Re-examining Pyrophosphate Metabolism in Clostridium thermocellum *through Genome-Scale Modeling leads to new Hypotheses*

Background

- The lignocellulolytic anaerobic bacterium *Clostridium thermocellum* possesses an atypical glycolytic pathway that substitutes GTP or pyrophosphate (PP_i) for ATP in some steps, including the energy-investment phase. As such, identification and manipulation of PP_i sources are key to engineering its metabolism. Prior recent *in vivo* efforts to identify the primary PPi source through targeted knockout experiments have been unsuccessful.

Approach

- A highly detailed genome-scale stoichiometric model of *C. thermocellum* metabolism, *i*CTH669, was reconstructed using the latest data, with a particular focus on transport, phosphate, and energy metabolism.
- The feasibility of hundreds of potential PP_i sources were investigated using *i*CTH669 (for yield/stoichiometry), reaction thermodynamics (through eQuilibrator), and available transcriptomics to identify a primary PP_i source.

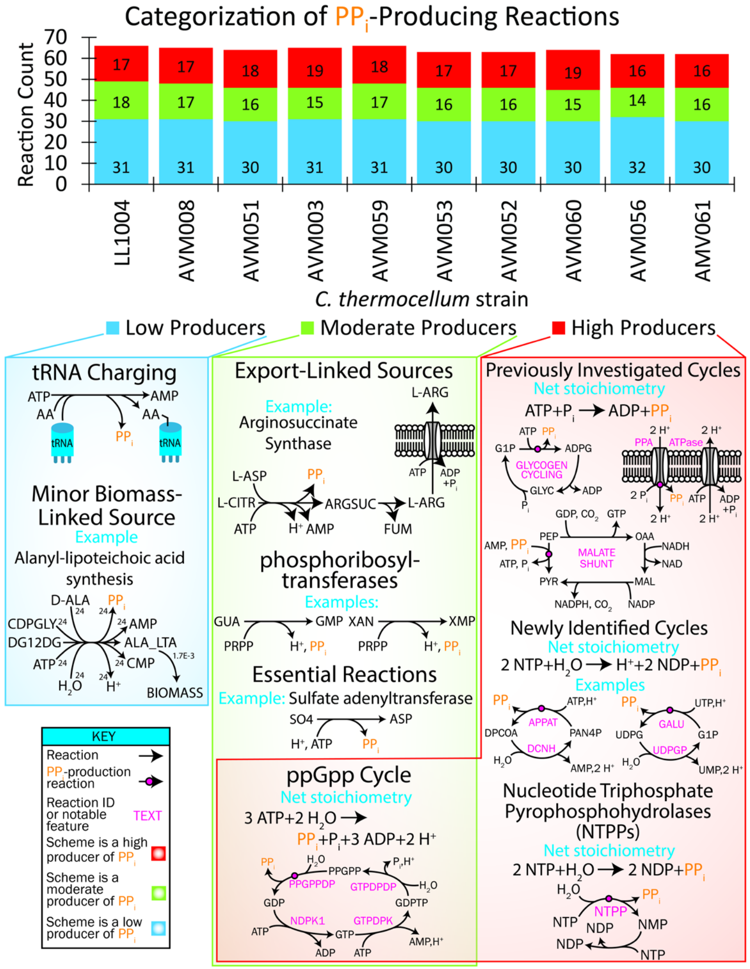
Results

- *i*CTH669 recapitulates biomass and fermentation product yield data across ten knockout strains.
- No clear-cut relationship between PP_i and ethanol yields was observed.
- The new genome-scale metabolic model (GSMM) indicates that the use of PP_i provides no *direct* energy advantage as, at best, its cost is equivalent to ATP.
- The GSMM and data indicate that there exists no "primary" PP_i source in wild-type, rather there are many contributing sources; previously investigated PP_i sources likely account for most PP_i production in wild-type strains. When previously investigated sources are knocked out, alternative metabolic pathways can collectively maintain PP_i levels. Nucleotide triphosphate pyrophosphohydrolysis (NTPP) plays a key role.

Significance

- The thorough re-examination of multiple existing datasets via a new GSMM leads to several new actionable hypotheses for the energy metabolism of *C. thermocellum*, by the elimination of hundreds of previously proposed PP_i sources allowing for systematic investigations by accumulating gene deletions.

Schroeder et al. *Metab Eng* (2023). doi.org/10.1016/j.ymben.2023.04.003.



Graph of reactions capable of producing PP_i in various knockout strains categorized by fraction of need it can meet as low (<5%), medium (5%-70%), and high (>70%). Example illustrations of reactions/cycles included in each category are given.