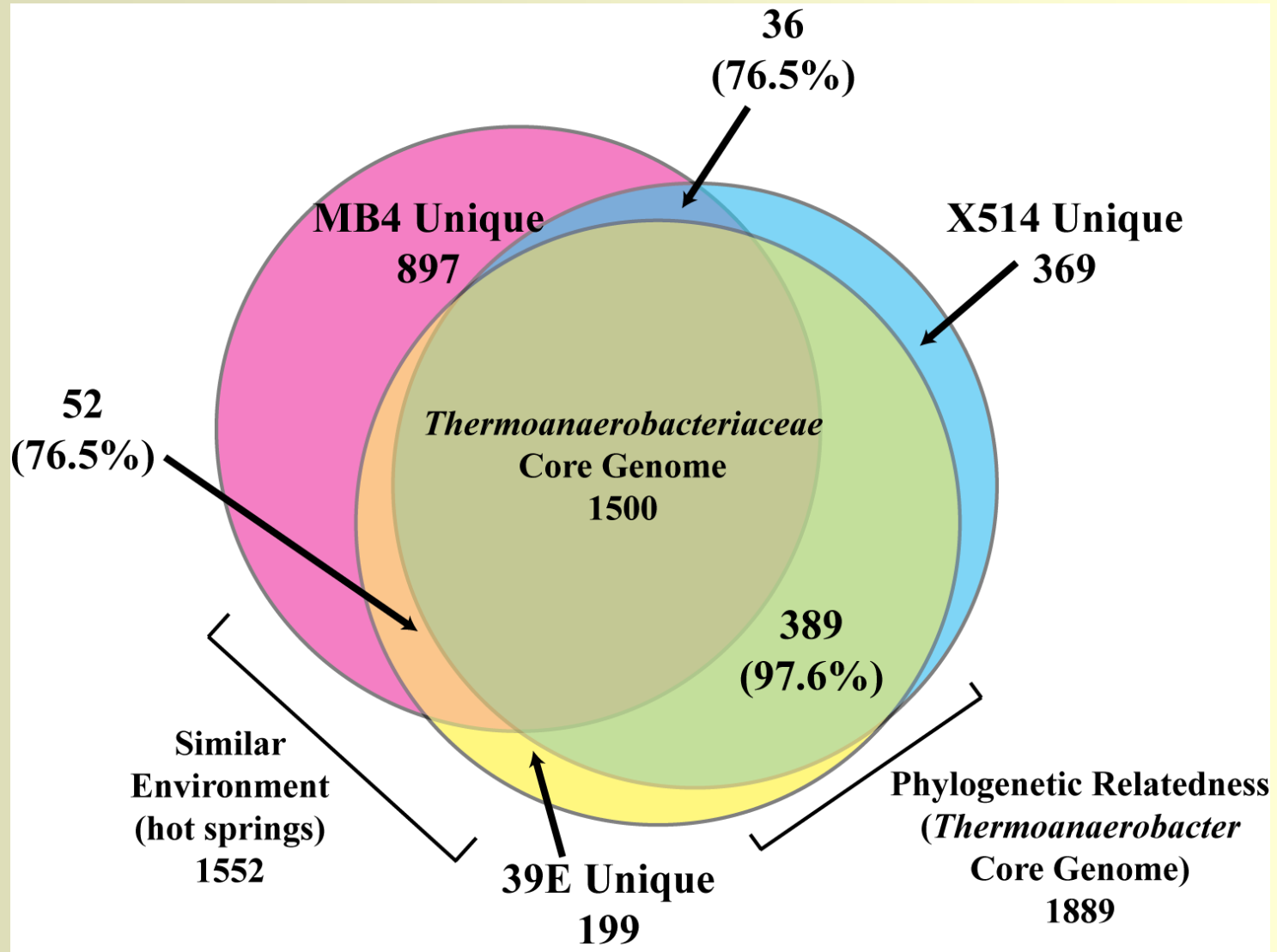


Thermoanaerobacter Genomics

- **Comparative genomics**
- **Correlation of genomics to physiology**
- **Development of genetic systems**



Thermoanaerobacter Core Genome



Results

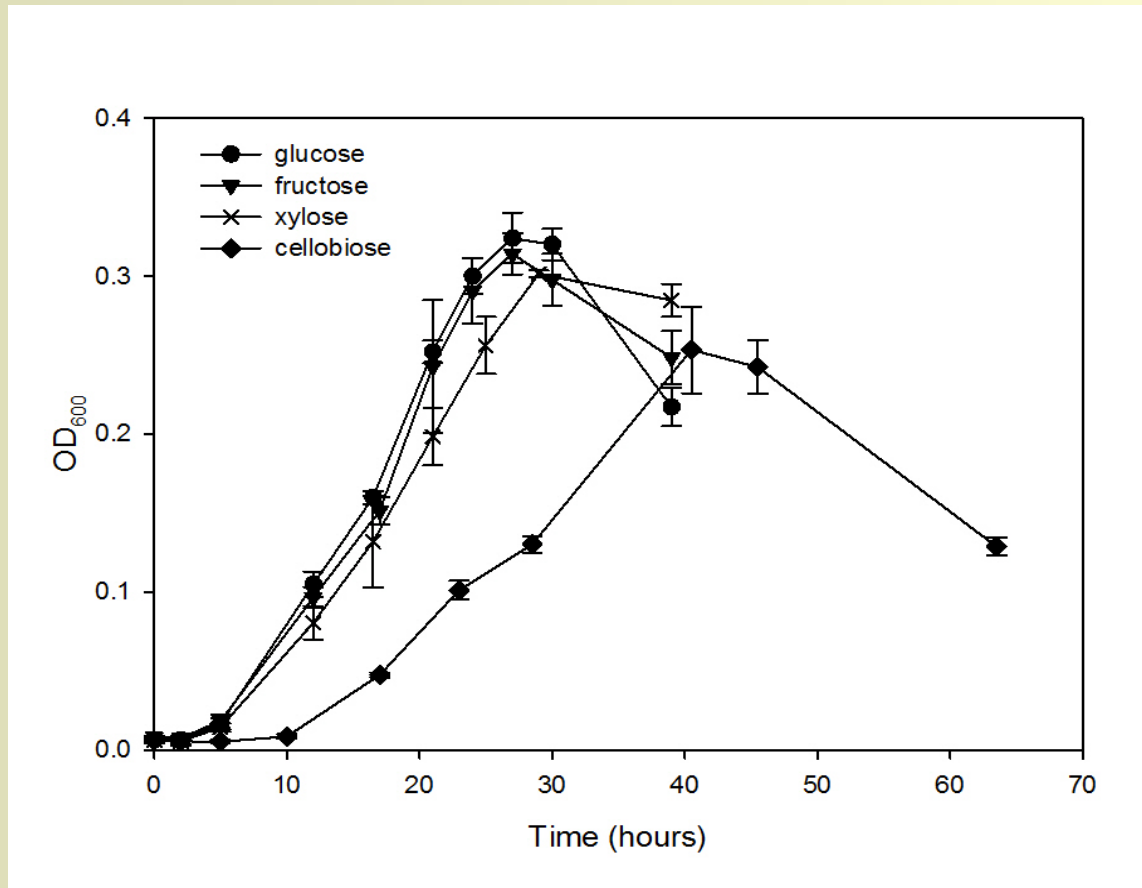


Fig. 1 Growth curves of *Thermoanaerobacter* sp. X514 grown in defined original medium with glucose, xylose, fructose and cellobiose as the sole carbon source.



