Analyzing Environmental Microbes for Genomic Regions Promoting Ionic Liquid Tolerance in E. coli

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ABSTRACT

Ionic liquids (ILs) are promising as solvents to increase the efficiency of biofuel production; however, ILs are toxic to microbes used in the fermentation of liquid fuels. To engineer IL resistant biofuels, environmental bacteria were screened for tolerance, and these were used to create gene libraries to test in E. coli. Future characterization of these libraries using molecular techniques will be used to identify genes that contribute IL-tolerance to transformed microbes.

BACKGROUND

The tough cell walls that protect plants in nature also make it difficult to produce biofuels from carbon sequestering crops like switchgrass. The crystalline structure of lignocellulose in cell walls provides resistance to chemical or biological degradation, even after grinding the tissue into fine particles (1). Ionic liquids (IL) are a class of salts that are liquid below 100°C, and some of these are capable of disrupting hydrogen bonds in the cell wall. IL pretreatment of biomass followed by enzymatic digestion of the cell wall offers a means of accessing sugars within the biomass (2). After pretreatment and washing, the biomass may contain between 1% to 5% of residual IL. This makes it problematic to isolate DNA between 30-50 kb for fosmid library construction.

RESULTS & CONCLUSIONS

Genomic regions that are found to be common among IL tolerant transformants expressing a gene library can be used to identify promising individual genes or groups of genes that promote IL tolerance. Further analysis of candidate genes can be extended using transcriptomics such as microarray or RNA-Seq, or through tracking growth in transformed bacteria using GFP-fluorescence.

For each library, clones (bars) selected by E. coli growth on 2% IL containing plates will be sequenced from fosmid insertion sites. Any common genomic regions among IL tolerant transformants will be further analyzed to identify individual genes or groups of genes (blue regions) able to confer IL tolerance.


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