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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Background

First described in 2009, Acute Oak Decline (AOD) is a disorder of native oak (*Quercus robur* and *Q. petraea*) in the UK. Of the biotic agents involved in the syndrome, bacteria are consistently isolated. Previous results revealed that there were many bacterial taxa present in symptomatic oak including members of the *Pseudomonadaceae* and *Enterobacteriaceae* as well as Gram-positive bacteria.

In the search for putative necrogenic biota, conventional isolation techniques were used and the consistent and frequent occurrence of *Brenneria goodwinii* and *Gibbsiella quercinecans* made them of particular interest as possible causal agents of the condition.

It would therefore be helpful if isolation methods that reduced recovery of taxa of no interest could be employed as part of the diagnostic procedure. Using a selective medium may streamline the process and be more time and cost effective in looking for the target species.

A literature search has shown commercially available products specifically aimed at isolating members of the *Enterobacteriaceae*. Gassner agar (GA) seemed to be a possible candidate medium due to the metachrome yellow dye it contained, which inhibits the growth of Gram-positive bacteria. Thus it was considered useful to determine the advantages that this medium would have on the isolation process.

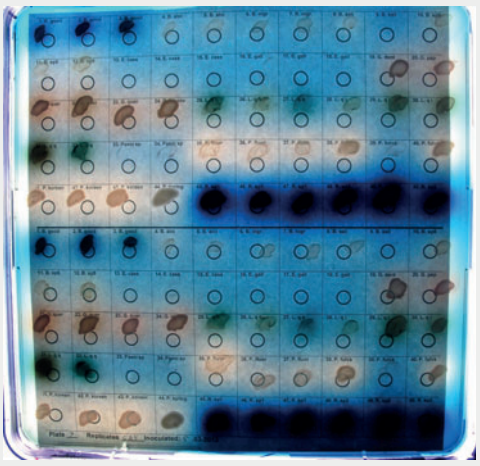

Figure 1: Growth and colour response of bacterial spp. tested on Gassner agar after 48 hours.

Aims

- To determine the effects of GA on the growth of enterobacteria associated with AOD, specifically *B. goodwinii* and *G. quercinecans*.
- To determine the inhibitory effects of GA on the growth of other taxa commonly isolated from AOD tissue samples, including *Enterococcus* spp., *Paenibacillus* sp. and *Pseudomonas* spp.

Materials and Methods

- 50 bacterial strains covering 21 species were used in the evaluation.
- A paper template dividing 250ml Q-Trays into 10 x 10 grid squares was attached to the base of the trays (Figure 1).
- Each strain was inoculated into a grid square.
- Both nutrient agar (NA) (4 reps - controls) and GA (10 reps) were tested.
- Q-Trays were incubated at 28°C for 24 hours before the first growth score was carried out.
- The score was based on qualitative analysis of each Q-Tray in turn. Strong growth = 3, moderate growth = 2, poor growth = 1 and no growth = 0.
- The growth score was repeated after 48 hours of incubation.

Statistical Analysis

The effects of medium (NA v GA), bacteria class (Gram-positive v *Enterobacteriaceae*) and time (24 hours v 48 hours) were analysed by fitting an ordinal response model to the growth score data. This model was defined with a multinomial error distribution and cumulative logit link function and interaction terms for the three main effects were included in the model fitting process.

Results

- All strains exhibited either medium or strong growth rates on NA.
- Individual bacterial strains exhibited a wide range of growth rates on GA $p<0.001$).
- The average growth rate for Gram-positive strains on GA was significantly lower than the average growth rate for *Enterobacteriaceae* ($p<0.001$).
- Results observed at 48 hours showed little difference from those at 24 hours.
- *G. quercinecans* growth was not affected by the selective medium (Figure 2).
- Only some *Brenneria* strains showed slightly reduced growth on GA.
- There were observed colour changes in the medium caused by different species.
- After 24 hours *G. quercinecans* produced an orange colour but *Rahnella* spp. produced a very dark blue colour.
- *Pseudomonas* spp. also produced an orange colour.
- *B. goodwinii* produced the same dark blue colour after 48 hours.

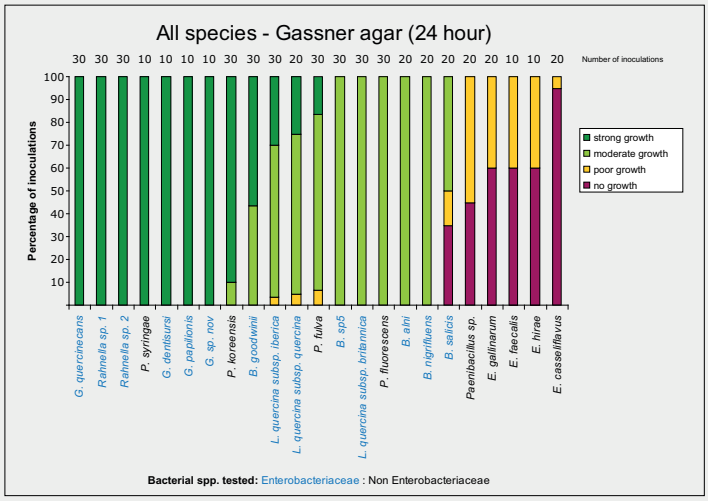

Figure 2: Effect of Gassner agar on growth of bacterial species associated with oak, particularly those with symptoms of Acute Oak Decline.

Discussion

The reason for carrying out this work was to obtain a method that would expedite isolation and detection of specific enterobacteria, especially *G. quercinecans* and *B. goodwinii* from symptomatic oak. GA had no effect on bacteria of interest, but the suppression of Gram-positive bacteria will reduce time spent isolating relevant species and avoid isolating bacteria of no interest thereby reducing the number of samples requiring DNA sequencing, which will reduce costs. Although this medium is not specific for the isolation of *G. quercinecans* or *B. goodwinii*, it is a useful starting point for the production of a more selective medium. Further work is required on suppressing the growth of *Pseudomonas* spp. in particular.

Acknowledgements
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