Results

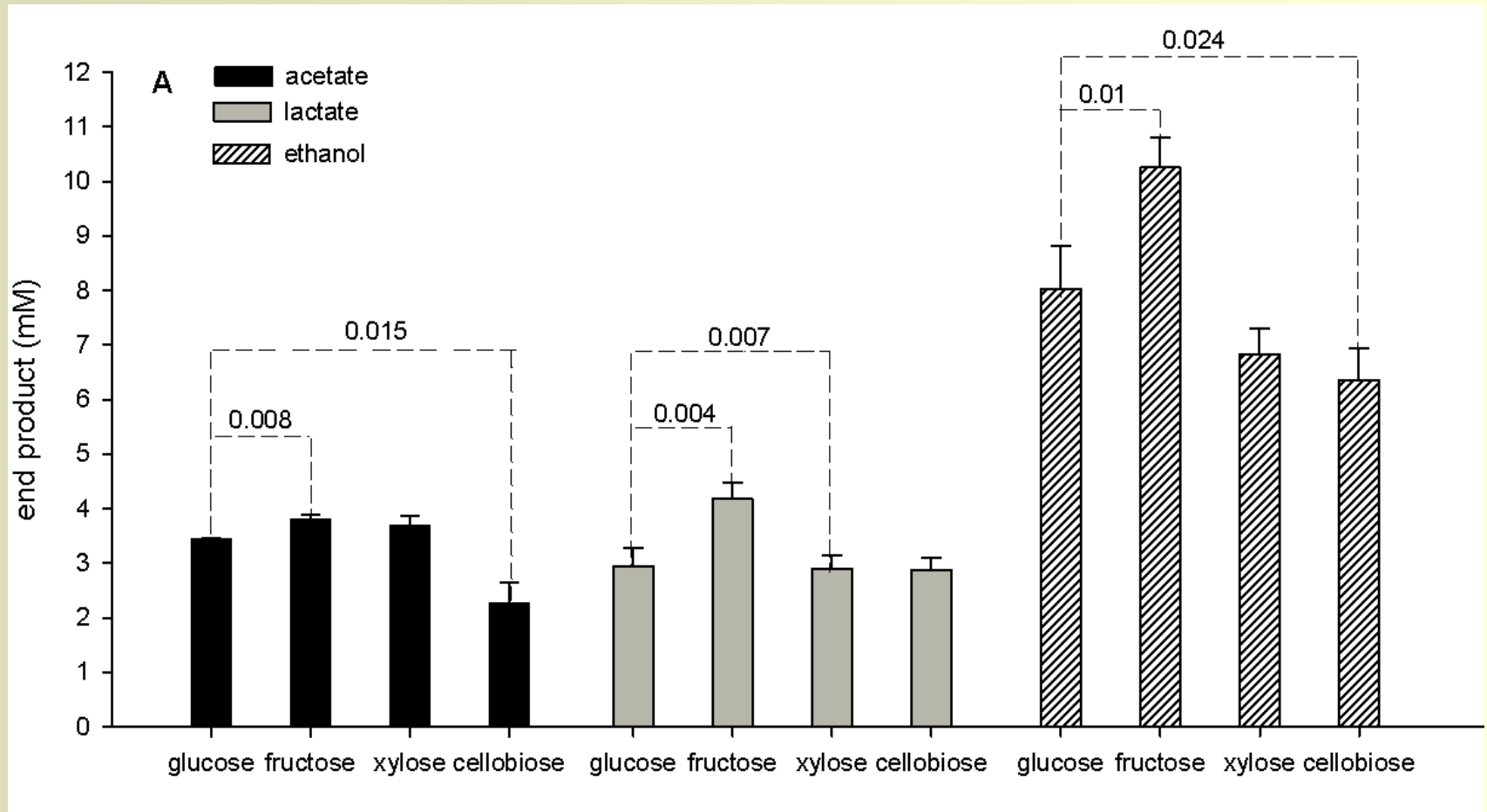


Fig. 2A Metabolite levels of *Thermoanaerobacter* sp. X514.

Metabolite production by *Thermoanaerobacter* sp. X514 in defined medium under different substrate conditions.



