Engineering Bacteria to Secrete Cellulases for Breaking Down Cellulose into Glucose

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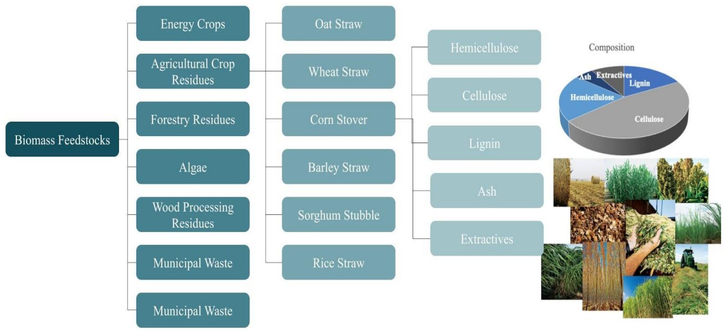
Abstract

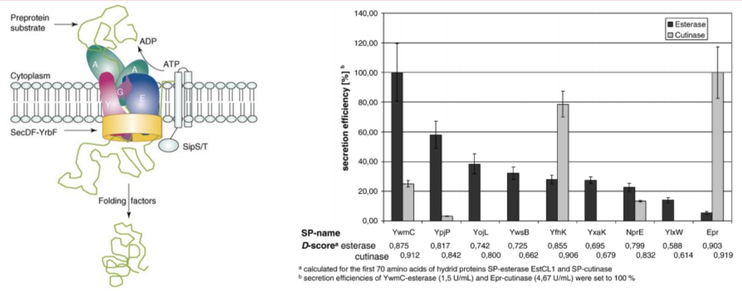
Cellulases are enzymes that are crucial for the breakdown of cellulose into glucose, the primary carbon source in bacterial systems. However, the current method of using commercial cellulases is very costly. Present work aims at engineering bacterial strains for efficient cellulase production. Bacteria was engineered for expression of beta glucosidase and the enzyme activity was studied using pNPG assay. As a first step, we were able to confirm the secretion of two glucosidase with 5 folds increase compared to wild type strain. The aim is to develop a consolidated bioprocess which converts cellulose biopolymer into utility chemicals.

Lignocellulosic Biomass



- Abundant and renewable natural resource
- Composed of carbohydrate polymers and aromatic polymers
- Contain different sugar monomers
- Can be used to produce biofuels and other useful chemicals with further treatment

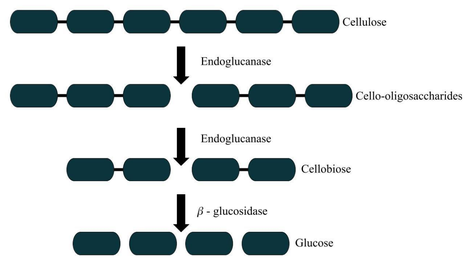
Signal Peptides and Protein Export



Figures from Freudl et al. Leaving Home ain't easy: protein export systems in Gram-positive bacteria (2013)

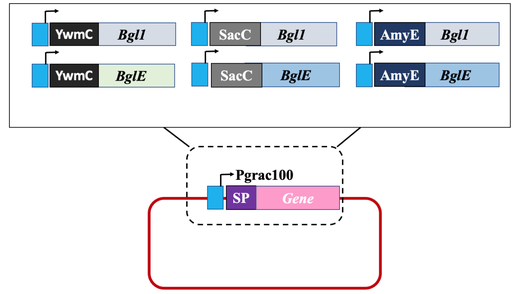
- Protein export is process where protein produced by cell is transferred to the surface of the cell
- Process is crucial to the survival of the Cell
- Signal peptides can cause increase in protein export

Synergistic Breakdown

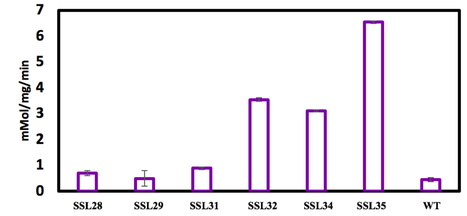


- Multiple enzymes in different steps required to break down the complex carbohydrate
- Desired enzymes are endoglucanase and β- glucosidase
- Developing signal peptides and gene pairings would result in the ideal production of these enzymes

Constructs Developed for β-Glucosidases Secretion



β glucosidase producing recombinant strains comparison



Next Steps

- Cellulose constructs must further be developed
- Co-cultures that secrete endoglucanases and β- glucosidase will be developed
- Bacterial strains will be constructed to secrete desired enzymes, allowing the full utilization of cellulose

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