Evaluation of a commercial semi-selective culture medium for detection and isolation of putative necrogenic *Enterobacteriaceae* associated with Acute Oak Decline

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First described in 2009, Acute Oak Decline (AOD) is a disorder of native Oak (*Quercus robur* and *Q. petraea*) in the UK. To determine possible causes, comparative investigations to elucidate putative fungal and bacterial causal agents were carried out on healthy and symptomatic trees. Pieces of symptomatic and non-symptomatic tissue were plated onto Proteose Peptone Yeast Glucose agar (PYGA) to isolate bacterial flora. PCR amplification of the Gyrase B gene region followed by DNA purification and sequencing enabled the identification of strains using BLAST®. Many bacterial taxa, both Gram-negative and Gram-positive, were present, but the frequent and consistent occurrence of *Gibbsiella quercinecans* and regular presence of *Brenneria goodwinii* (both *Enterobacteriaceae*) in only symptomatic oak tissue identifies them as having a possible causal role in the condition. It is therefore of key importance that detection and identification of these bacteria is completed rapidly, even when dealing with large numbers of isolations. Since conventional isolation techniques are used in the first step towards identification and in research work, a less labour intensive method that facilitates this process and makes it more cost effective would be useful. One way of accelerating identification is through the use of a selective medium.

A search for commercially available products highlighted Gassner agar (GA) as a suitable candidate for testing. GA is used for the detection and isolation of pathogenic *Enterobacteriaceae* from organic samples. It contains metachrome yellow (1.25 g L^{-1}) which inhibits Gram-positive microbial flora. Many bacteria identified in oak tissue are Gram-positive, including *Enterococcus* spp. and *Paenibacillus* spp. This medium may remove strains of little interest, with respect to AOD, that are normally isolated.

The effectiveness of GA to permit the selective growth of bacteria relevant to AOD (*Enterobacteriaceae*) was investigated. Fifty strains representing 21 species were used to test the selective medium including members of the *Enterobacteriaceae*: *Brenneria*, *Gibbsiella*, *Lonsdalea* and *Rahnella*. There was a highly significant reduction in the growth of all the Gram-positive bacteria compared to the members of the *Enterobacteriaceae* ($P<0.001$), with only some of the *Brenneria* strains showing slightly reduced growth. Interestingly each species produced different colour changes in the selective medium: After 24 h *G. quercinecans* produced an orange colour but *Rahnella* spp. produced a very dark blue colour while *B. goodwinii* produced the dark blue only after 48

h. Although this medium is not specific for the isolation of *G. quercinecans*, it is useful to suppress species of no interest thereby reducing time and effort in the identification of relevant species. Further tests are required, but it may be a starting place for the production of a species specific selective medium.

Keywords: *Brenneria*, *Enterobacteriaceae*, *Gibbsiella quercinecans*, selective medium, AOD,