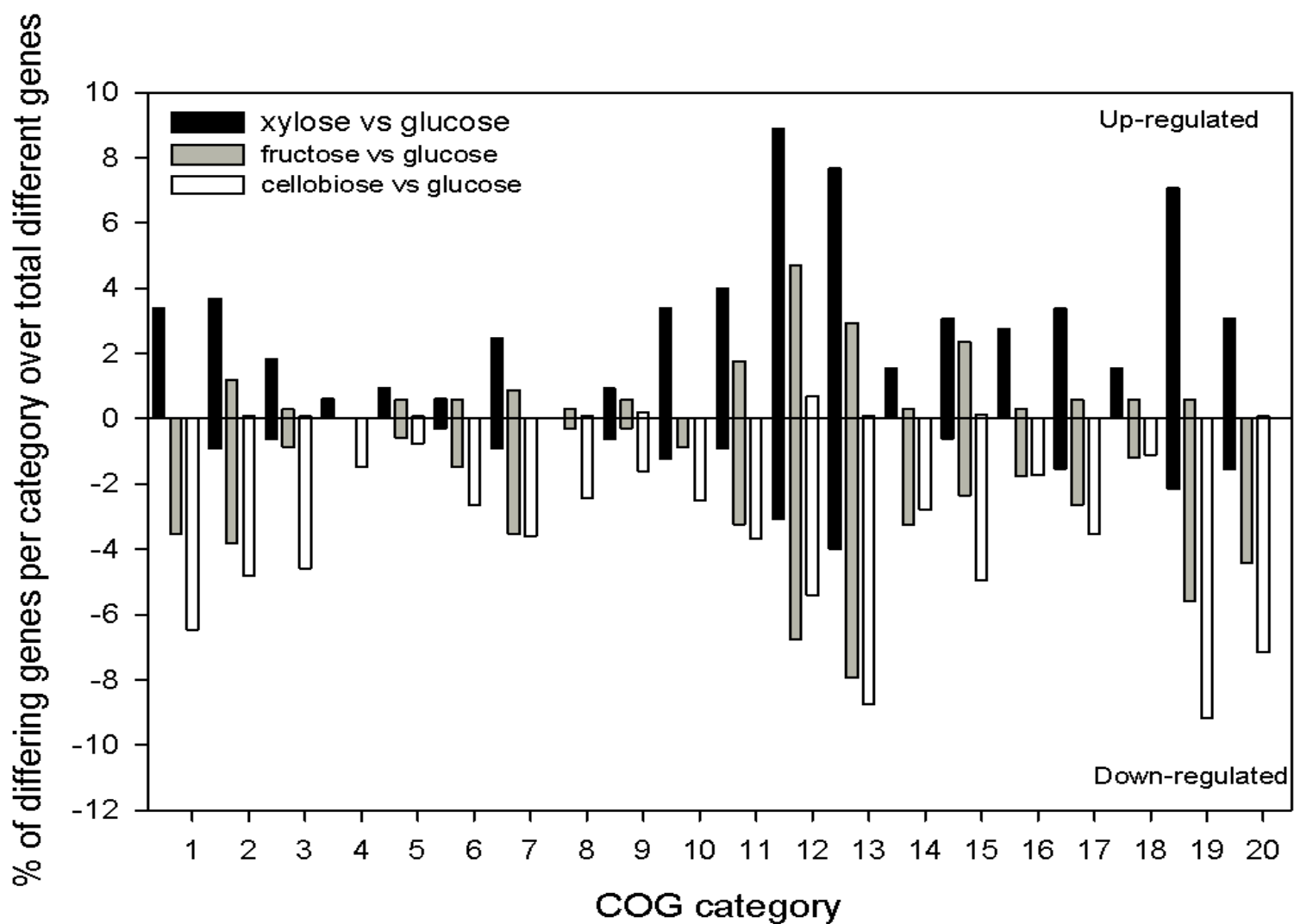
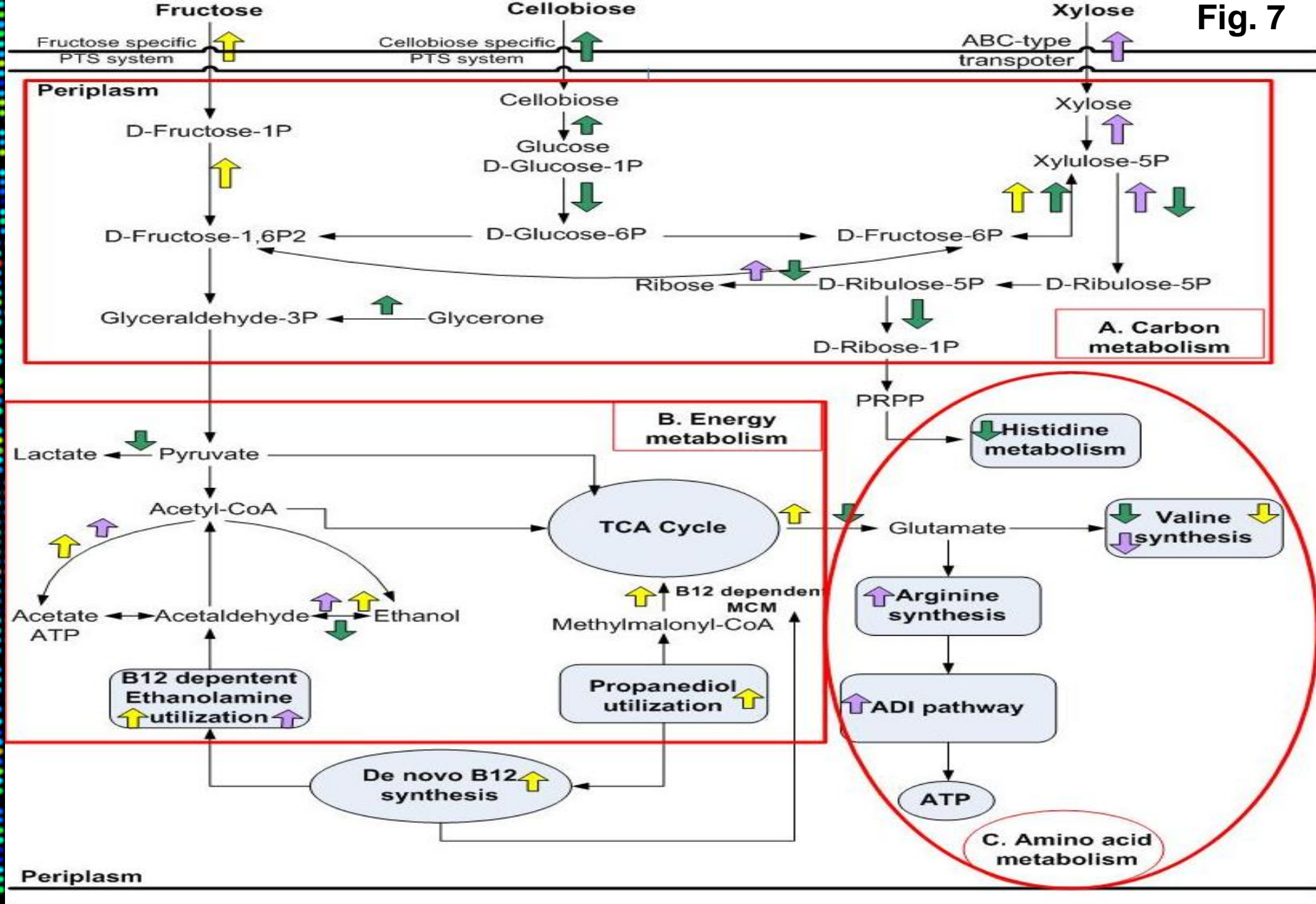


