Speedy Breedy - Lab Memo 30

Experiments to Investigate a Culture Medium for Rapid Detection of Yeasts in Speedy Breedy

Author: Darren Hermes Date: March 2014

Background

Yeasts can be both a friend and a foe of a food manufacturer. Some products rely on the fermentation activity of yeasts such as the rising of bread or the production of beers and wines. The ability to ferment can also be problematic, with excess gas production in food packaging being an aesthetic issue as well as a consumer safety issue, should the contents of packaging be forcibly expelled when opened.

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 was that using an appropriate medium, Speedy Breedy would be able to identify the yeast species Candida albicans and Saccharomyces cerevisiae whilst selectively inhibiting the growth of bacterial contaminants. We also hypothesised that Speedy Breedy would exhibit increasingly rapid detection times when challenged with increased yeast contamination in samples.

Aim of Study

The aim of this study was to correlate data for detection of C. albicans and S. cerevisiae in artificially contaminated samples of sterile water, with increasing levels of contamination. Detection would be achieved using the portable microbial respirometer Speedy Breedy with culture vessels containing a modified Yeast and Mould (YMC) medium.

At the same time, selective detection would be challenged by artificially contaminating samples of sterile water with heavy inocula of both Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa) and Gram-positive bacteria (Staphylococcus aureus, Enterococcus faecalis).

Materials & Methods

In order to measure Time to Detection (TTD) against varying bacterial load in sample, stock cultures of C. albicans and S. cerevisiae as well as the organisms to be used for challenging the selectivity of the medium (Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis and Staphylococcus aureus) were first required and through serial dilution, a number of samples of each organism with decreasing microbial load created.

Initial cultures were cultivated using either Selectrol discs (NCPF 3178 S. cerevisiae, TCS Biosciences Ltd), Vitroid discs (ATCC 9027 P. aeruginosa, ATCC 11175 E. coli Sigma-Aldrich) or Lenticule discs (NCTC 6571 S. aureus, NCTC 775 E. faecalis, NCPF 3255 C. albicans, Public Health England). Following serial dilution, 100µl of each dilution was used to create a spread plate culture (PO0160A Sabouraud Dextrose Agar, Oxoid / Thermo Scientific). After 48 hours incubation at 30°C, counts were taken of colony forming units (CFU) and from this, CFU / ml of serial dilution calculated.

Speedy Breedy culture vessels initially containing no culture medium were filled with 49ml of modified YMC medium. 1ml of prepared organism dilution was then used to inoculate the vessel. This process was repeated for five different dilutions of C. albicans and S. cerevisiae and for a single dilution of each of the bacteria used for selectivity testing.

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

All vessels were incubated using Speedy Breedy instruments with a 48 hour test protocol at a 30°C incubation temperature. Pressure over time results from Speedy Breedy instruments were reviewed after the 48 hour test protocol completed to ascertain the TTD.

Results

Tables 1 and 2 below show data recorded for TTD with varying CFU loads of C. albicans and S. cerevisiae in culture vessels tested using Speedy Breedy as outlined above.

Figures 3 and 4 show the data from Tables 1 and 2 plotted as a curve of TTD against CFU in the culture vessel.

Table 1: Initial sample C. albicans load (CFU) and corresponding Time to Detection (TTD).

CFU in Vessel	4.20×10^6	3.40×10^5	3.10×10^4	2.82×10^3
TTD (Minutes)	641	918	1032	1363
TTD (Hours)	10.68	15.30	17.20	22.72

CFU in vessel	3.20×10^2	48
TTD (Minutes)	1589	1961
TTD (Hours)	26.48	32.68

Table 2: Initial sample S. cerevisiae load (CFU) and corresponding Time to Detection (TTD).

CFU in Vessel	7.10×10^6	5.20×10^5	4.90×10^4	5.30×10^3
TTD (Minutes)	432	991	1072	1614
TTD (Hours)	7.20	16.52	17.87	26.90

CFU in Vessel	4.20×10^2	35
TTD (Minutes)	1817	2475
TTD (Hours)	30.28	41.25

Figure 3: Initial sample C. albicans load (CFU) and corresponding Time to Detection (TTD).

