

Systems Level Comparison of Medium Chain Fatty Acid Production

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Project Goals: Our aims are to (1) elucidate the genetic elements that enable members of the Clostridia class of Firmicutes to produce medium chain fatty acids (MCFAs) via reverse beta oxidation and (2) identify environmental conditions that maximize MCFA production by anaerobic microbial communities grown on complex organic residues from different industrial processes.

Medium chain fatty acids (MCFAs), 6-12 carbon saturated monocarboxylic acids, are high-value compounds that can be produced from a variety of industrial residues by fermentative microbial communities. Residues from lignocellulosic biorefineries, starch ethanol plants, and the dairy industry are examples of carbon-rich residues considered low-value co-products that are typically sent to anaerobic digesters for biogas generation [1] or concentrated and sold as animal feed [2]. Following the model of the petroleum industry, diversifying product formation from the primary feedstock can help offset operating costs, reduce the selling point of the primary products (e.g. biofuel), and ultimately make these industries more economically viable [3].

MCFA-producing microbial communities contain microorganisms predicted to perform two main general functions within the communities [4]. One set of organisms hydrolyses and ferments energy-rich substrates such as carbohydrates (monomeric and oligomeric) to low-carbon fermentation products such as acetate, ethanol, and lactate, or to low-carbon intracellular metabolites such acetyl-CoA and propionyl-CoA, while the second group uses these fermentation products to produce higher-carbon products such as MCFA by “chain-elongation” via the reverse beta-oxidation pathway.

We are interested in elucidating the genetic elements necessary for MCFA production by chain elongation to enable accurate predictions of MCFA production using genome-scale metabolic models of Clostridia. We have characterized and described two MCFA-producing Clostridia, *Candidatus Weimeria bifida*, gen. nov., sp. nov., and *Ca. Pseudoramibacter fermentans*, sp. nov. [5], which were enriched in a continuously stirred tank reactor (CSTR) fed conversion residue from a lignocellulosic biorefinery and operated with a 6-day residence time, pH 5.5, and 35°C. Metatranscriptomic analyses of the microbial community predicted that these two organisms had

high expression of gene products in the reverse beta-oxidation pathway, including an electron bifurcating Acyl-CoA dehydrogenase (ACD) and associated electron transfer flavoproteins (EtfA, EtfB). In addition, both organisms were predicted to have energy conserving mechanisms via ion motive force generation using the RNF and the energy conserving hydrogenase (Ech) complexes. The metatranscriptomic data also predicted a difference in preferred organic substrates for both organisms. Whereas *Ca. W. bifida* had high transcript abundance for genes involved in carbohydrate metabolism, *Ca. P. fermentans* had high transcript abundance for genes associated with lactate and glycerol utilization. These primary metabolic features were sufficient to assemble metabolic models representing MCFA production from carbohydrates and lactate, respectively [4].

The ability to enrich for MCFA-producing Clostridia from the same inoculum source (acid digestion bioreactor from wastewater treatment plant) but fed different organic-rich substrates is currently under investigation. Identification of microorganisms using 16S rRNA gene amplicon sequencing of communities enriched on a synthetic xylose-rich medium, on ultra-filtered milk permeate (UFMP) coproducts from the dairy industry, on lignocellulosic biorefinery residues, and on thin stillage (TS) from a starch ethanol biorefinery has revealed high abundance of Clostridia in all enrichments. The synthetic xylose-rich substrate resulted in the enrichment of a *Ca. Weimeria* strain; the UFMP substrate enriched for organisms related to the recently defined *Agathobacter* genus [6] within the Lachnospiraceae and members of the Clostridiales_Incertae Sedis XIII; the lignocellulosic residues enriched for strains of *Ca. Weimeria* and *Pseudoramibacter*; the TS enriched for *Butyrivibrio*, *Dialister*, *Pseudoramibacter*, and Lachnospiraceae in the class Clostridia. Metagenomic analyses of these microbial communities is underway.

Comparative genomic analyses of these enriched members of the Clostridia class of Firmicutes, along with the available genomes for related Clostridia will help us improve metabolic models for MCFA production, which will guide future investigations on how to optimize MCFA yields with either self-assembled or synthetically-created microbial communities.

References

1. Gerbrandt K, et al. 2016. Curr Opin Biotechnol **38**:63–70. doi:10.1016/j.copbio.2015.12.021
2. Lupitskyy R, et al. 2015. Biomass and Bioenergy **72**:251–255. doi:10.1016/j.biombioe.2014.10.029
3. Scarborough MJ et al. 2018. Biotechnol Biofuels **11**:1–17. doi:10.1186/s13068-018-1193-x
4. Scarborough MJ, et al. 2019. bioRxiv **11**:200–36. doi:10.1101/759548
5. Scarborough MJ, et al. 2019. Appl Environ Microbiol **1**–44. doi:10.1128/AEM.02242-19
6. Rosero JA, et al, 2016. Int J Syst Evol Micr **66**:768–773. doi: 10.1099/ijsem.0.000788

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