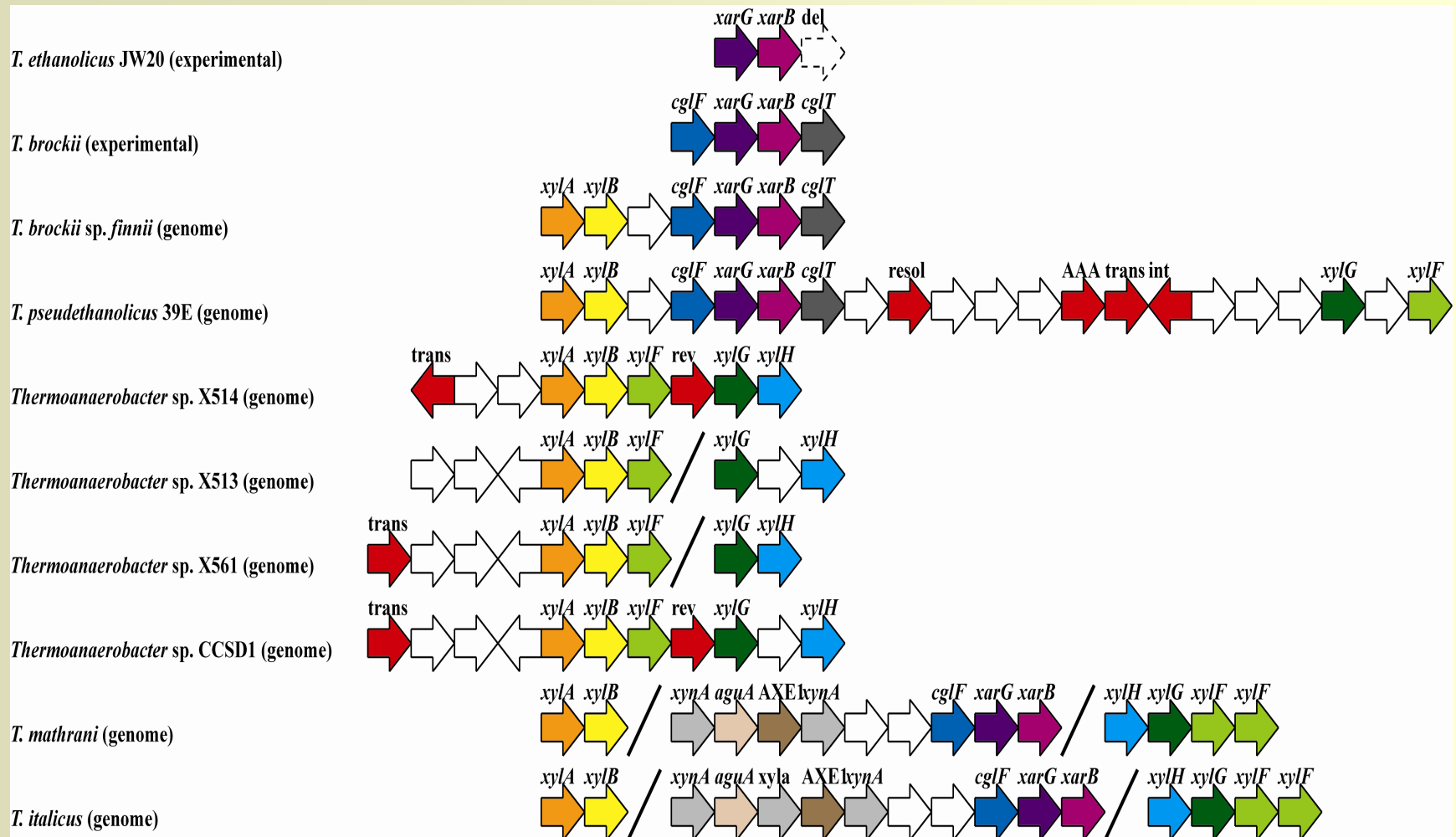
Fig. 3 Number of differentially expressed genes represented as a percentage of the total genes per functional classification according to JGI. Columns: 1, J; 2 K; 3 L; 4 D; 5 V; 6 T; 7 M; 8 N; 9 U; 10 O; 11 C; 12 G; 13 E; 14 F; 15 H; 16 I; 17 P; 18 Q; 19 R; 20 S.

Fig. 7

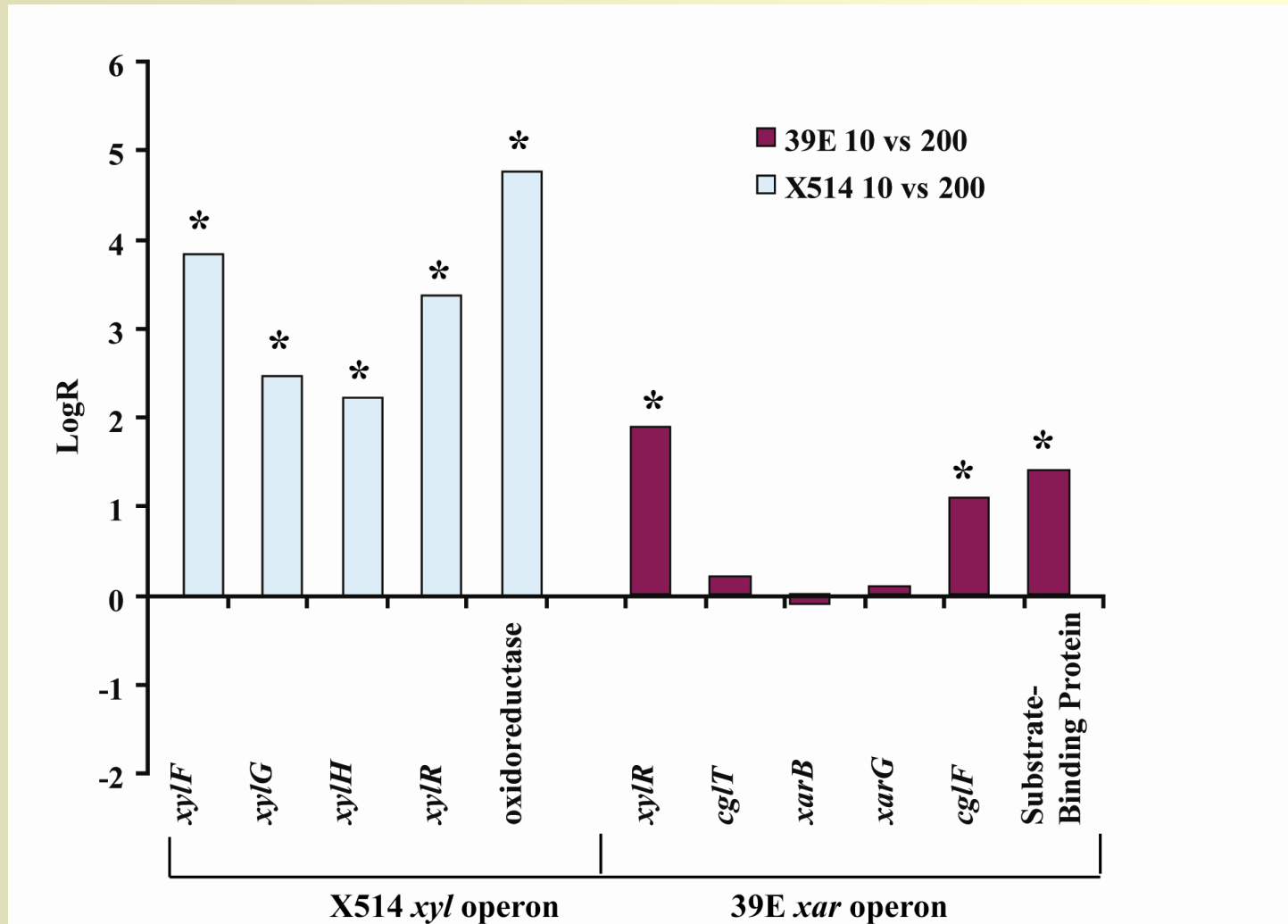


Notes: up arrow: up-regulation, down arrow: down-regulation
yellow: fructose, green: cellobiose, purple: xylose

