Speedy Breedy - Lab Memo 24

Experiments to Investigate a Culture Medium for Selective Detection of Lactic Acid Bacteria in samples contaminated with *Lactobacillus brevis*.

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Principle & Background

Flavour, aroma and appearance are all key characteristics that make a beer distinct and individual. The product of carefully selected hops, malt and yeast types, some beer brands are decades and centuries old, with consumers loyal to the experience of drinking a given beer. Brewers of new beers rely on individuality to encourage drinkers to try something new which they would enjoy.

All of these characteristics can be significantly affected by microbial contamination during the brewing process. Whilst many types of bacteria are unable to withstand the low pH and high alcohol conditions of the process, some bacteria are well known to brewers as being capable of spoiling a brew. Even with sterility processes in place, contamination can still occur and the rapid identification and resolution of contamination is essential to prevent costly losses.

Arguably the most significant bacterial cause of spoilage are the so-called 'lactic acid' bacteria. Producing undesirable compounds, these bacteria, notably Lactobacillus and Pediococcus, can cause significant changes in flavour and appearance of a brew.

Speedy Breedy confirms microbial contamination by the sensitive monitoring of pressure changes within a closed vessel. Containing a culture medium, vessels promote microbial replication. As part of a closed system, microbial respiration leading to changes in gas presence in the vessel can be monitored. An internal algorithm defines a significant pressure event associated with detection of contamination and the length of time from inoculation of sample to pressure event is the Time to Detection (TTD).

Hypothesis

Our hypothesis is that using a selective medium, Speedy Breedy will be able to identify Lactobacillus brevis in samples whilst selectively excluding other, non-relevant organisms that may be present (such as brewing yeast). We also hypothesise that Speedy Breedy will exhibit increasingly rapid detection times when challenged with increased L. brevis contamination in samples.

Aim of Study

The aim of this study is to correlate data for detection of L. brevis in artificially contaminated samples of sterile water, with increasing levels of contamination. Detection will be achieved using the portable microbial respirometer Speedy Breedy with culture vessels containing Nocive Brewers Bacteria (NBB) medium.

At the same time, selective detection will be challenged by artificially contaminating samples of sterile water with heavy inocula of Pseudomonas aeruginosa and Escherichia coli (to ensure no Gram-negative organisms produce false positive results, lactic acid bacteria of concern to brewers being Gram-positive) as well as Saccharomyces cerevisiae (to ensure no yeasts produce false positive results).

Materials & Methods

In order to measure Time to Detection (TTD) against varying bacterial load in sample, stock cultures of L. brevis, E. coli, P. aeruginosa and S. cerevisiae were first required and through serial dilution, a number of samples of each organism with decreasing bacterial load created.

Initial cultures were cultivated using Vitroid discs (RQC12002 P. aeruginosa and RQC01702 E. coli, Sigma-Aldrich) or Selectrol discs (MM73-10 S. cerevisiae and MM76-10 L. brevis, TCS Biosciences Ltd). Following serial dilution, 100µl of each dilution was used to create a spread plate culture (PB0122A Columbia Blood Agar for the Gram-negative organisms, PO0231 MRS Agar for L. brevis and PO0160A Sabouraud Dextrose Agar for the S. cerevisiae, Oxoid / Thermo Scientific). After 24 hours incubation at 36°C, counts were taken of colony forming units (CFU) and from this, CFU / ml of serial dilution calculated for the bacteria grown. For the yeast, incubation was carried out at 30°C for 48 hours prior to CFU counts being obtained.

Speedy Breedy culture vessels initially containing no culture medium were filled with 49ml of NBB culture medium. 1ml of prepared organism dilution was then used to inoculate the vessel. This process was repeated for six different dilutions of L. brevis and single, heavy inocula of known CFU level for each of the other organisms.

The medium used is comprised of the commonly used brewing medium Nocive Brewers Bacteria (NBB) broth (50725 NBB Broth, Sigma-Aldrich) with the addition of the anti-Gram-negative compound Sodium Azide (71290 Sodium Azide, Sigma-Aldrich) and the antifungal compound Actidione (01810 Cycloheximide, Sigma-Aldrich). As a further addition, a strong reducing agent was added to the medium to aid in generating anaerobic conditions within the culture vessel, favourable for lactic acid bacteria.

Control vessels containing 50ml sterile NBB medium were incubated to demonstrate that no detection activity is derived from uninoculated vessels.

All vessels were incubated using Speedy Breedy instruments with a 72 hour test protocol at a 36°C incubation temperature. Pressure over time results from Speedy Breedy instruments were reviewed after the 72 hour test protocol completed to ascertain the TTD.

Results

Table 1 below shows data recorded for TTD with varying CFU loads of L. brevis in culture vessels tested using Speedy Breedy as outlined above.

