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U.S. Department
of Transportation



Title: Discovery of Novel Lignin-Degrading Genes in Bacteria Using Metagenomic and Proteomic Approach for Enhanced Saccharification of Plant Biomass

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Project Goal

The primary goal of this research is to identify novel genes and enzymes in bacteria that have high potential to be useful for lignocellulose bioconversion when introduced into an appropriate expression system for enzyme production. The objectives are: (i) Perform in-silico analysis of Yellowstone metagenome and generate a protein database, (ii) Identify extracellular proteins in cultures grown on lignin as the sole carbon source, and (iii) Express and purify lignin-degrading enzymes using a recombinant production system.

Project Outcomes

- A lignin degrading bacterial consortium was developed from decaying wood from a thermal pond in Yellowstone National Park (YS-Enrichment). This enrichment is capable of degrading native lignin in alfalfa and switch grass.
- YS Metagenome revealed an inventory of lignin degrading genes including laccase, peroxidase, chloroperoxidase, C-alpha dehydrogenase, beta-etherase, feruloyl esterase, carboxylesterase, vanillate demethylase, vanillyl-alcohol oxidase, vanillin dehydrogenase, syringate O-demthylase, and many genes for ring oxidation and cleavage.
- YS Metagenome revealed a high abundance of sequences related to Proteobacteria, Actinobacteria, and Firmicutes.
- *Pseudomonas* sp., *Rhizobium* sp., *Acromobacter* sp., *Bravibacillus* sp., and *Novospingobium* sp., capable of growing solely on lignin and lignin-derived compounds were isolated from the enrichments.
- Genome analysis of the pure cultures revealed the presence of lignin degrading genes
- Secretome analysis of YS-enrichment as well as pure cultures grown on switchgrass or alfalfa detected lignin degrading extracellular enzymes.
- **Conclusion:** Bacteria have the genes/enzymes that degrade lignocellulose.



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Oklahoma State
University - *Microbiology
and Molecular Genetics*

Funded: \$75,000

Start Date: 07/01/11

End Date: 06/30/13

Other Sources of

Funding: Oklahoma State University covered the cost sharing portion of this award.