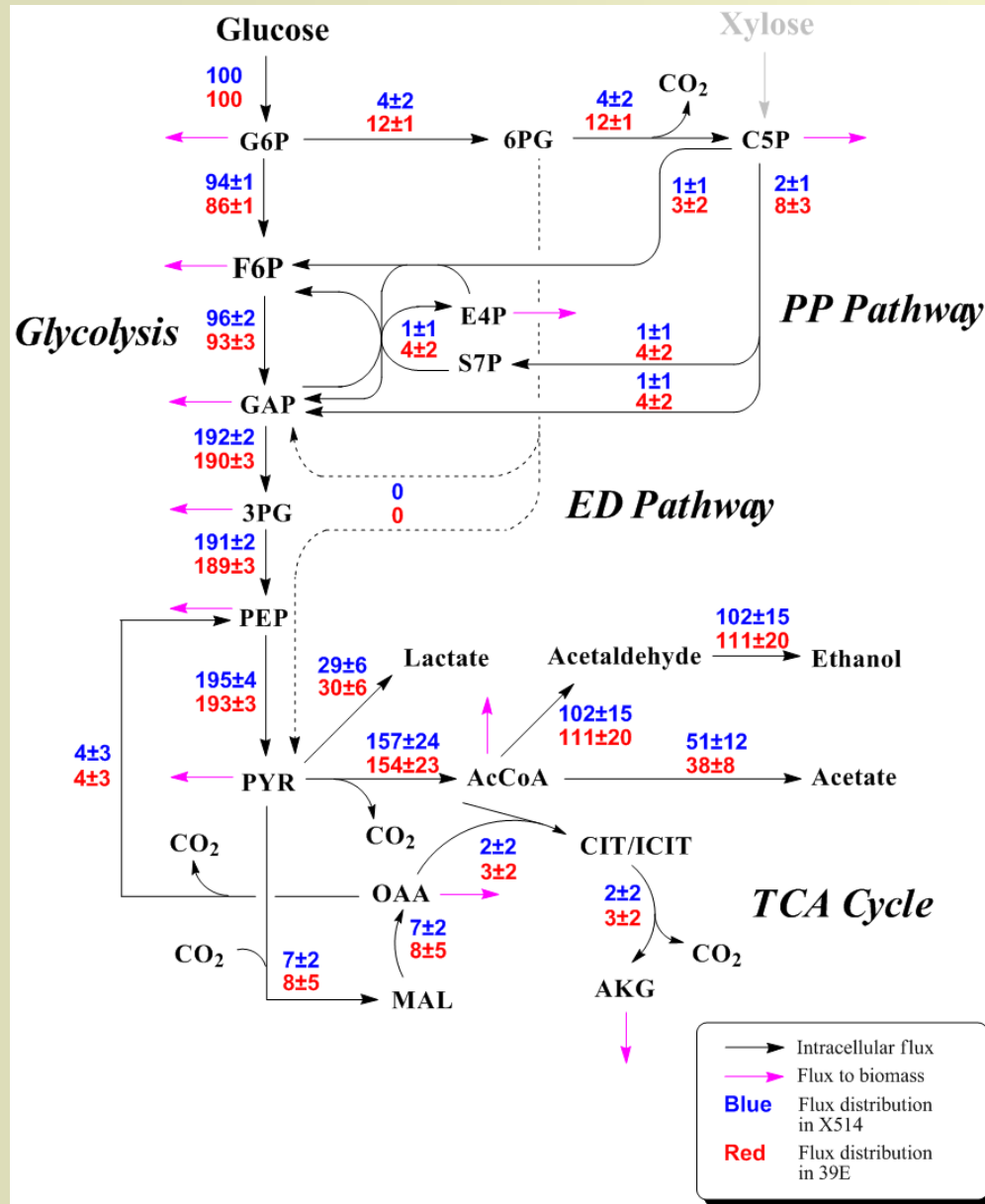
Xylose and Xylan Metabolism and Transport in *Thermoanaerobacter*



Expression of Xylose Transport Genes



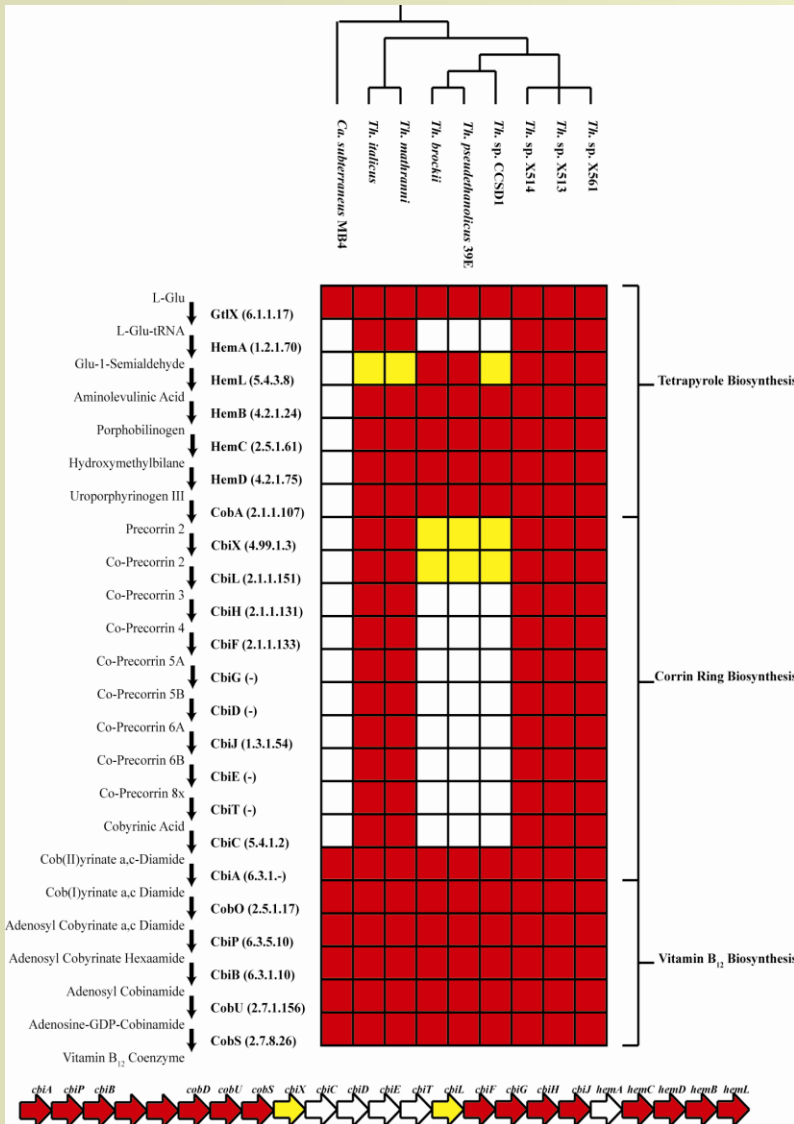
Metabolic Flux Analysis



- Relative carbon flux from xylose the same in both strains
- Absolute carbon flux from xylose greater in X514



