METHODS FOR THE CULTURE OF C. ELEGANS AND S. CEREVISIAE IN MICROGRAVITY

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To support the study of the effects of microgravity on biological systems, our group is developing and testing methods that allow the cultivation of C. elegans and S. cerevisiae in microgravity. Our aim is to develop the experimental means by which investigators may conduct peer reviewed biological experiments with C. elegans or S. cerevisiae in microgravity. Our protocols are aimed at enabling investigators to grow these organisms for extended periods during which samples may be subcultured, collected, preserved, frozen, and/or returned to earth for analysis. Data presented include characterization of the growth phenotype of these organisms in liquid medium in OptiCells™ (Biocrystal, LTD).

Subculture and sampling activities of C. elegans and S. cerevisiae in microgravity are more manageable when the organisms are grown in a closed liquid culture system. We have therefore examined the growth of C. elegans in C. elegans minimal medium (CeMM) (Lu et al. 1993, Szewczyk et al. 2003) utilizing OptiCells™ as growth chambers. The OptiCells™ are under investigation as a containment system for liquid C. elegans culture since they are amenable to flight subculture and sampling procedures. Our data show that C. elegans grow at a rate comparable to that in a standard culture flask, reach densities of up to 1 x 10⁶ worms/ml in liquid medium in OptiCells™, and may be initiated with as few as 10 worms/ml (Figs 1 and 2). Similarly, S. cerevisiae cultured in standard YPD medium in OptiCells™ grow at a comparable rate to those in a static flask (Fig. 3). Note that the Opticells apparently mimic a static flask condition rather than a shaking flask condition. These results suggest that our culture conditions support the growth of C. elegans and S. cerevisiae in a manner comparable to conventional culturing methods.

Characterization of the required conditioned transport and storage of CeMM is important as it affects the hardware, power, and space requirements for the experiment. CeMM is known to contain components that are light and temperature sensitive and is therefore typically stored at 4 °C in the dark. To characterize the effect of storage at elevated temperature, we grew worms in medium that had been stored at room temperature in the dark for 10 months. As shown in Figure 4, this severely affected ability of the medium to support C. elegans growth.

Figure 1. Growth of C. elegans in OptiCells™ compared to flasks. Animals were inoculated 10 ml of CeMM in OptiCells™ or T75 vented flasks. Samples were incubated at 20 °C and worms were periodically counted. Error bars show the standard deviation from the mean. N = 3.

Figure 2. Growth of C. elegans in OptiCells™ with various starting densities. Animals were inoculated to a density of 10, 100, or 1000 worms/ml 10 ml of CeMM in OptiCells™ and incubated at 20 °C. Worms were periodically withdrawn and counted. Error bars show the standard deviation from the mean. N = 3.

Figure 3. Growth of S. cerevisiae in Opticells. Stationary phase S. cerevisiae BY4743 were diluted 1:1000 in YPD broth, transferred to shaker flasks or Opticells™ and incubated at 30 °C. The flasks were incubated either with shaking or statically. The Opticells™ were incubated statically with a 2mm space between replicate Opticells. Growth was monitored over time by measuring the optical density at 600 nm. Error bars depict the standard deviation from the mean. N = 3.
As expected, during growth testing of *S. cerevisiae* in YPD medium in OptiCells™, we observed the formation of large bubbles, CO₂, within the OptiCells™ (data not shown). Since bubbles are undesirable during flight experiments, and dextrose is a substrate converted to CO₂, we sought to mitigate bubble formation by reducing the dextrose in the YPD medium, which typically contains 2.0% dextrose. To this end, we grew samples in OptiCells™ in YPD medium modified to contain 2.0%, 1.5%, 1.0%, or 0.5% dextrose. With lower dextrose concentrations, we found a slightly reduced growth rate and final cell density (data not shown), yet a dramatic reduction in bubble formation (Fig. 5). This suggests that modulation of the glucose concentration in YPD medium may be used to reduce bubble formation in OptiCells™ by yeast.

*S. cerevisiae* cells must be held in stasis during launch to allow controlled initiation of a flight experiment. We have examined methods that may allow us to prevent yeast growth yet maintain viability prior to experiment initiation. It is common practice to freeze cells long term in YPD containing 15% (v/v) glycerol. Although 15% (v/v) glycerol affects the growth rate of the cells, we found that lower concentrations of glycerol, such as 5% (v/v) do not significantly affect the *S. cerevisiae* growth rate (data not shown). We assessed the use of the lower glycerol concentration for preservation of *S. cerevisiae* at -20 °C. As shown in Figure 6, we found that 5% glycerol in YPD medium was sufficient to significantly improve the long term viability of *S. cerevisiae* cells at -20 °C. These data suggest that YPD supplemented with 5% glycerol may serve as an ideal freezing and growth medium for *S. cerevisiae* during flight experiments.

The experiments described support the cultivation of *C. elegans* and *S. cerevisiae* in microgravity utilizing OptiCells™. In each instance the OptiCell(tm) containers provided growth rates similar to standard laboratory vessels. Our data suggest that OptiCells, coupled with the appropriate media, may provide a means to culture *C. elegans* and *S. cerevisiae* in a manner conducive to space flight experiments.

**REFERENCES**
