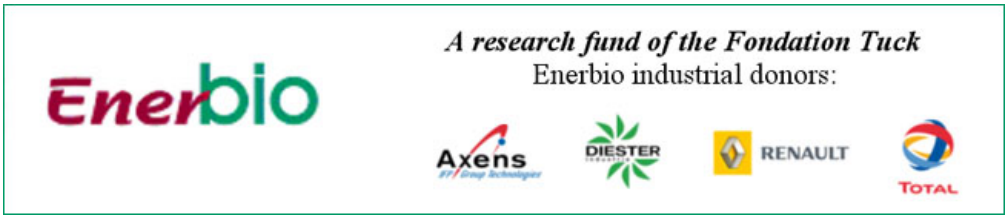
Isolation of environmental lignin-degrading bacteria and identification of extracellular enzymes



THESE 2009

Title of the project	Isolation of environmental lignin-degrading bacteria and identification of extracellular enzymes
PhD student	Charles Robert Taylor
University-Doctoral school	University of Warwick, Coventry CV4 7AL, UK
Supervisor	Dr. Timothy D H Bugg, Professor of Biological Chemistry, Department of Chemistry, University of Warwick
Laboratory of welcome	- Department of Chemistry, University of Warwick, UK
Responsible for thesis	- Timothy D H Bugg, Professor of Biological Chemistry, Department of Chemistry, the University of Warwick
Duration	Three years (end 2009 to end 2012). PhD thesis submitted to the University of Warwick on 21st December 2012

Summary

Breakdown of the lignin aromatic polymer component of lignocellulose is a major limitation in second generation bioethanol production from lignocellulose. Novel lignin-degrading enzymes or novel bacterial strains that can break down lignin could be used as a bacterial pretreatment for second generation bioethanol production, thereby increasing the bioavailability of cellulose, while requiring less energy input, and at the same time generating useful small molecule aromatic chemical by-products.

The objectives of this project are:

To identify novel lignin-degrading bacterial strains, and purify novel lignin-degrading enzymes
To use directed evolution to optimize the catalytic activity, thermostability, and selectivity of lignin-degrading enzymes
To examine whether the lignin-degrading enzymes, or the parent bacterial strains, can be used to convert lignin into high-value aromatic chemicals

Results

Two recently developed assay methods have been optimized for the high-throughput screening of environmental bacteria for activity towards lignin degradation. Seven mesophilic lignin-degrading strains have been isolated from woodland and heathland soils, and three moderately thermo-tolerant strains from

composted wheat straw. The most active was *Sphingobacterium sp.*, approximately ten-fold more active than the other strains.

The strains isolated in this project were found to be capable of depolymerizing high-molecular weight forms of Kraft lignin into lower-molecular weight material.

Significant variations between the abilities of the different strains to metabolise selected aromatic carbon sources and cellulose were observed: *Sphingobacterium sp*. and *Rhodococcus erythropolis* were found to grow well on biphenyl, vanilic acid and veratryl alcohol whilst most of the other strains appeared to be more selective, growing well on only one or two of the carbon sources. *Sphingobacterium sp*., *R.erythropolis* and *Microbacterium phyllosphaerae* were found to be capable of degrading wheat straw, Organosolv lignin and Kraft lignin into soluble and insoluble phenolic compounds.

These organisms produce a variety of enzymes that are directly or indirectly linked to lignin degradation. Four different forms of superoxide dismutase and an oxidoreductase have been identified from the culture supernatant of *Sphingobacterium*. Future work is required to develop our understanding of these enzymes in the context of lignin degradation. Cloning and expression of genes for superoxide dismutase is currently being carried out in the lab of Pr. Bugg. Ultimately, purified lignin-degrading enzymes from the strains isolated in this project could be commercially applied to the pretreatment of lignocellulosic biomass and more specifically to the degradation of lignin.

Livrables

Manuscript of the thesis

Publications

Development of novel assays for lignin degradation: comparative analysis of bacterial and fungal degraders. M.Ahmad, C.R. Taylor, D. Pink, K.Burton, G.Bending and T.D.H. Bugg, Molecular Biosystems, 2010, 6, 815-821

Isolation of bacterial strains able to metabolize lignin from screening of environmental samples. C.R. Taylor, E.M. Hardiman, M. Ahmad, P.D. Sainsbury, P.R. Norris and T.D.H. Bugg. Journal of Applied Microbiology, 2012, 3, 521-530

Contact

Timothy D H Bugg
University of Warwick

Coventry CV4 7AL

United Kingdom
t.d.bugg@warwick.ac.uk