Vitamin B₁₂ Biosynthesis in *Thermoanaerobacter*

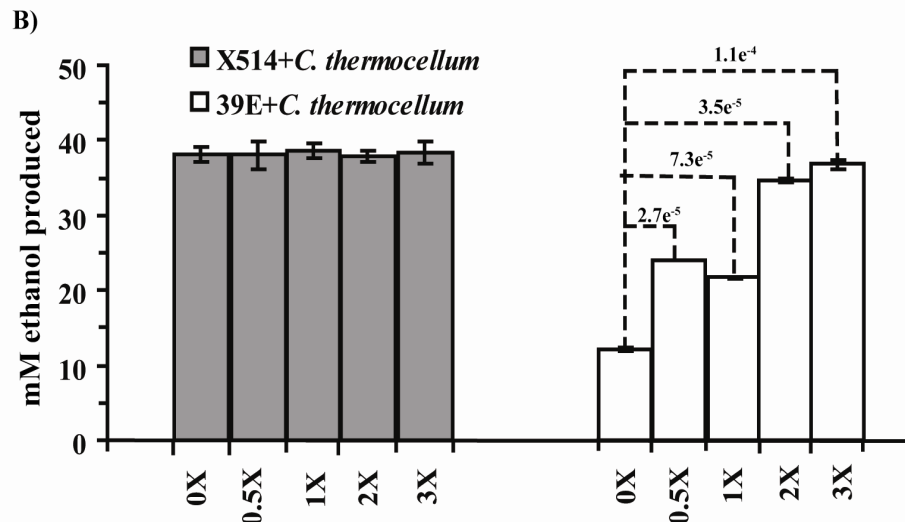
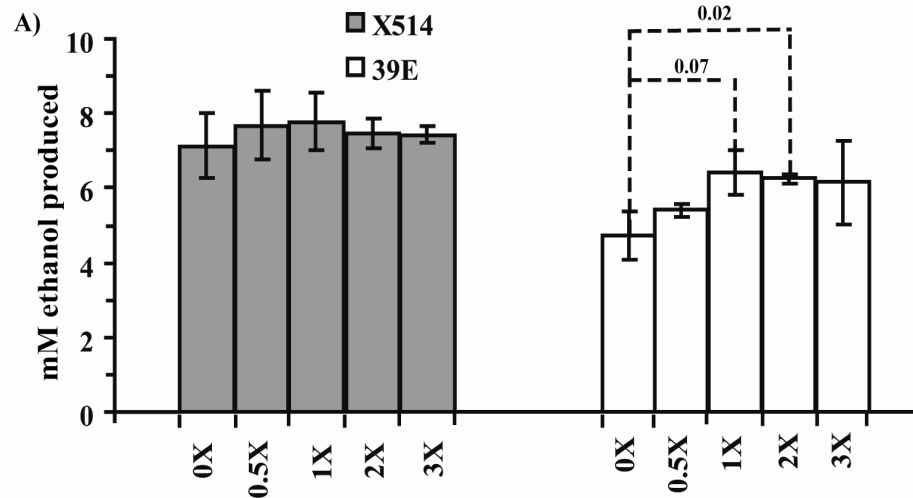


- Complete *de novo* vitamin B₁₂ biosynthesis pathway in X514 and *mathranii* lineages
- Hypothesis
 - B₁₂ produced by X514 enhances cellulose degradation and ethanol yields in coculture w/ LQRI
- Experiments
 - Growth assays w/ LQRI monoculture grown on cellulose w/ B₁₂
 - Growth assays w/ 39E-LQRI coculture grown on cellulose w/ B₁₂



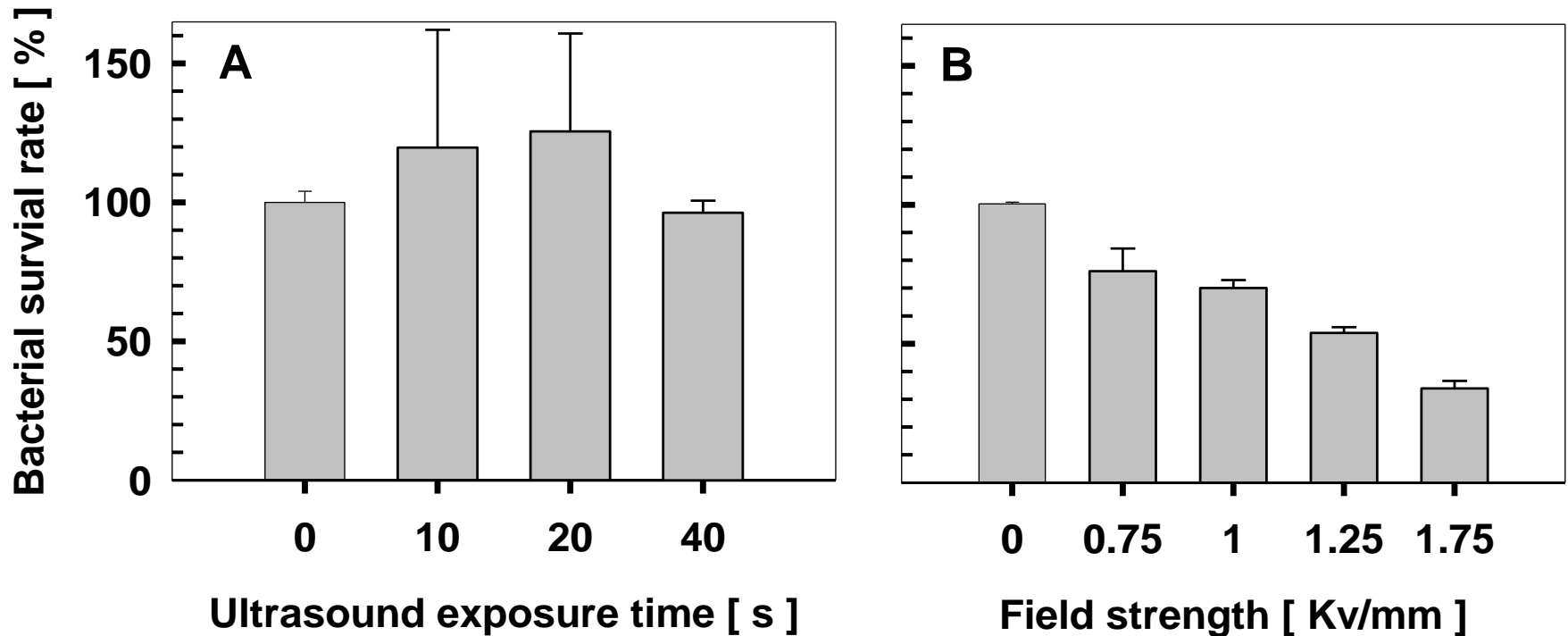
Effects of Vitamin B₁₂ on Ethanol Production in Mono- and Co-Culture

- X514 is insensitive to exogenous B₁₂
- 39E is very sensitive to exogenous B₁₂



Results

- ❖ Survival rate of *Thermoanaerobacter* sp. X514 after sonoporation and electroporation.

