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Evaluating the performance of carboxylate platform fermentations across diverse inocula originating as sediments from extreme environments

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HIGHLIGHTS

- 501 geographically diverse sediments varied for carboxylate platform performance.
- Multivariate analysis revealed complex relationships among parameters measured.
- Established trade-offs among fermentation outcomes.
- Identified optimal sediment characters for desirable fermentation outcomes.

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ABSTRACT

To test the hypothesis that microbial communities from saline and thermal sediment environments are pre-adapted to exhibit superior fermentation performances, 501 saline and thermal samples were collected from a wide geographic range. Each sediment sample was screened as inoculum in a 30-day batch fermentation. Using multivariate statistics, the capacity of each community was assessed to determine its ability to degrade a cellulosic substrate and produce carboxylic acids in the context of the inoculum sediment chemistry. Conductance of soils was positively associated with production of particular acids, but negatively associated with conversion efficiency. *In situ* sediment temperature and conversion efficiency were consistently positively related. Because inoculum characteristics influence carboxylate platform productivity, optimization of the inoculum is an important and realistic goal.

1. Introduction

The MixAlco™ process, which was developed at Texas A&M University, is an example of the carboxylate platform for biofuel production. It involves the conversion of lignocellulosic biomass into carboxylate salts by fermentation with a mixed microbial community isolated from sediment (Hollister et al., 2011; Fu and Holtzapple, 2010b). Carboxylate platform fermentations produce small-chain (C2–C7) carboxylic acids, which convert to ethanol, gasoline, jet fuel, or industrial chemicals via well-established chemistry (Holtzapple and Granda, 2009). As feedstocks, the carboxylate platform can use many non-food biomass materials including landfill-targeted wastes such as yard clippings and kitchen waste, agricultural residues such as sugarcane bagasse (Fu and Holtzapple, 2011), and industrial byproducts such as paper fines and industrial biosludge (Domke et al., 2004). Furthermore, unlike the more common sugar platform, the carboxylate platform is a non-sterile fermentation process in which an initial mixed microbial inoculum overtakes microbial populations in the feedstock and nutrient sources used in the fermentation, eliminating any energy or material costs associated with sterilization.

In the carboxylate platform, carboxylic acids are buffered to carboxylate salts (Aiello-Mazzarri et al., 2006). Prior to initiating this project, the few attempts to manipulate the inoculum successfully improved fermentation performance of the carboxylate platform. Terrestrial inocula from environments expected to favor rapid degradation of biomass (e.g., compost pile or ruminant gut) (Fu, 2007) were the original microbial communities for the platform. A noteworthy aspect of these early studies of carboxylate platform fermentations is that the productivity of these original

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communities slows as the fermentation reaches high product concentration, a well-established issue in industry (Taylor et al., 2008). Therefore, it seems reasonable to expect that the original terrestrial microbial communities are sensitive to product concentrations. Because the fermentation products are salts, initial attempts to optimize inocula included microbial communities from saline environments. Specifically, switching inocula to a marine community from Galveston Island, TX sediment resulted in more than double acid yield relative to the original terrestrial (non-saline) soil community (Thanakoses et al., 2003a,b). Furthermore, a community from a hypersaline environment (Great Salt Lake, Salt Lake City, UT) boosted performance another 20% relative to the Galveston Island community (Fu, 2007). Thus, it is reasonable to expect that other microbial communities from natural environments with conditions similar to those in industrial fermentations will exhibit continued optimization of fermentation performance.

Microbes found in extreme environments have physiological adaptations that allow them to live normally in adverse conditions, including high temperatures and high salt concentrations (Mesbah et al., 2007; Meyer-Dombard et al., 2005; Porter et al., 2007). As a general rule, industrial bioprocesses operating at higher temperatures run at faster rates, thus providing shorter residence times and greater profitability (Aitken and Mullennix, 1992). Microbes in industrial processes tend to perform optimally at lower product concentrations (Heipieper et al., 2007; Taylor et al., 2008); however, efficient recovery requires high product concentrations. Thus, it seems reasonable to expect that microbial communities from thermal (Rastogi et al., 2010) and high-salinity (Mesbah and Wiegel, 2008) natural environments should possess adaptations that favor superior performance in high-temperature industrial processes that also accumulate high concentrations of salts. Recent studies of mixed microbial communities from soils that degrade lignocellulose (Haruta et al., 2002; DeAngelis et al., 2010, 2012) and from compost (Izquierdo et al., 2010; Sizova et al., 2011) reveal that fermentation-capable communities are stable after prolonged fermentation (Werner et al., 2011) including maintenance of cellulose degradation despite exposure to heat, cold, and sub-culturing (Haruta et al., 2002). Further, a direct study of community acclimation in the presence of elevated salts and ammonia reveal improved waste hydrolysis rates, leading to the suggestion that microbial communities from natural saline environments allow for additional optimization (Wilson et al., 2013).

