Gene targets found to improve *Clostridium thermocellum* LL1210 tolerance to acidic pH

Background

- *C. thermocellum* LL1210 is an engineered strain which produces the highest reported ethanol titers and yields, at neutral pH, directly from lignocellulosic feedstocks in Consolidated Bioprocessing (CBP) fermentations.
- The development of CBP fermentations at **acidic pH** would reduce process-costs, as result of decreasing the addition of neutralizing agents, and would enable the co-cultivation with other microbes that lead to process productivity enhancements. Thus, this study aims to understand the pH limits and homeostasis mechanisms of *C. thermocellum* at lower pHs than neutral.

Approach

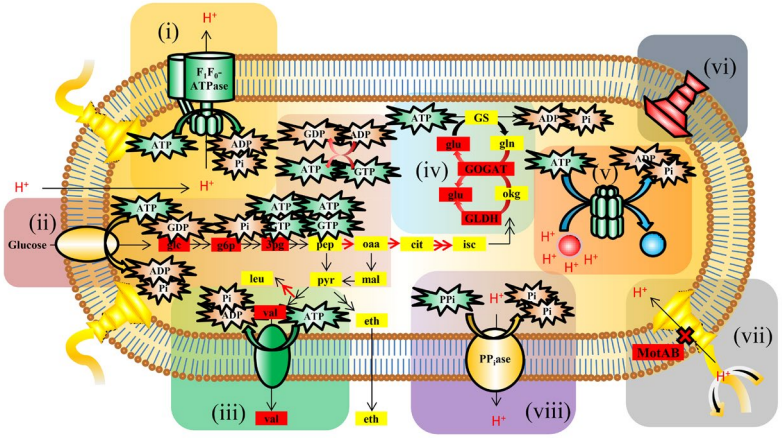
- *C. thermocellum* was grown in a chemostat at different pHs, ranging from 5.9 to 6.98, at various dilution rates. To study metabolic fluctuations, transcriptomic and metabolomic analyses were performed when cell growth reached steady-state at pH 6.15, 6.25, 6.5, and 6.98.

Outcomes

- Chemostat experiments showed bacterial growth limitations at pH $\leq$ 6.24 at a dilution rate of 0.1 h^{-1}.
- Differential **transcriptomic** studies revealed that ATP utilizing enzymes and pathways were downregulated at pH ≤ 6.24.
- **Metabolomic** analyses showed that long-chain fatty acids, glycolysis intermediates and some amino acids accumulated intracellularly at pH ≤ 6.24.
- In addition, genes encoding **proton-pumping PP_i-ases, glutamate decarboxylases, and ureases** are **potential targets** for further metabolic engineering in *C. thermocellum*.

Significance

- This study provides a series of **genetic targets** to further test tolerance improvements to acidic pH in *C. thermocellum* LL1210.
- This foundational dataset will be a valuable source of information to other researchers who investigate pH homeostasis mechanisms and search for targets to enhance the tolerance to lower pH in a variety of organisms.



Overview of the metabolic imbalances and key expression differences at growth-limiting pH values. (i) A proton-pumping F_1F_0-ATPase maintains ΔpH. **(ii)** Glycolysis intermediates accumulate indicating inhibition of flux. **(iii)** Valine transport, **(iv)** glutamine synthetase activity, and **(v)** chaperones consume ATP. ATP is preserved **(vi)** directly via downregulation of flagella biosynthesis and motility and other ATP-utilizing functions, and **(vii)** indirectly by downregulation of proton-channeling motility genes MotAB and **(viii)** ATP-independent PP_iase proton pump. Green and red proteins are the ones whose genes were up and downregulated, respectively – such as the F_1F_0-ATPase. Dark red metabolites were the ones that accumulated. Yellow proteins or metabolites were unchanged.

Whitham JM *et al*. *Clostridium thermocellum* LL1210 pH homeostasis mechanisms informed by transcriptomics and metabolomics. *Biotechnology for Biofuels*. (accepted, 2018)

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