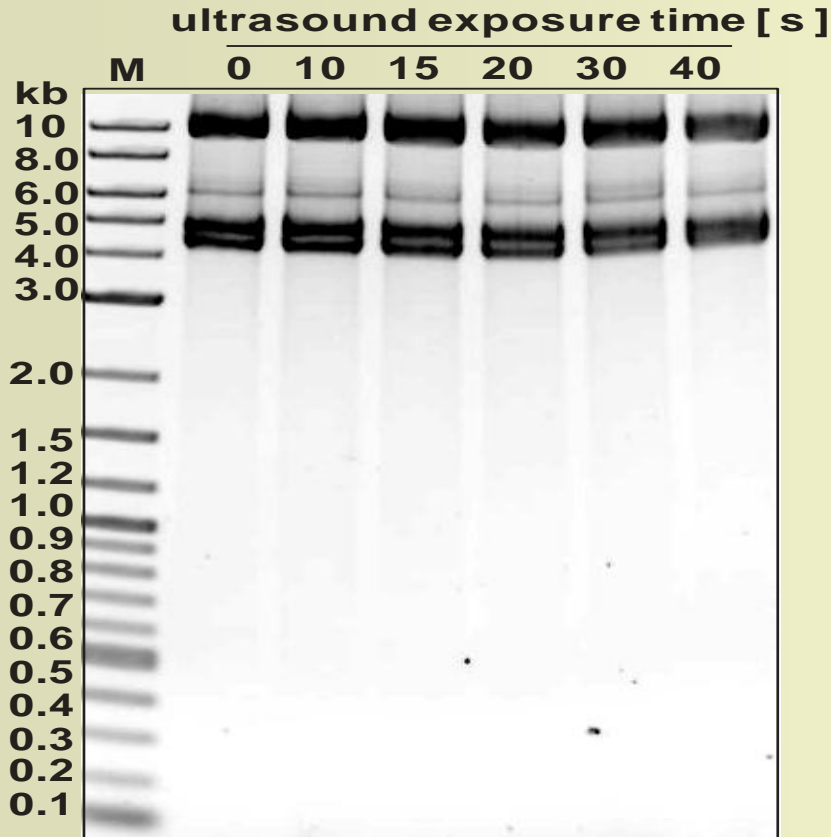


The result indicated ultrasound can be used to efficiently incorporate macromolecules into X514 without significant cell damage.



Results

❖ Effect of ultrasound exposure times on plasmid integrity

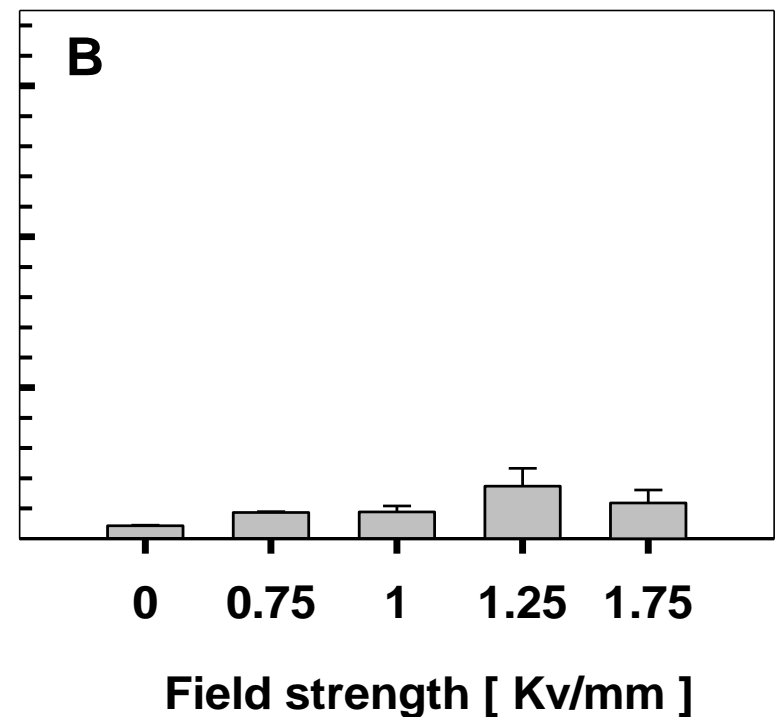
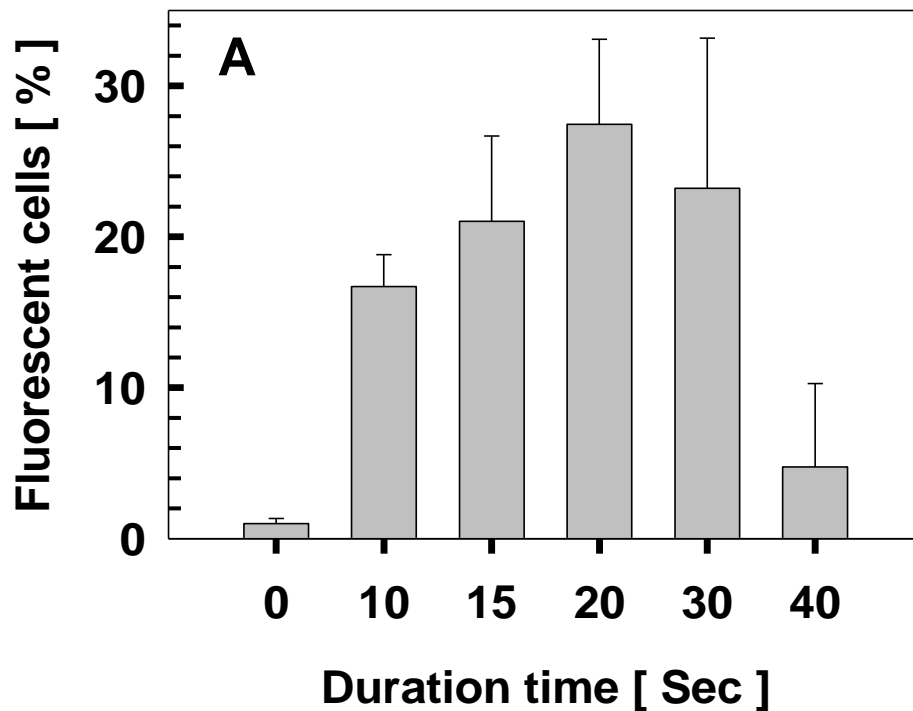


The plasmid pIKM1 was separated on 1% agarose gel after ultrasound exposure with times ranged from 0 to 40 s in Branson B200 sonifier.

The result showed plasmid integrity was not significantly impacted by sonification at the exposure times investigated.



