# Speedy Breedy - Lab Memo 25

## Experiments to Investigate a Culture Medium for Selective Detection of 'Wild Yeasts' in Speedy Breedy

Author: Darren Hermes  Date: January 2014

### *Principle & Background*

The term 'wild yeast' is used in the brewing industry to describe any yeast strain that has not been deliberately introduced into the brewing process. Contamination by wild yeast can occur through poor sanitary conditions or through contaminated ingredients – even the pitching yeast used for brewing may be contaminated with wild types.

Wild yeast contamination can cause significant problems for a brewer, creating undesired fermentation products (including off-flavours and odours), out-competing brewing yeast for nutrients during fermentation and significantly affecting turbidity and introducing 'haze' to 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).

Yeast detection systems, when screening for wild yeasts, must incorporate a process by which brewing yeasts do not produce false positive results. In this study the suitability of Speedy Breedy for rapid detection of the wild yeast Saccharomyces diastaticus, using a Yeast and Mould with Copper Sulphate culture medium, commonly used in brewing microbiology.

### *Hypothesis*

Our hypothesis is that using a selective medium, Speedy Breedy will be able to identify S. diastaticus in samples whilst selectively excluding Saccharomyces cerevisiae. We also hypothesise that Speedy Breedy will exhibit increasingly rapid detection times when challenged with increased S. diastaticus contamination in samples.

### *Aim of Study*

The aim of this study is to correlate data for detection of S. diastaticus 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 a Yeast and Mould with Copper Sulphate (YMC) medium.

At the same time, selective detection will be challenged by artificially contaminating samples of sterile water with a heavy inoculums of the brewing yeast S. cerevisiae.

## Materials & Methods

In order to measure Time to Detection against varying yeast load in the sample, stock cultures of S.diastaticus and S. cerevisiae were first required and through serial dilution, a number of samples of each organism with decreasing bacterial load were created.

Initial cultures were cultivated using either Selectrol discs (MM73-10 S. cerevisiae, TCS Biosciences Ltd) or a slope culture (S. diastaticus, Campden BRI). 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 the YMC medium. 1ml of prepared organism dilution was then used to inoculate the vessel. This process was repeated for six different dilutions of S. diastaticus and a single, heavy S. cerevisiae with inocula of known CFU concentration.

The YMC medium used is comprised of a yeast and mould enrichment medium (271120 YM Broth, Difco / BD) with the addition of copper sulphate (209198 Copper (II) Sulfate Pentahydrate, Sigma-Aldrich). Copper sulphate is commonly used in broth and agar culture media by microbiology laboratories undertaking brewing-related testing as a means to differentiate brewing yeasts (sensitive to copper sulphate) and wild yeasts (resistant to copper sulphate).

Control vessels containing 50ml sterile 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 96 hour test protocol at a 30°C incubation temperature. Pressure over time results from Speedy Breedy instruments were reviewed after the 96 hour test protocol completed to ascertain the TTD.

## Results

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

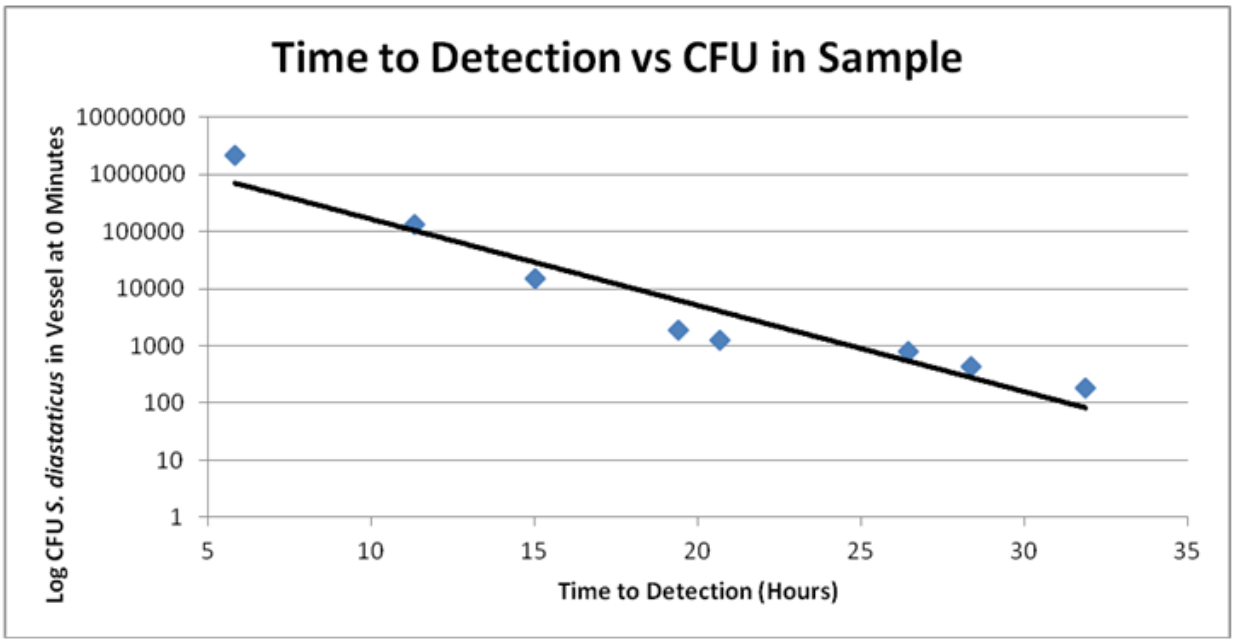
Figure 2 shows the data from Table 1 plotted as a curve of TTD against Log CFU in the culture vessel.

### *Table 1: Initial sample S.diastaticus load and corresponding Time to Detection (TTD).*

| **CFU in vessel** | $2.20 \times 10^6$ | $1.34 \times 10^5$ | $1.50 \times 10^4$ | $1.90 \times 10^3$ |
|---|---|---|---|---|
| **TTD (Minutes)** | 350 | 680 | 900 | 1165 |
| **TTD (Hours)** | 5.83 | 11.33 | 15.00 | 19.42 |

| **CFU in vessel** | $1.28 \times 10^3$ | $7.80 \times 10^2$ | $4.30 \times 10^2$ | $1.80 \times 10^2$ |
|---|---|---|---|---|
| **TTD (Minutes)** | 1240 | 1586 | 1701 | 1912 |
| **TTD (Hours)** | 20.67 | 26.43 | 28.35 | 31.87 |

### *Figure 2: Initial sample S. diastaticus load (Log CFU) and corresponding Time to Detection (TTD).*




Control vessels inoculated with S. cerevisiae, at the high cell concentration of 5.2 x 107 CFU in a 50ml volume in the culture vessel, showed no detection event during the course of the experiment. 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 S. cerevisiae suggests that the YMC medium has selectively excluded the organism. The viability of the inocula used is confirmed by the successful agar plate culture.

Vessels inoculated with S. diastaticus 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 and selectively detect the wild yeast S. diastaticus whilst selectively controlling the activity of the brewing yeast S. cerevisiae.
- The use of the YMC medium provides a good selective solution when wanting to screen samples for wild yeasts without producing false positive results courtesy of brewing yeasts 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 180 CFU of S. diastaticus in a 50 ml working volume (equating to less than 4.0 CFU / ml) in a little under 1.5 days in comparison to standard culture methods requiring up to 5 days, shows Speedy Breedy to be a rapid, sensitive and selective tool for wild yeast detection.