Project title: Understanding microbial lignin degradation in soil

Host institution: University of Warwick

Theme: Organisms and Omics

Key words: lignin degradation, soil bacteria, enzymes

Supervisory team: Prof Timothy DH Bugg, Department of Chemistry, University of Warwick, T.D.Bugg@warwick.ac.uk; Prof Elizabeth MH Wellington, School of Life Sciences, University of Warwick, E.M.H.Wellington@warwick.ac.uk

Project Highlights

- Understand microbiology of lignin degradation in soil
- Identify novel lignin-degrading bacteria and enzymes
- Application to resource recovery from landfill waste

Overview

The lignin heteropolymer in plant cell walls is highly resistant to microbial breakdown, but a number of bacteria and fungi have been identified that can metabolise lignin. Recent studies in TDHB’s group have identified about 20 lignin-degrading bacteria, found mostly in the alpha- and gamma-proteobacteria and actinobacteria. Several enzymes responsible for lignin oxidation have been identified: DyP-type peroxidases with activity for lignin oxidation have been identified in Rhodococcus jostii and Pseudomonas fluorescens, while an unusual extracellular manganese superoxide dismutase enzyme with activity for lignin oxidation has been identified in Sphingobacterium sp. However, genome sequencing of lignin-degrading bacteria has revealed a diversity of lignin-degrading enzymes, raising questions about how lignin degradation takes place in soil:

1. Do communities of bacteria operate together to break down lignin in soil? Since lignin is a very complex and inert substrate, and different lignin-degrading bacteria produce different lignin-oxidising enzymes, it seems likely that consortia or whole communities of bacteria work together to attack lignin. Do communities of bacteria in soil share extracellular enzymes to oxidise lignin? What types of bacteria are present in these consortia? Are there specialists for different substructures present in lignin?

2. Are there different types of lignin-degrading communities present in aerobic topsoil vs microaerobic subsoil? Are there different types of bacteria found in different scenarios, or common features? Are similar types of enzyme used to attack lignin? Recent data has shown that lignin-degrading facultative anaerobes can promote gas generation in soil, by anaerobic methanogenic bacteria, so are there interactions between lignin-oxidising bacteria and methanogenic bacteria in subsoil?

3. Can consortia of lignin-degrading bacteria be used to promote lignin breakdown in landfill soil, and hence assist resource recovery from landfill waste?

Methodology:

1. We will use ‘omics techniques to study communities of lignin-degrading bacteria in soil. Soil samples from a range of environments will be enriched by growth on minimal media containing lignocellulose or lignins, and then the bacterial population analysed by metagenomic DNA sequencing. The extracellular lignin-degrading enzymes will be analysed by proteomic analysis of extracellular proteins.
2. Sampling of soil from different depths of soil, followed by growth on aerobic vs micro-aerobic conditions will be carried out in order to assess the consortia formed under aerobic and micro-aerobic conditions. The metabolic fate of lignin carbons will be assessed by preparation of specifically 13C or 14C-labelled lignins, followed by incubation with lignin-degrading consortia, and sampling of biomass vs gaseous fractions.

3. Lignin-degrading consortia will be applied as a pretreatment for lignocellulose in soil, and tested for a) enhanced gas release b) improved anaerobic degradation capability.

Training and skills
The project will provide training in:
- **Environmental microbiology** – environmental sampling, culturing & isolation of novel microorganisms, identification via 16S rRNA sequencing
- **Molecular cloning techniques** – gene cloning, polymerase chain reaction, construction of DNA libraries
- **‘Omics techniques** – metagenomic DNA sequencing, proteomic analysis of secreted enzymes, bioinformatics analysis
- **Enzymology & applied microbiology** – expression & purification of recombinant enzymes, enzyme kinetics, application of recombinant enzymes & bacterial strains for delignification of lignocellulosic biomass

Partners and collaboration (including CASE)
Through the NERC-funded project INSPIRE (In situ processes in resource extraction), TDHB has links to the groups of Dr Devin Sapsford & Dr Peter Cleall at Cardiff University with interests in resource recovery from landfill waste. Industrial partners in the INSPIRE project (Ricardo-AEA, CIRIA), and Clearfleau Ltd interested in anaerobic digestion applications, may be interested in collaborating as CASE partners.

Possible timeline
**Year 1:** Sampling of environmental samples, identification of lignin-degrading bacteria and consortia
**Year 2:** ‘Omics analysis of environmental samples, identification of bacterial lignin-degrading communities and extracellular lignin-degrading enzymes
**Year 3:** Application of lignin-degrading bacteria and consortia for delignification of lignocellulosic biomass. Characterisation of novel recombinant lignin-degrading enzymes.

Further reading

Further details
Applicants from the UK or the EU are eligible. Applicants should hold a BSc and/or MSc degree in relevant subjects. Informal enquiries can be made to Prof Tim Bugg (T.D.Bugg@warwick.ac.uk).

Details of how to apply can be found at [https://warwick.ac.uk/fac/sci/lifesci/study/pgr/studentships/nerc-centa/](https://warwick.ac.uk/fac/sci/lifesci/study/pgr/studentships/nerc-centa/)