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U.S. Department of
Transportation



Project Title: *Development of Enrichment Cultures that Degrade Lignin for Enhanced Biofuel Production*

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Project Goals

The overall objective of this project was to develop bacterial enrichment cultures and assess their potential to degrade lignin for enhanced bioavailability of cellulose and hemicellulose in plant residues. The long-term goal of this study is to identify novel enzymes and express them in a heterologous system.

Project Outcomes

- Four enrichment cultures including the Cow creek (CC), Yellowstone (YS), Greenstream (GS), and Rumen-Termite (RT) were developed that degraded lignin as the sole source of carbon.
- Analysis of 16S rRNA clone libraries generated from genomic DNA of the enrichments showed a large percentage of clones belonging organisms known to degrade aromatic compounds including members of the order Pseudomonadales, Burkholderiales, Bacillales, Xanthomonadales, Spingomonadales, and Actinobacteria.
- All enrichment cultures grew well on lignin as well as lignin-derived monomers and dimers as the sole sources of carbon.
- Ring-cleaving genes: catechol 1, 2- dioxygenase, catechol 2, 3- dioxygenase, and protocatechuate 3, 4 dioxygenase were amplified from the genomic DNA using degenerate primers & confirmed by cloning and sequencing PCR products.
- Cow Creek and Yellowstone enrichments degraded significant amount of lignin in alfalfa within 30 days and almost complete degradation within 80 days. On the other hand, degradation of lignin in switchgrass was minimal (9 to 15%) and occurred at slow rates.

Conclusion:

Bacterial enrichments established from various decaying biomass sources were dominated with aromatic compound degrading bacteria.



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Photo courtesy of Oklahoma State University