49. Halophilic Communities as a Source for Novel Lignocellulolytic Enzymes

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Project Goals:
Characterize halophilic communities from saline environments as sources of novel halophilic microorganisms, genes, and enzymes for biofuel feedstock deconstruction.

Selectively enrich microbial populations from complex microbial communities from saline environments on biofuel feedstocks (Miscanthus, Pine and Eucalyptus) to obtain candidates potentially capable of deconstruction of feedstocks under high salinity conditions.

Describe the metabolic potential and gene expression patterns in both natural saline communities and feedstock enrichments by sequencing and screening of metagenomes, metatranscriptomes and metaproteomes.

Use functional metagenomics to express a library of genes that potentially represent novel mechanisms for deconstruction of biomass that are currently underrepresented in gene catalogues.

Formulate (by synthesis, cloning and expression of genes characterized above) and verify activity of a cocktail of halophilic enzymes for deconstruction of biomass in the presence of ionic liquids.

Lignocellulose presents a challenge to next generation biorefineries due to its recalcitrance to microbial degradation. Ionic liquid (IL)-based pretreatment has been successful in preparing biomass for enzyme saccharification, but the most common ILs used for pretreatment inhibit many downstream enzymatic and microbial processes mediated by mesophilic enzymes. Halophiles, by definition, are adapted to high-salt environments and are thus a potential source for IL tolerant enzymes. Here we sought to discover & recover novel lignocellulolytic enzymes from environmental and feedstock-enriched halophilic bacterial communities. We collected both liquid and sediment samples from different saline environments in Puerto Rico and San Francisco including salt flats, saltern ponds and turtle grass beds. For each of the environmental samples we obtained 16S rRNA gene sequences, metagenomes and metaproteomes. The data revealed an increase in relative abundance of haloarchaea and genes and proteins implicated in a hypersaline lifestyle with increasing salinity. In addition, a fosmid library was constructed in an expression vector for high throughput functional metagenomics screening using the robotics platform at JBEI.

Samples from a turtle grass bed (3.5% salinity) and a high salinity saltern pond (33.2% salinity) were selected for enrichment on the potential biofuels feedstocks: miscanthus (M), eucalyptus (E) or pine (P) under aerobic and anaerobic conditions and followed through three 2---week passages. At the end of each passage cells were harvested, specific enzyme activities were measured and DNA, RNA and proteins were extracted. Data collected include enzyme activities for B glucosidase, cellobiohydrolase and xylanase, 16S rRNA gene sequences, metagenomes metatranscriptomes and metaproteomes. We found that enzyme activity was typically highest after the first passage, with the aerobic turtle grass enrichments having consistent activity on each feedstock. After the third passage, metagenomes were constructed and binned using MaxBin, a binning algorithm developed at JBEI. Bins were subsequently searched against the CAZY/dbCAN HMMs. In addition, expressed transcripts from 11 metatranscriptomes were identified by either alignment to the reference metagenomes or *de novo* assembled. To date we have identified over 1000 expressed candidate carbohydrate active enzymes from the enrichments and obtained reconstructed genomes for >100 feedstock-enriched archaea/bacteria. Heterologous expression of a diverse collection of 29 putative glycoside hydrolases is ongoing. The next step will be to validate and incorporate these candidate enzymes into a halophilic deconstruction enzyme mixture with high activity and IL tolerance.

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