

Taxonomy and phylogeny of bacteria associated with Acute Oak Decline (AOD)

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An episode of Acute Oak Decline (AOD) has recently been identified in Britain on native oak (*Q. robur* and *Q. petraea*) and has a rapid effect on tree health. Affected trees are characterized by extensive bleeding of dark, sticky exudates from vertical cracks between the bark plates. Tissues underlying the stem-bleed are stained and necrotic. A similar condition has been noted on Mediterranean oak (*Q. pyrenacia* and *Q. ilex*) in Spain, on *Q. robur* in Belgium, France, Germany, Poland and the Czech Republic and on black oak (*Q. kelloggii*) in the USA. Since 2008, numerous bacterial strains, representing several novel species, have been isolated from necrotic lesions and fluid exudates of symptomatic trees in Britain. The most frequently isolated species belong to *Gibbsiella quercinecans* and *Brenneria goodwinii*, both identified as novel species and in the case of *Gibbsiella*, a novel genus. The majority of bacterial strains isolated from symptomatic oak are Gram-negative and belong to the family *Enterobacteriaceae*. Species belonging to this family are typically difficult to identify and classify, owing to a high degree of both phenotypic and phylogenetic similarity. Partial sequencing of the 16S rRNA gene can possibly assign strains to the correct genus, but this gene lacks resolving power at the species level. In contrast, MLSA (multilocus sequence analysis), based on partial sequencing of 3-5 protein-encoding genes, is a robust and reliable method for identification, classification and phylogenetic studies of the *Enterobacteriaceae*. Symptomatic oak samples are currently screened by sequencing a single gene, *gyrB*, which is commonly included in MLSA schemes. Phylogenetic analyses of the *gyrB* sequences have indicated that the bacteria recently isolated from symptomatic oak may belong to several additional novel species within the *Enterobacteriaceae*. The aim of this study was to characterize the strains recently isolated from symptomatic oak in the UK and USA using a polyphasic approach based on MLSA of partial *gyrB*, *rpoB*, *infB* and *atpD* sequences, DNA relatedness studies and phenotypic assays. Phylogenetic trees generated from the concatenated MLSA data revealed that the strains form seven discrete clusters, five within the *Rahnella* clade and one each in the *Gibbsiella* and *Brenneria* clades, suggesting that these strains constitute seven novel species. Initial DNA relatedness data confirm the phylogenetic results and support the proposal of novel species. Additionally, the potential novel species from each genus can be differentiated from their closest phylogenetic neighbours on the basis of several phenotypic characteristics. Following further DNA relatedness tests, seven novel species will be formally described and classified in three genera in the family *Enterobacteriaceae*. The role of these novel species in the current episode of AOD has yet to be determined.

Keywords: oak, Acute Oak Decline, *Gibbsiella*, *Enterobacteriaceae*