Consolidated bioprocessing of hemicellulose by engineered coculture consortia

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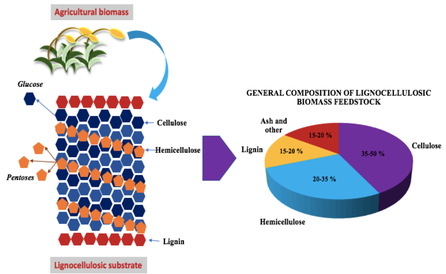
Abstract

Increasing global concerns about limited reserves of conventional fuel sources has led to exploration of alternative feedstock for the renewable production of fuels and chemicals worldwide. Lignocellulosic feedstock is a fascinating alternative which mainly constitutes of cellulose, hemicellulose and lignin. Efficient and cost-effective methods are essential for breakdown of cellulosic materials into sugars that can be fed to microbes for bioconversion. Present work aims at development of *Bacillus subtilis* strains for heterologous expression and extracellular export of endoxylanases for extracellular depolymerization of hemicellulose. Different combinations of signal peptides with genes were verified for optimal functionality. Three signal peptides namely YwmC, SacC and AmyE; two xylanases from *Trichoderma resei* and *Neosartorya fischeri* (Nf); were selected based on compatible functionality with respect to pH and temperature conditions. The recombinant and wild type strains of *B. subtilis* WB800N were screened for extracellular hydrolysis of the substrates and comparative analysis was performed on the basis of DNSA assay. All the recombinants demonstrated increase xylan depolymerization over wild type. The engineered strain with YwmC-Xy(Nf) displayed 2 fold increase in activity compared to SacC and AmyE signal peptides. *In situ* depolymerization of Xylan showed 15 g/L of xylose yield which is 4 folds higher than published works. *B. subtilis: E. coli* Coculture process was developed to break down xylan and produce succinate in a single pot achieving a succinate titer of 3.7 g/L. This approach can be further explored for effective degradation of complex polymers like cellulose and hemicellulose to obtain substrates for production of wide variety of fermentation by products.

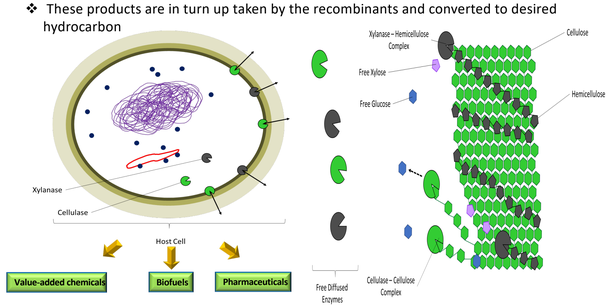
Lignocellulosic biomass: a carbon rich resource



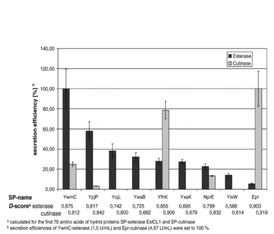
- The Agricultural biomass mainly constitutes biopolymers *viz*. cellulose, hemicellulose and lignin
- Cellulose is widely explored as a source of glucose through bio/chemical hydrolysis for production of biofuels
- Hemicellulose has random and amorphous structure formed mainly from pentoses
- To circumvent limitations of chemical hydrolysis biocatalysts like proteinaceous enzymes are promising alternative (cellulases, hemicellulases, xylanases etc) to leverage process efficiency.