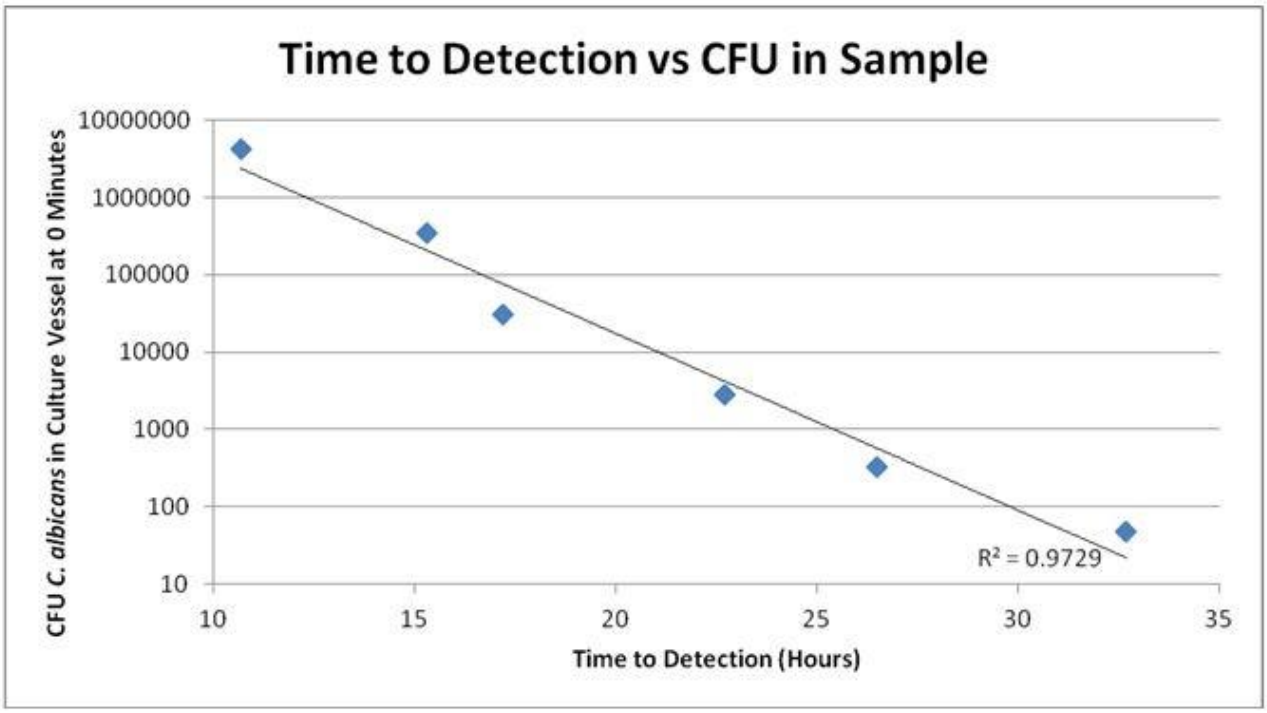
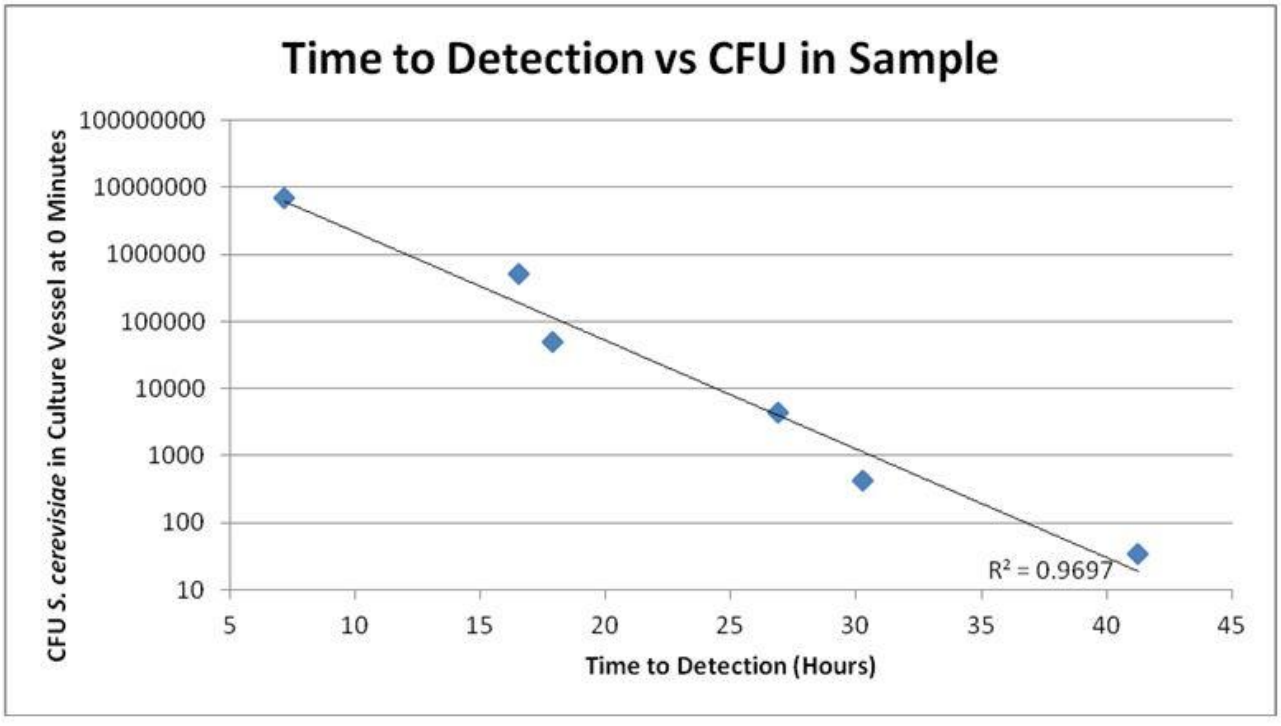


Figure 4: Initial sample S. cerevisiae load (CFU) and corresponding Time to Detection (TTD).



Control vessels inoculated with Gram-negative or Gram-positive bacteria at concentrations of greater than 1000 CFU all showed no detection event during the course of the experiment. Control vessels containing only sterile medium also showed no detection during the course of the experiment.

Interpretation

The lack of microbial activity in vessels inoculated with bacteria suggests that the modified YMC medium has selectively excluded the organisms. The viability of the inocula used was confirmed by successful agar plate culture.

Vessels inoculated with *C. albicans* or S*. cerevisiae* show rapid detection and a strong correlation between microbial load and Time to Detection.

Conclusions & Observations

- As per our hypothesis, Speedy Breedy can be used to rapidly detect yeasts whilst ensuring no false positives are detected from bacterial contamination.
- The use of the modified YMC medium provides a good selective solution when wanting to screen samples for yeasts without producing false positive results courtesy of bacteria present in samples.
- 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 the Time to Detection recorded.
- The successful detection of less than 50 CFU of either yeast in a 50 ml working volume (equating to less than 1.0 CFU / ml) in less than 2 days in comparison to standard culture methods requiring up to 5 days, shows Speedy Breedy to be a rapid, sensitive and selective tool for yeast detection.