To address the hypothesis that microbial communities from saline and thermal environments should exhibit improved fermentation performance, samples from saline and thermal sediments across a wide geographic range (Supplemental Tables 1 and 2) were collected and screened for performance in a 30-day batch carboxylate platform fermentation. The capacity of each community to degrade a cellulosic substrate and produce carboxylic acids was measured. Further, with the resulting performance data, the features of particular soil environments that favored the most successful communities were identified. Specifically, a multivariate statistical analysis was performed to examine soil chemistry data and fermentation performance data, including acid profiles and biomass conversion. Using this large-scale analysis of communities, the prediction was that associations among soil characteristics and process performance parameters would inform efforts to optimize the carboxylate platform for producing biofuels and industrial chemicals.

2. Methods

2.1. Study design and site selection

This study was a large-scale effort to examine variation among soil microbial communities as inocula for fermentations in the carboxylate platform. Frequent collection trips were conducted from October 2008 to May 2010. In most cases, at a given geographic location (site) multiple samples were collected; sample locations were chosen based on variation in physical and ecological features or presumed gradients (e.g., moisture, visible salt accumulation, temperature). In total, 501 samples (Supplemental Table 1) from 75 sites (Supplemental Table 2) were collected. Sites were identified via literature, database (Boyd, 2002), internet searches, and by personal communications with site stakeholders.

This study had two stages (hereafter Stage I and Stage II) with distinct site selection criteria and fermentation experimental conditions (Supplemental Tables 1 and 2). In Stage I of this study, sampling sites were from within the southern central region of the United States with a history of salt accumulation or commercial salt production and/or sites known to be high in total dissolved solids (TDS). Stage I involved evaluating 102 inoculum samples from four collection trips to 17 sites conducted in 2008 (Supplemental Table 2). In Stage II of this study, site selection criteria were expanded to include greater ecological and geographic diversity and specific addition of sites with thermal features (Supplemental Table 2). Stage II involved evaluating of 399 inoculum samples from 58 sites and 14 collection trips across the continental United States, Puerto Rico, and Hawaii conducted in 2009 and 2010 (Supplemental Table 2).

2.2. Sediment sampling

In most cases, a single sample involved collecting three adjacent cores using a standard garden bulb-planting tool to a depth of 10–12 cm and with a width of 6.5 cm. Sediment temperatures (Splashproof Thermometer, VWR, PA, USA) at a depth of 5 cm were recorded. Each of the three cores was sealed in a separate zip-top plastic bag with the air removed. As soon after collection as possible, one core from each sample was flash frozen with dry ice and subsequently stored at −80 °C for use in future studies. The remaining two cores were vacuum-sealed (Sunbeam Products, Inc., FoodSaver Model V2220, DE, USA) and stored in insulated coolers allowing them to reach ambient temperature during transport to the lab. These ambient-temperature cores were used as inoculum sources for fermentation and as material for sediment characterization.

2.3. Sediment characterization

Upon return to the laboratory and immediately prior to inoculating the fermentation vessel, one sample core was homogenized by hand. Approximately 30 cm^3 (or around two tablespoons) of homogenized sediment mixture was used to measure volatile solids and moisture content and followed the method of Fu and Holtzapple (2010a). Additionally, sufficient sediment from this homogenized core was submitted to the Soil, Water, and Forage Testing Laboratory at Texas A&M University for chemical and physical characterization following the methods described by Hollister et al. (2010a). As long as the sample size permitted, these soil samples were analyzed for the following: (1) electrical conductivity of soluble salts; (2) soil water pH; (3) detailed salinity measures of potassium, calcium, magnesium, and sodium; (4) plant-available phosphorus and sulfur; (5) analysis of organic carbon, total carbon, total nitrogen; and (6) texture. All sediment remaining after this procedure was stored under vacuum-seal at 4 °C for use in further studies.

2.4. Fermentation experiments

Table 1 details the fermentation screens employed in Stage I and Stage II. Because the fermentation broth contained $\geqslant$2% carboxylate salt concentration, both approaches evaluated inoculum