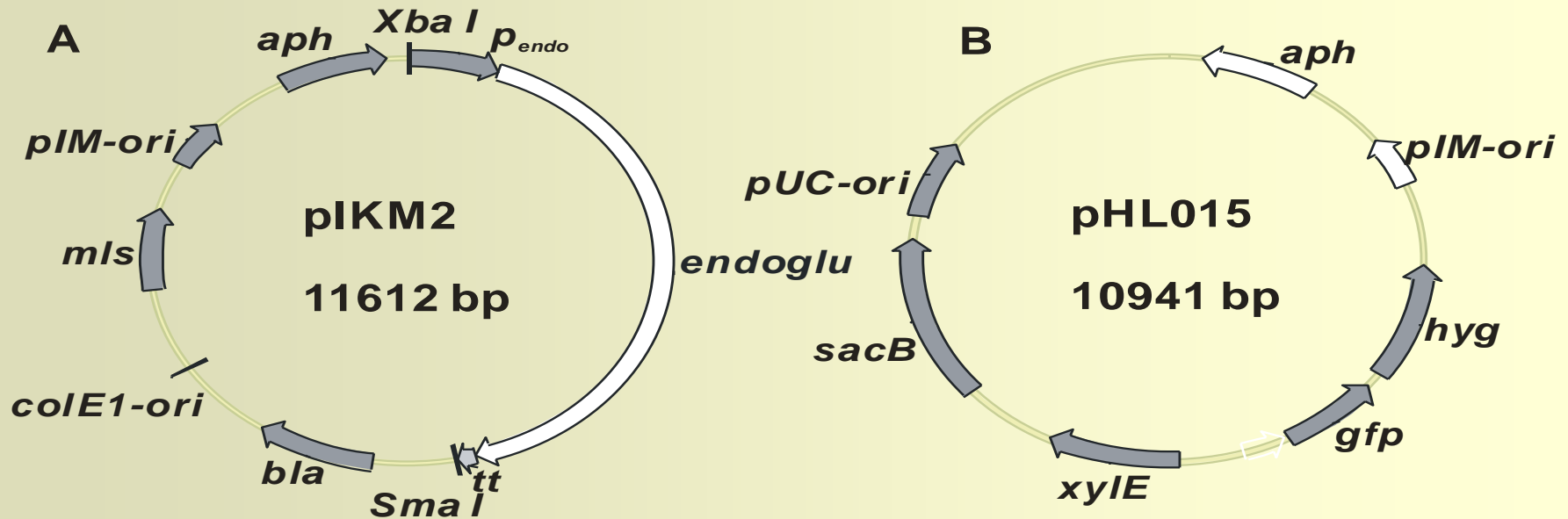
Results



The optimal sonoporation parameters: 40kHz,19W,20S
(Branson 200)



Transformation of methylated pKM2 and pHL015 into *Thermoanaerobacter* sp. X514 by sonoporation and electroporation



Physical maps of plasmid pKM2 and pHL015.

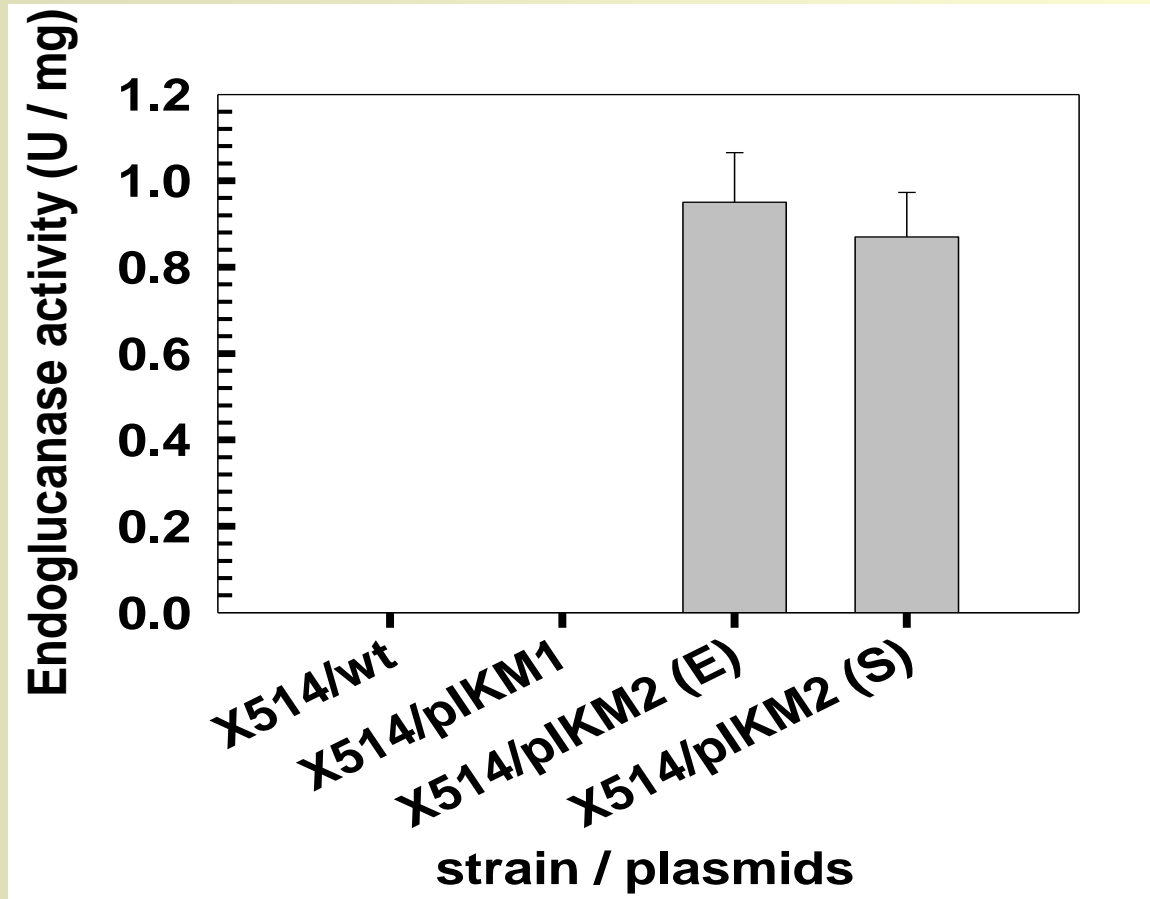
A: pKM2. pKM2 is a derivative of pKM1 (22) by inserting the beta-1, 4-endoglucanase gene into *Xba I* / *Sma I* sites of pKM1 as described in text.

B: pHL015. pHL015 is a derivative of construct by inserting the *Nco I* / *Nsi I* flanked fragment of pKM1 into similarly digested plasmid pML523.

The pHL015 contains a kanamycin resistance gene (*aph*, from pKM1), a *Bacillus subtilis* replicon (*pIM-ori*, from pKM1), a hygromycin resistance gene (*hyg*), a green fluorescence protein gene (*gfp*), a catechol 2,3-dioxygenase gene (*xyIE*), a levansucrase gene (*sacB*) and an *E. coli* replicon (*pUC-ori*).



Endoglucanase activity of *Thermoanaerobacter* sp. X514 transformants



The X514 was transformed by electroporation (E) and sonoporation (S), respectively. Wild-type X514 and pIKM1 transformants were used as negative control.



Future Directions

- Establishment and refinement of genetic and mutagenic systems for Clostridia
- Long-term evolution of *Thermoanaerobacter*
- H₂ production from biomass
- More genomic sequencing and comparative genomics



Acknowledgements

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