METAGENOMIC ANALYSIS OF MICROBIAL COMMUNITIES ASSOCIATED WITH ACUTE OAK DECLINE IN THE UK

Melanie Sapp[†*], John Elphinstone[†], Richard Thwaites[†], Neil Parkinson[†], Susan Kirk[‡] Gavin Hunter[‡] and Sandra Denman[‡*]

[†]*Food and Environment Research Agency (Fera), Sand Hutton, York, YO41 1LZ, UK*
[‡]*Forest Research (FR), Alice Holt Lodge, Gravel Hill Road, Farnham, GU10 4LH, UK*

*Melanie.sapp@fera.gsi.gov.uk ; sandra.denman@forestry.gsi.gov.uk

A novel metagenomic approach was used to supplement investigations into the causes of an episode of acute oak decline (AOD) which has spread rapidly through the Midlands and South East England since 2006. DNA extraction and next generation (Roche 454) amplicon sequencing procedures were optimised to identify potential plant pathogens and insect pests exclusive to symptomatic oak tissues. Symptoms included lesions in the inner bark which also often extend to surrounding galleries left by boring larvae of the buprestid beetle *Agrilus biguttatus*. In this study primers targetted bacteria (16S rRNA gene; Gyrase B (*GyrB*) and DNA-J genes), fungi (ITS) and insects (Co-A). In the initial investigation, two healthy and symptomatic trees each, were identified at a site in Oxfordshire and Berkshire, central England. After tree felling, equivalent sets of symptomatic and asymptomatic tissue pieces were dissected. One set was processed by conventional isolation on a large number of selective and general culture media plating pieces from the inner bark, outer bark, sapwood, heartwood and the larval galleries, which were thought to be created by *A. biguttatus*. The second set of tissues was used for DNA extraction and next generation (Roche 454) amplicon sequencing. Work presented here reports bacterial results only. The bacterial community structure was assessed based on sequence diversity in the V1-V3 region of the 16S rRNA gene. Clustering and BLAST analyses were used to compare sequences with those in the curated Ribosomal Database Project (RDP) and *gyr*B sequences was used as an alternative taxonomic marker. 16S rRNA gene sequencing resulted in 119,757 sequences compared with 73,566 *gyr*B sequences. Clustering and BLASTn analyses with those in the curated Ribosomal Database Project (RDP) were carried out. The results indicated that bacterial diversity was higher in symptomatic than in healthy tissue. The families *Sphingobacteriaceae*, *Pseudomonadaceae*, *Enterobacteriaceae* and *Rhodobacteraceae* were represented. Where similarities to known genera could be established, it appeared that most bacteria were of likely environmental origin, many associated with soil, water or woodland plants. Of most interest to date are the potential oak pathogens *Gibbsiella quercinecans* and *Brenneria goodwinii*, (*Enterobacteriaceae*), found only in symptomatic tissues at different sites, and which were also isolated from the AOD-affected tissues. Baseline data on bacterial communities present in healthy oak tissue are also emerging, of which there is little published information available to date. Our results showed the presence of *Acetobacteraceae* in particular. Similar approaches are being developed to assess fungal community diversity in diseased and healthy oak tissues according to Internal Transcribed Spacer (ITS) sequence diversity.

Keywords: Acute oak decline, metagenomics, microbial communities