Endogenous carbohydrate esterases of *Clostridium thermocellum* are identified and disrupted for enhanced isobutyl acetate production from cellulose

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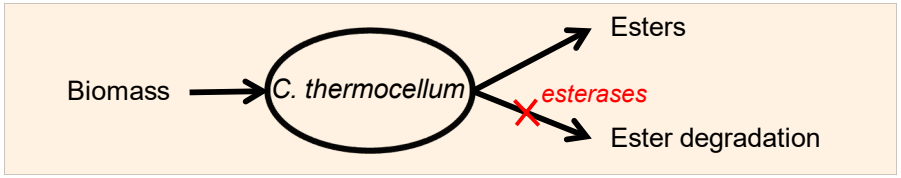
Background

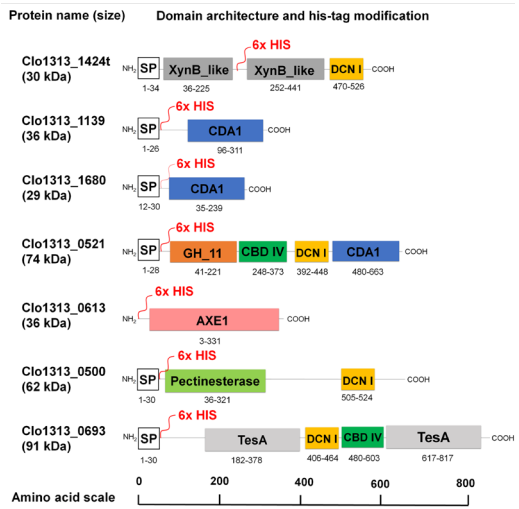
- Medium chain (C6-C10) esters are potential drop-in biofuels and industrial chemicals.
- Cellulolytic bacteria are attractive platforms for direct conversion of lignocellulosic biomass into products via consolidated bioprocessing (CBP) where biomass degradation and fermentation take place in a single step.
- *C. thermocellum,* a cellulolytic bacteria, has multiple carbohydrate esterases (CEs) which help deconstruct lignocellulose, but it is unknown which CEs hydrolyze the medium chain esters.

Approach

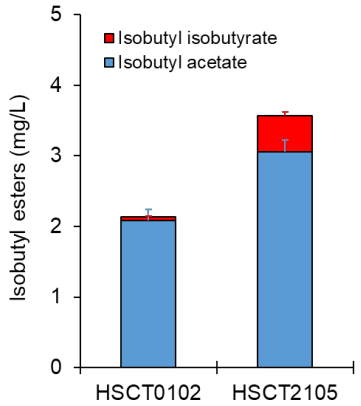
- We used bioinformatic analysis with detailed enzyme and strain chacterization to identify, characterize, and disrupt endogenous esterases responsible for the isobutyl acetate degradation in *C. thermocellum*.



Identification of carbohydrate esterases hydrolyzing isobutyl acetate in *C. thermocellum*



Enhanced ester production by disruption of the esterases



Outcomes and Impacts

- We discovered the key esterases responsible for hydrolyzing isobutyl acetate in *C. thermocellum.*
- We created an esterase-deficient, ester-producing *C. thermocellum* that significantly reduces ester degradation and hence improves ester biosynthesis while not affecting the strain's capability to effectively utilize cellulose.

Significance

- This study shows a path for metabolic engineering of a cellulolytic microorganisms to produce a value-added class of chemicals. Esters have immediate use in industrial chemicals such as solvents, additives, and fragrances as well as a potential advanced biofuel.

Seo, H., Nicely, P.N. Trinh, C. T., Endogenous carbohydrate esterases of Clostridium thermocellum are identified and disrupted for enhanced isobutyl acetate production from cellulose *Biotechnol Bioeng*, (2020) doi.org/10.1002/bit.27360

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