

# Toward improving tolerance of thermophilic microorganisms to pretreatment inhibitors



## Background

- While BESC platform organisms such as *Clostridium thermocellum* and *Caldicellulosiruptor bescii* exhibit excellent cellulose utilization, they can be susceptible to inhibitors released in biomass pretreatment.
- Yet other thermophilic anaerobes such as *Thermoanaerobacter pseudethanolicus* 39E display robust tolerance to common pretreatment inhibitors such as furfural and 5-hydroxymethyl furfural although the mechanisms of resistance are not known.

## Approach

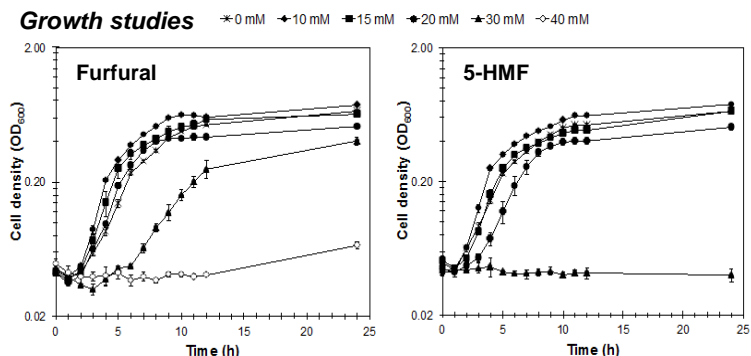
- Apply shotgun proteomics to determine cell-wide changes in protein expression for cultures of *T. pseudethanolicus* 39E exposed to a 15 mM furfural challenge.
- Identify putative functions involved in furan aldehyde detoxification.
- Clone, overexpress and assay candidate genes/enzymes in *E. coli* and test for furfural/5-HMF reduction to less toxic alcohols.

## Outcome

- 225 proteins were differentially regulated in response to furfural (152 upregulated vs. 73 downregulated).
- Two oxidoreductases were upregulated >6-fold by furfural and were targeted for further investigation.
- One of the two enzymes, a butanol dehydrogenase (BdhA) encoded by Teth39\_1597, showed significant NADPH-dependent furfural and 5-HMF reduction activity.

## Significance

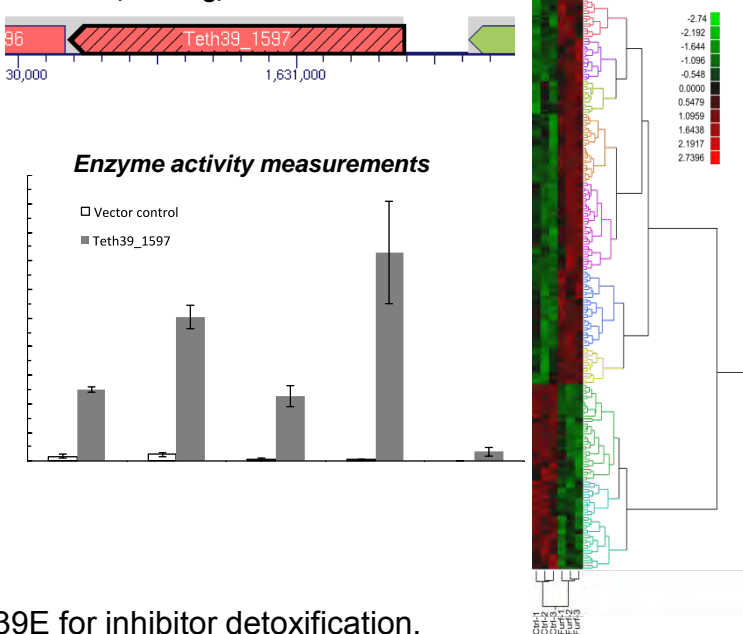
- A heat-stable furfural reductase has been identified in *T. pseudethanolicus* 39E for inhibitor detoxification.
- It promises rational improvement in tolerance and has already shown promising results when expressed in the cellulolytic thermophile, *C. bescii* (in preparation).



## Gene i.d., cloning, characterization



## LC-MS/MS proteomics



## Enzyme activity measurements

