

# Identification of LacI repressor-based regulatory network activity targeting hemicellulases in *C. thermocellum*

## Background

- Understanding *C. thermocellum* gene regulation is of importance for improved fundamental knowledge of this industrially relevant bacterium.
- To date, the majority of genetic engineering studies have focused on improving fuel production, but little genetic work has targeted understanding regulatory pathways.

## Approach

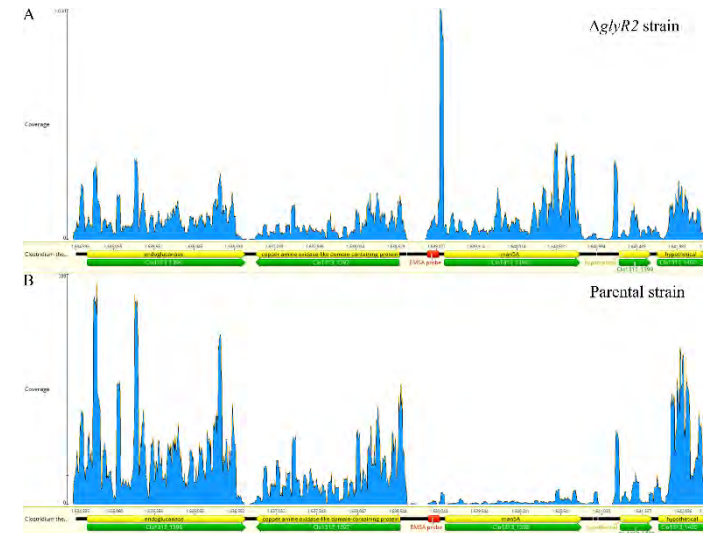
- We combined use of three *lacI* gene deletions with transcriptomics and DNA binding assays to gain insights into LacI regulatory networks.

## Outcomes

- Several hemicellulases, including cellosomal enzymes, were highly up-regulated (5 to 100 fold) in the absence of each LacI regulator.
- This study indicates there are small specific *C. thermocellum* LacI regulons.
- Higher expression for the Man5A mannanase was observed in a  $\Delta glyR2$  strain and  $\alpha$ -mannobiose was identified as a probable inducer for GlyR2 regulated genes.

## Significance

- The identification of LacI repressor activity for hemicellulase gene expression is a key result of this work and will add to the small body of existing literature on the area of gene regulation in *C. thermocellum*.



RNA-Seq analysis for *man5A* (mannanase) region in  $\Delta glyR2$  mutant and parent strains



GlyR2 has decreased DNA binding in the presence of  $\alpha$ -mannobiose