

# The pentose phosphate pathway of cellulolytic clostridia relies on 6-phosphofructokinase instead of transaldolase

## Background

- Most cellulolytic clostridia do not contain transaldolase genes, which help connect pentose metabolism to the rest of metabolism
- Here we show that the PPI-dependent phosphofructokinase (PPI-Pfk) can fill in for the transaldolase in two cellulolytic clostridia species

## Approach

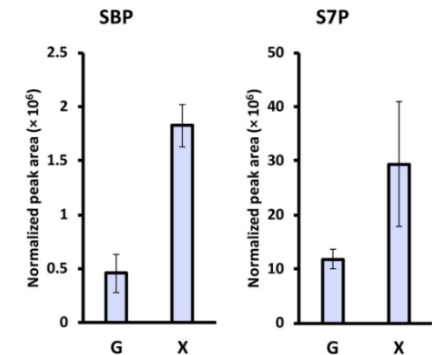
- Formation of sedoheptulose-1,7-bisphosphate (SBP) would provide evidence for the existence of this pathway
- Hence, we sought to document formation of SBP, both in batch cell cultures, and also with *in vitro* assays using purified PPI-Pfk protein

## Outcome

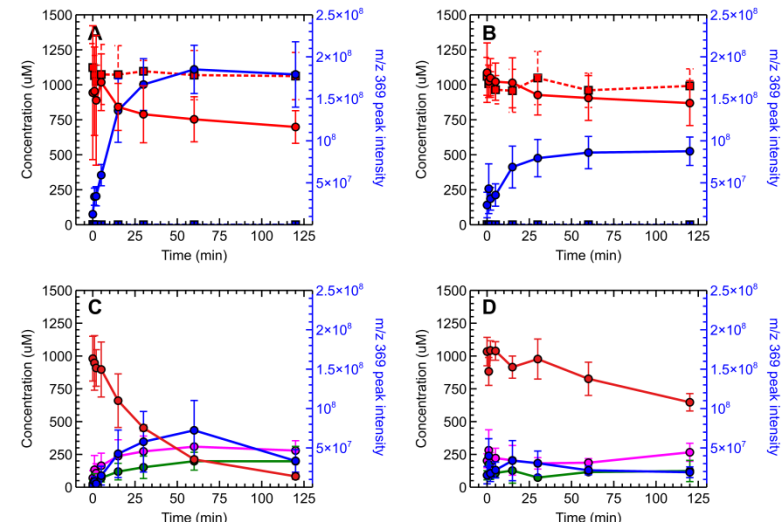
- *C. thermosuccinogenes* were shown to accumulate SBP; accumulation of SBP was much higher when grown on xylose
- Purified PPI-Pfk proteins from *C. thermosuccinogenes* and *C. thermocellum* were shown to be able to phosphorylate sedoheptulose-7-phosphate (S7P) to SBP.

## Significance

- We provide experimental evidence supporting the theory that the PPI-dependent phosphofructokinase participates in the non-oxidative pentose phosphate pathway in addition to glycolysis
- This finding should help improve the engineering of glycolysis.



SBP and S7P accumulate in cells of *C. thermosuccinogenes* grown on glucose (G) and xylose (X)



Purified PPI-Pfks (solid lines) can convert S7P to SBP *in vitro*; without the added protein (dashed lines) no conversion happens