Development of a real-time PCR assay for the identification of *Brenneria goodwinii,* associated with Acute Oak Decline (AOD) of *Quercus* spp. in the United Kingdom

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Acute Oak Decline (AOD) is a complex disorder of native Oak species, *Quercus robur* and *Q. petrea* in England. Recent studies have identified two Enterobacterial species, *Gibbsiella quercinecans* and *Brenneria goodwinii* and a buprestid, *Agrilus biguttatus* associated with AOD. Over the past 5 years, reports submitted to Forest Research Advisory Service revealed that the prevalence and distribution of AOD in England has steadily increased. Techniques used to identify the bacteria associated with AOD rely on culturing techniques and DNA sequencing of informative housekeeping genes. However, these techniques are labour intensive and expensive. The aim of this study was therefore to develop a rapid, specific and reliable molecular diagnostic assay for the identification of *B. goodwinii*. In order to achieve this, a large and comprehensive DNA sequence dataset based on gyraseB gene sequences was established for species of *Brenneria* and related genera within the *Enterobacteriaceae*. This DNA sequence dataset was used to identify potential species-specific DNA sequence targets for *B. goodwinii*. Using a commercial facility (PrimerDesign) *B. goodwinii* specific oligonucleotide primers and fluorescently labeled hydrolysis probes were developed. To validate their specificity for *B. goodwinii* a series of specificity tests were conducted on the LightCycler® real-time PCR platform using DNA from representative species of *Brenneria* including *B. alni*, *B. nigrifluens*, *B. rubrifaciens*, *B. salicis*, and *Brenneria sp. nov.* for comparison. Additionally, the limit of detection (LOD) and quantification framework for the assay was developed by preparing standard curves based on *B. goodwinii* samples of known DNA concentrations. Results from these studies indicate that the primers and hydrolysis probe have good specificity toward *B. goodwinii* and that the assay is able to detect very low amounts of *B. goodwinii* DNA. It is envisaged that the assay will be put into routine diagnostic use within the laboratory of Forest Research (FR) and will be added to the existing molecular diagnostic pipeline employed by FR staff in order to identify forestry pathogens.

Keywords: *Brenneria*, Enterobacteriaceae, Gammaproteobacteria, Hydrolysis probes, LightCycler, molecular diagnostics, real-time PCR.