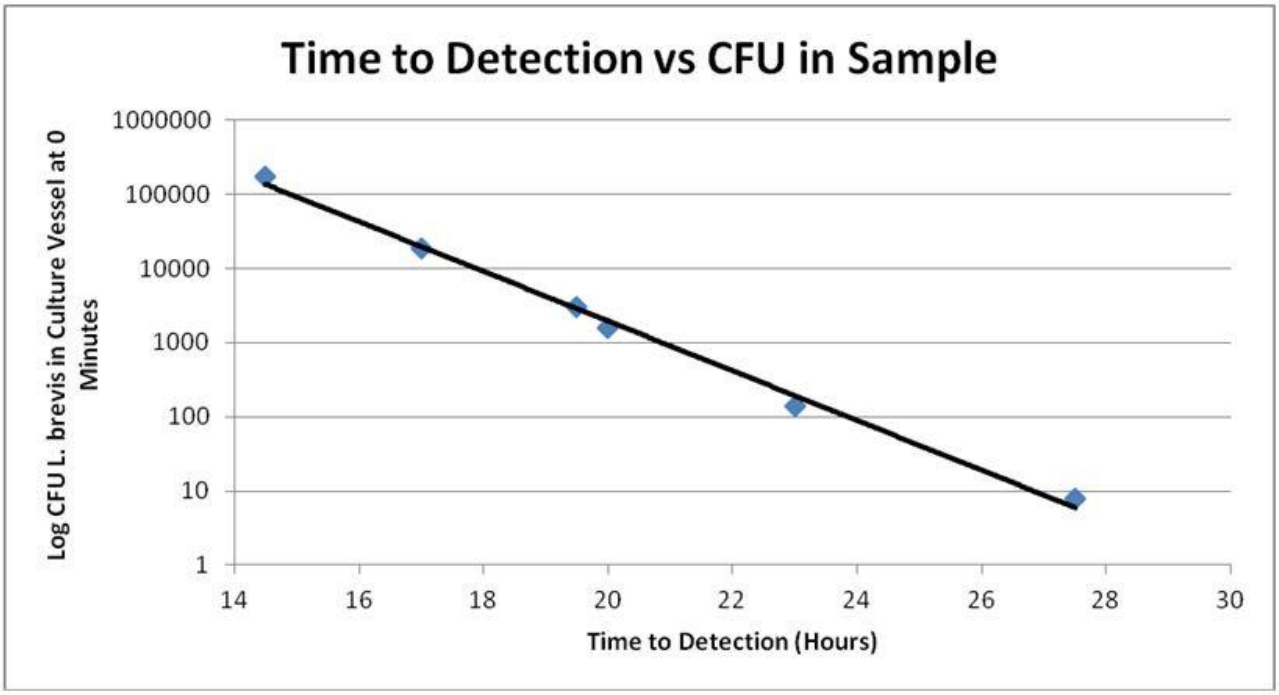
Figure 2 shows the data from Table 1 plotted as a curve of TTD against Log CFU in the culture vessel.

Table 1: Initial sample L. brevis load and corresponding Time to Detection (TTD).

CFU in Vessel	1.75×10^5	1.84×10^4	3.07×10^3
TTD (Minutes)	870	1020	1168
TTD (Hours)	14.50	17.00	19.50

CFU in Vessel	1.57×10^3	1.40×10^2	8.00×10^0
TTD (Minutes)	1200	1380	1650
TTD (Hours)	20.00	23.00	27.50

Figure 2: Initial sample L. brevis load (Log CFU) and corresponding Time to Detection (TTD).



Control vessels inoculated with E.coli (7.8 x 105 CFU), P. aeruginosa (1.6 x 106 CFU) and S. cerevisiae (3.3 x 104 CFU) all showed no detection event during the course of the experiments.

Control vessels containing only sterile medium all showed no detection during the course of the experiment.

Interpretation

The lack of microbial activity in vessels inoculated with potential contaminating organisms other than L. brevis suggests that the bespoke medium has selectively excluded these species. The viability of the inoculums used is confirmed by the successful detection on agar plates.

There is a marked reduction in Time to Detection with Speedy Breedy as L. brevis contamination of the original sample is increased and there is a strong correlation between bacterial load and time to detection. Very low CFU levels (8) were detected within twenty eight hours of experimentation commencing.

Conclusions & Observations

- As per our hypothesis, Speedy Breedy can be used to rapidly and selectively detect the wild yeast S. diastaticus whilst selectively controlling the activity of the brewing yeast S. cerevisiae.

- The use of the NBB medium provides a good selective solution when wanting to screen samples for Lactobacillus without producing false positive results courtesy of other potential contaminants.

- The strong correlation between Time to Detection and CFU levels in the inoculated samples suggests that Speedy Breedy can be used for quantitative analysis of samples based on Time to Detection recorded.

- The successful detection of 8 CFU of L. brevis in a 50 ml working volume (equating to less than 1 CFU / ml) in a little over 24 hours in comparison to standard culture methods requiring up to 7 days, shows Speedy Breedy to be a rapid, sensitive and selective tool for detection.