Characterization of carboxylate producing microbial communities

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HIGHLIGHTS:

- Enriched for Clostridiales and Lactobacillales putatively involved in production of industrially relevant carboxylates, independent of growth feedstock.
- Alteration of operational conditions of bioreactors, e.g. increased temperature, can select for a different profile of fermentation products.
- Two recently described MCFA-producing strains, *Ca.* Weimeria bifida, and *Ca.* Pseudoramibacter fermentans [1], enriched from lignocellulosic residues, have also been identified as prominent community members in the current experiments utilizing different feedstocks.

BACKGROUND: Carboxylic acids, including succinic acid, lactic acid, and medium chain fatty acids (MCFAs), are valuable chemicals that can be produced from a variety of industrial residues by fermentative microbial communities. Residues from lignocellulosic biorefineries (conversion residue; CR), starch ethanol plants (thin stillage; TS), and the dairy industry (ultra-filtered milk permeate; UFMP) are examples of carbon-rich, low-value co-products that are typically sent to anaerobic digesters for biogas [2] or sold as animal feed [3]. Diverse product formation from primary feedstocks can help offset operating costs, reduce the selling point of the primary products (e.g. biofuel), and ultimately make these industries more economically viable [4].

RESULTS & DISCUSSION: The CR-fed bioreactor was stable for over 100 d and produced primarily C4 and C6 fatty acids. The UFMP-fed bioreactor initially produced C6 and C8 fatty acids, and then, shifted to producing primarily butyrate (C4). TS-fed bioreactors produced a mixture of C5-C8 fatty acids. Decreasing the retention time in the TS-fed bioreactor induced a shift to succinate production. Furthermore, increasing temperature of the

TS-fed bioreactor to 55°C induced shifts to lactic and propionic acid as the primary fermentation products. Identification of microorganisms using 16S rRNA gene amplicon sequencing revealed high abundance of Clostridiales and Lactobacillales in communities enriched on CR, TS, and UFMP. The UFMP bioreactor enriched for organisms related to the recently defined *Agathobacter* genus [5] within the Lachnospiraceae. In the TS reactors, *Prevotella* (*phyl.* Bacteroidetes), *Lactobacillus*-relatives, and the Clostridia *Pseudoramibacter* were prominent under MCFA-producing conditions and a disappearance of *Butyrivibrio* occurred when retention time decreased (Figure 1). The Clostridia were absent from the thermophilic TS bioreactor, and *Actetobacter* were abundant in addition to the *Lactobacillus*-relatives. Metagenomic analyses of these microbial communities is underway

CONCLUSION: The ability to enrich for carboxylate-producing taxa from the same inoculum source, but fed variety of organic-rich substrates is promising for the future of sustainable production of commodity chemicals, and is not tied to the residues of a single industry. The ability to adjust bioreactor conditions to control for certain microbial communities could help industries adapt to switches on prices or demand for chemicals produced by fermentation.

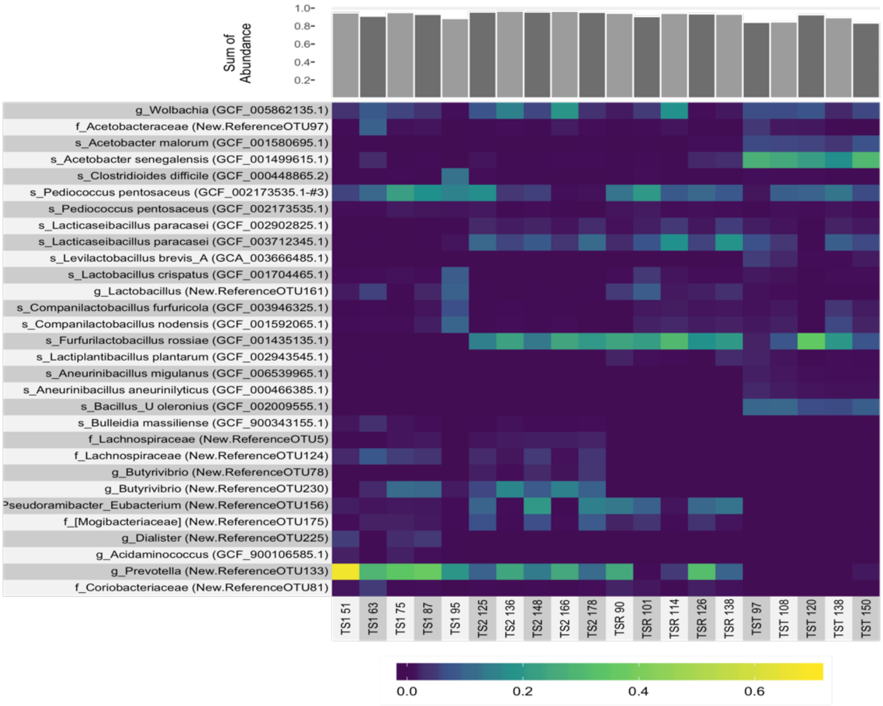

Figure 1. 16S rRNA gene amplicon based operational taxonomic units with greater than 1% relative abundance. OTUs represent microbial community during periods of stable bioreactor conditions (ca. 50 d) for four different bioreactor operational conditions. TS1, unaltered thin stillage, pH 5.5, 35°C, 6 d SRT; TS2, solids-removed thin stillage (SRTS), pH 5.5, 35°C, 6 d SRT; TSR, SRTS, pH 5.5, 35°C, 1 d SRT; TST, SRTS, pH 4.5-5.0, 55°C, 6 d SRT.

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