Metabolic Fluxes of Nitrogen and Pyrophosphate in Chemostat Cultures of *C. thermocellum* and *T. saccharolyticum* may control excess amino acids

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Background

Clostridium thermocellum and *Thermoanaerobacterium* saccharolyticum are organisms of interest for the generation of biofuels from lignocellulose. Their metabolisms are incompletely understood in the role of pyrophospate (PPi) in central metabolism and nitrogen.

Approach

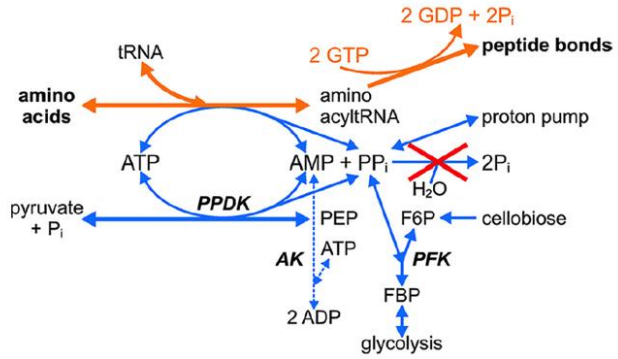
- *C. thermocellum* and *T. saccharolyticum* were grown in cellobiose and nitrogen-limited chemostat cultures. Cell concentration and 27 metabolites were measured to estimate 24 metabolic reactions.

Outcome

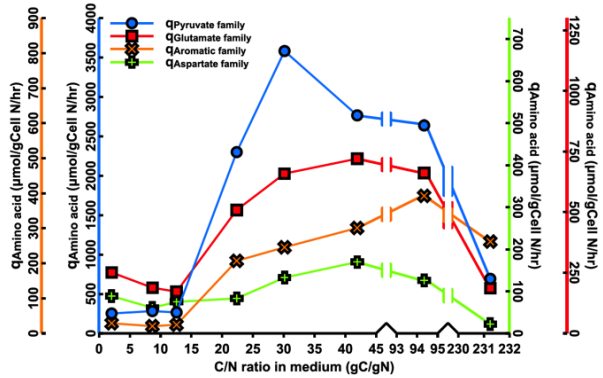
- In *C. thermocellum,* glycolysis proceeds via PPi-dependent phosphofructokinase (PFK), pyruvate-phosphate dikinase (PPDK), as well as a malate shunt for the conversion of phosphoenolpyruvate (PEP) to pyruvate. Pyruvate kinase (PK) activity was not detectable.
- In *T. saccharolyticum*, ATP but not PPi served as cofactor for the PFK reaction. High activities of PK and PPDK were present, whereas the activities of a malate shunt enzymes were low.
- In *C. thermocellum,* glycolysis via PPi-PFK and PPDK obeys the equation: glucose + 5 NDP + 3 PP_i $\Rightarrow$ 2 pyruvate + 5 NTP + P_i. Metabolic flux analysis of chemostat data with the wild type and a deletion mutant of the proton-pumping pyrophosphatase showed that a PPi-generating mechanism must be present that operates according to ATP + P_i $\Rightarrow$ ADP + PP_i.
- Both organisms excrete significant amounts of amino acids in cellobiose-limited cultures. Nitrogen limited cultivation of wild-type *C. thermocellum* revealed a bottleneck in pyruvate oxidation, as large amounts of pyruvate and amino acids, mainly valine, were excreted.

Significance

The fate of PPi is critical in the metabolism of two thermophilic anaerobes that lack a soluble irreversible pyrophosphatase but instead use a reversible membrane-bound proton-pumping enzyme. This may contribute and should provide strategies to reduce the observed secretion of amino acids during cellobiose fermentation and increase ethanol yield.



Fate of pyrophosphate in the formation of peptide bonds in the absence of irreversible PPi hydrolysis. The AMP and PPi produced in the charging of tRNA can be used by PPDK provided that sufficient PEP is converted to pyruvate in the anabolic network.



Specific rates of excretion of amino acids in four families as a function of the C/N ratio during growth on cellobiose and urea by *C. thermocellum*.

THE CENTER FOR BIOENERGY INNOVATION

Holwerda *et al.*, AEM, 2020, doi.org/10.1128/AEM.01795-20.