Consolidated bioprocessing of biomass components

- In principle extracellular enzymes secreted upon induction hydrolyse the biopolymer substrate
- These products are in turn up taken by the recombinants and converted to desired hydrocarbon

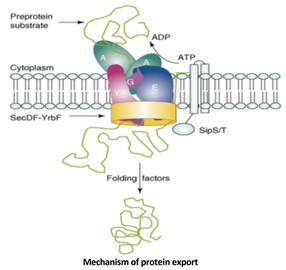


Signal peptides improve protein secretion



Increase in protein export after using signal peptides

(Brockmeier et al., 2006)

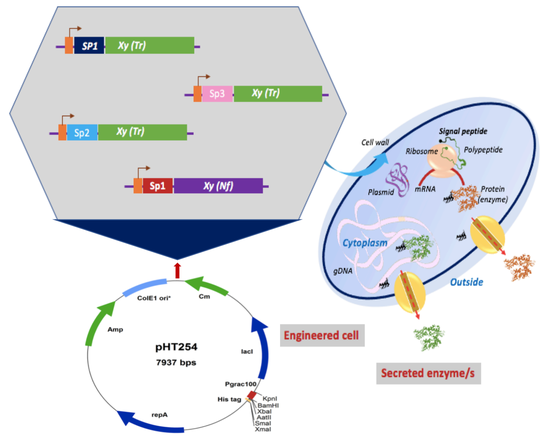


Mechanism of protein export

(Harwood and Cranenburgh, 2008)

Signal peptides play important role in protein translocation and export in gram negative and gram positive bacteria. Signal peptides fused with heterologous enzyme are unique combination and it is not possible to predict optimal signal peptide for certain enzyme.

Developed Signal peptide-gene constructs

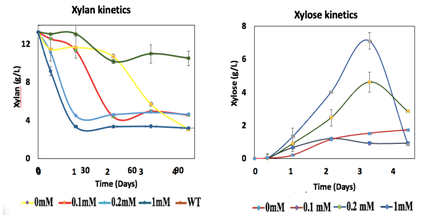


- The project aimed at developing signal peptide and enzyme combinations in a pHT254 vector.
- The signal peptides selected for present work were mainly SacC, AmyE and YwmC.
- The Enzymes selected for present study were Xylanase A from *N. fischeri (Xy(Nf))*, Xylanase 2 from *T. Resei (Xy(Tr))* .

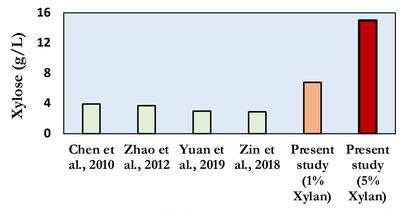
Conclusion

- We have demonstrated Xylose yield of 6 g/L from 1% Xylan in *in situ* system
- Xylan reduction by 65% in *in situ* system
- Highest xylose yield of 15 g/L was obtained from 5% Xylan
- First work on *B. subtilis* and *E. coli* consortia for biomass breakdown and succinate production with 3.7 g/L of succinate production

In situ breakdown of Xylan

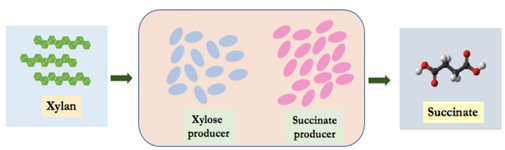


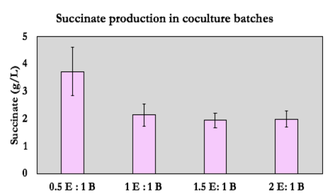
Comparison with literature



- Efficient secretion and appropriate folding of enzymes is important for successful *in situ* breakdown of Xylan.
- In present study higher IPTG concentration did not show high product yield and this can be because of inefficient export of enzymes due to high intracellular enzyme concentration.
- Xylose yield of 6.7 g/L was observed using 1% Xylan substrate and 15 g/L of xylose yield was observed in 5% Xylan substrate.
- Xylose yield of 1% Xylan was 2 folds higher than literature

Succinate production in *B. subtilis*: *E. coli* coculture





E: B – *E. coli* OD_{600} / *B. Subtilis* OD_{600}

- In proposed coculture system Xylan is degraded into xylose using Xylose producer (*B. subtilis*) in aerobic fermentation and the produced xylose is assimilated by Succinate producer (*E. coli*).
- Different combinations of E. coli and B subtilis were used to optimize the coculture ratio and *E. coli* : *B subtilis* ratio of 0.5 showed highest succinate yield of 3.7 g/L

Acknowledgements