

Taxonomy of Bacteria Associated with Acute Oak Decline

Carrie Brady¹, Ilse Cleenwerck¹, Sandra Denman², Stephanus Venter³, Teresa Coutinho³ and Paul De Vos¹

¹ BCCM/LMG Bacteria Collection, Ghent University, Belgium ² Forest Research, United Kingdom

³ University of Pretoria, South Africa

Background

There are very few bacterial pathogens reported to cause disease on oak. Recently a novel genus and species, *Gibbsiella quercinecans* (Brady *et al.*, 2010), were described to accommodate enterobacterial strains isolated from native oaks in Britain and Spain suffering from acute oak decline (AOD). The exact role played by *G. quercinecans* in the current episode of AOD in Britain is presently under investigation. Other isolates, recovered in lower numbers together with *G. quercinecans* from the infected oak tissue, were tentatively identified as *Brenneria quercina* (the causal agent of drippy nut disease) based on 16S rRNA gene sequencing. Because of the polyphyletic nature of *Brenneria*, it was not possible to assign these strains to a species using 16S rRNA gene sequencing. Following the successful resolution of taxonomic issues within *Pantoea* and *Tatumella*, multilocus sequence analysis (MLSA) was performed on all *Brenneria* species and oak isolates from Britain and Spain to determine their taxonomic position within the family *Enterobacteriaceae*.

Methods

MLSA, based on partial sequences of the housekeeping genes *gyrB*, *rpoB*, *infB* and *atpD*, was performed on the British and Spanish oak isolates and on reference strains of the species belonging to the genera *Brenneria*, *Pectobacterium* and *Samsonia* (Brady *et al.*, 2008). Maximum likelihood trees were constructed in PhyML for each of the four housekeeping genes, and for the concatenated sequence data of all four genes using the models and parameters determined by Modeltest. Bootstrap analysis with 1000 replicates was performed on all trees to assess the reliability of the clusters generated.



Figure 1: Maximum likelihood tree based on concatenated partial sequences of *gyrB*, *rpoB*, *infB* and *atpD* of strains from species of *Brenneria*, *Pectobacterium*, *Samsonia* and oak isolates from Britain and Spain.

Results

In the maximum likelihood tree based on concatenated multilocus sequencing data, the validly published species of *Brenneria* and its closest phylogenetic neighbours, *Pectobacterium* and *Samsonia*, are clearly differentiated. The oak isolates from Britain form two clusters while the Spanish oak isolates are contained in another cluster, all with strong bootstrap support of 100 %.

The larger cluster of British oak isolates falls within the *Brenneria* clade indicating that these strains belong to a novel species. This has been confirmed with DNA-DNA hybridizations and the name *Brenneria goodwinii* is proposed for this species. The second cluster of British oak isolates groups with the Spanish oak isolates and *B. quercina* reference strains. This clade is on a separate branch far removed from the remaining *Brenneria* species, suggesting that *B. quercina* should be transferred to a novel genus as these strains are phenotypically and biochemically different to other *Brenneria* species. The DNA-DNA hybridization values between *B. quercina* strains and the British and Spanish oak isolates are on the border of the species delineation cut-off point and can therefore not be described as novel species. It is proposed to transfer *B. quercina* to a novel genus, *Lonsdalea*, as *Lonsdalea quercina* with two novel subspecies, *Lonsdalea quercina* ssp. *britannica* and *Lonsdalea quercina* ssp. *iberica* to accommodate the oak isolates from Britain and Spain respectively.

Conclusions

MLSA can clearly differentiate species of *Brenneria*, *Dickeya* and *Samsonia* and confirmed the existence of a novel species and two novel subspecies. Additionally, the phylogenetic position of *B. quercina* was re-examined and found to be distant to the genus *Brenneria*. This study has demonstrated the diversity of enterobacteria isolated from oak suffering from AOD. The role these bacteria play in AOD must still be elucidated.

References

- Brady, C.L., Cleenwerck, I., Venter, S.N., Vancanneyt, M., Swings, J. & Coutinho, T.A. (2008). Phylogeny and identification of *Pantoea* species associated with plants, humans and the natural environment based on multilocus sequence analysis (MLSA). *Syst Appl Microbiol* **31**, 447-460.
- Brady, C.L., Denman, S., Kirk, S., Venter, S.N., Rodríguez-Palenzuela, P. & Coutinho, T.A. (2010a). Description of *Gibbsiella quercinecans* gen. nov., sp. nov., associated with Acute Oak Decline. *Syst Appl Microbiol* **33**, 444-450.