Speedy Breedy - Lab Memo 41

Growth and quantitative determination of *Corynebacterium glutamicum* using Speedy Breedy.

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Principle & background

Corynebacterium glutamicum is an important organism in biotechnology because it has characteristics that make it easy to grow and handle while generating many useful biochemical compounds. Amongst other things, it grows rapidly, has relatively few growth requirements, is not pathogenic, does not form spores, and has a relatively stable genome.
C. glutamicum produces several useful compounds and enzymes including glutamate, amino acids, such as lysine, threonine, and isoleucine, as well as vitamins like pantothenate.
Further possible uses for C. glutamicum include bioremediation, particularly for arsenic removal as glutamicum contains two operons in its genome (ars1 and ars2 operons) that are resistant to arsenic.
In this study we investigated the ability of Speedy Breedy to grow C. glutamicum for research and industrial purposes and assess the correlation between Time to Detection (TTD) in Speedy Breedy and numbers of C. glutamicum organisms present in the sample.

Hypothesis

It was hypothesised firstly that Speedy Breedy could be used for the rapid culture of C. glutamicum for research and industrial purposes and secondly that the number of organisms in a sample could be determined, making Speedy Breedy a useful tool in both environments. A calibration was performed over six logs to determine the potential to monitor industrial processes using this organism.

Speedy Breedy detects bacterial growth by measuring sensitive pressure changes within a closed vessel due to microbial respiration and assigns a Time to Detection (TTD) using an internal algorithm that detects a significant pressure event reflecting growth. The significant pressure event is created when organisms (often growing exponentially from low numbers), reaches a critical mass, respiring rapidly, that rapidly influences the gaseous composition and internal pressure within the culture vessel.

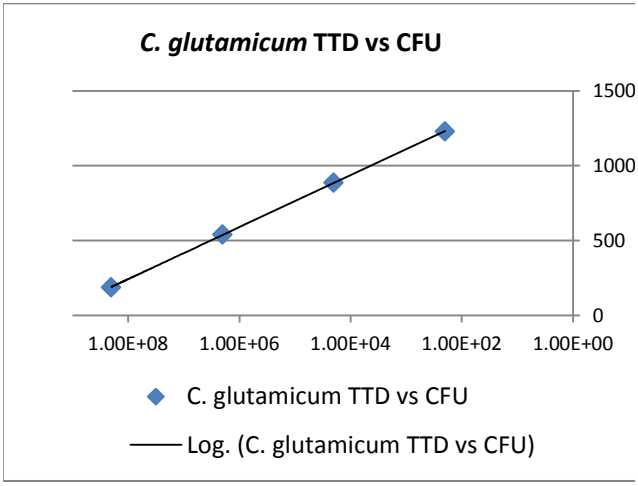
Experiment

- 8 Tryptose Soya Broth (TSB) culture vessels (product code BAC021) were reconstituted with 49 ml of sterile RO water.
- Four serial dilutions of C. glutamicum were prepared in TSB to give target values of 108, 106, 104 and 102 per ml.
- 1 ml of each dilution was aseptically added to the pre-prepared Speedy Breedy vessels in duplicate and cultured at 30 °C using Bactest's "Corynebacterium glutamicum 30 C" protocol.
- ml of the 104 and 102 samples were spread onto Tryptose Soya Agar (TSA) plates and the numbers of colonies counted after 24 hours @ 30 °C.
- The actual inoculum added to each Speedy Breedy vessel was calculated from the plate counts.
- Speedy Breedy detection times for were noted and plotted against inoculum (cfu/vessel)

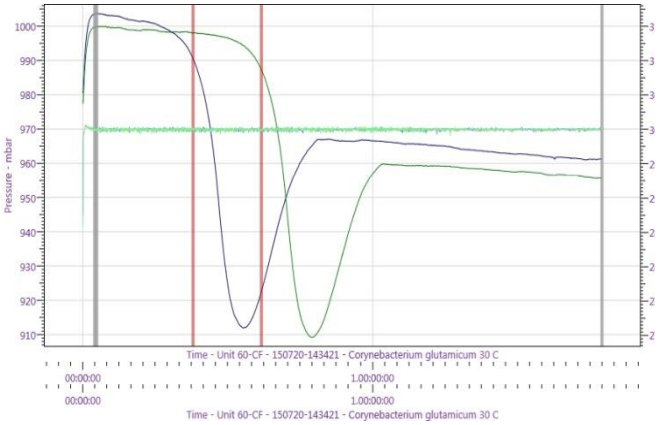
Results

Plate count					Detection Time
cfu/ml - Vessel	TTD Rep 1 (h:m)	TTD Rep 1 (min)	TTD Rep 2 (h:m)	TTD Rep 2 (min)	Mean TTD (min)
2.00E+08	3.12	192	3.05	185	189
2.00E+06	8.52	532	9.09	549	541
2.00E+04	14.49	889	14.46	886	888
2.00E+02	20.14	1214	20.44	1244	1229

Time to Detection (TTD) in minutes was plotted against the starting cfu/vessel value in Speedy Breedy.



Typical growth & Detection curves in Speedy Breedy



Conclusions

C. glutamicum is readily detectable in Speedy Breedy using TSB vessels (product code BAC021) and the "Corynebacterium glutamicum 30 C" protocol.
Excellent growth & pressure curves were generated with highly defined detection points.
Reproducibility of replicates was good
Speedy Breedy demonstrates a high degree of correlation between the Time to Detection and the number of C. glutamicum organisms in the test sample.
Speedy Breedy is highly suitable for culturing and monitoring this organism.

Note

The use of higher temperatures and more suitable culture medium may improve time to detection beyond the excellent results obtained in this study.

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