Protein Synthesis is Required for Recovery of *Escherichia coli* from Desiccation Stress

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Introduction

The stress of desiccation is fatal to many cells and can induce significant water loss, which can impact the structure and function of membranes (1,2). When put under the stress of desiccation, ***E. coli*** cells are thought to enter a Viable But Nonculturable (VBNC) state (unpublished results). The VBNC state is brought on by various stresses and leads to absence of cell division, reduced activity, but cells remain viable (3).

By adding chloramphenicol to cells, the importance of protein synthesis during desiccation and rehydration was investigated.

Understanding the mechanism required for recovery from the VBNC state is important in determining ways to improve microbial inhabitants in clinical and agricultural settings.

Project Goal

The goal of the project was to investigate the importance of protein synthesis during desiccation and rehydration of *E. coli* using the bacteriostatic antibiotic chloramphenicol.

By determining the importance of proteins synthesis, it is hoped that more can be learned about *E. coli's* mechanism of desiccation tolerance.

1. Potts, M, S.M. Slaughter, F.U. Hunneke, J.F. Garst, and R.F. Helm. 2005. Integr.. Comp. Biol. 45:800-809
2. Crowe, J.H., L.M. Crowe, and D. Chapman. 1984. Science 223:701-703
3. Oliver, J.D. 2005 J. Microbiol. 43:93-100

Figure 1. Desiccation of cell samples at constant relative humidity.

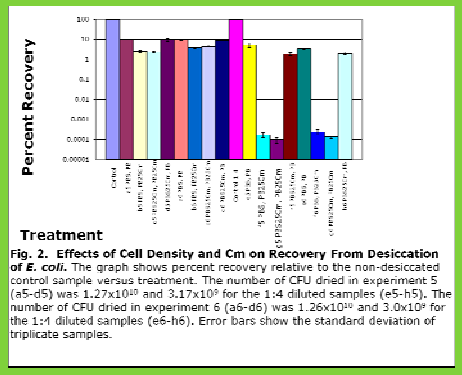

Fig. 2. Effects of Cell Density and Cm on Recovery From Desiccation of *E. coli*. The graph shows percent recovery relative to the non-desiccated control sample versus treatment. The number of CFU dried in experiment 5 (a5-d5) was 1.27×10^{10} and 3.17×10^{9} for the 1:4 diluted samples (e5-h5). The number of CFU dried in experiment 6 (a6-d6) was 1.26×10^{10} and 3.0×10^{9} for the 1:4 diluted samples (e6-h6). Error bars show the standard deviation of triplicate samples.

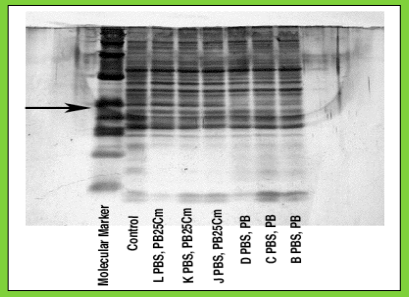

Figure 3.12% Tris-tricine gel showing a difference in protein levels between samples treated during rehydration compared to untreated samples.

Methods

- A culture of *E. coli* strain JM109 was grown to stationary phase, harvested, and washed with phosphate-buffered saline, and concentrated about 30-fold.
- Cells were typically dried for two weeks in a 12 well microtiter dish at room temperature in around 37% relative humidity.
- Cells were rehydrated in pyruvate buffer for two to three days.
- 25 µg/ml Cm was used to treat cells
- SDS-PAGE analysis was used for protein sample detection

Results

- Chloramphenicol inhibited recovery from desiccation when added during rehydration.
- Chloramphenicol had little effect when added during desiccation.
- Removal of antibiotic from rehydration buffer drastically increased cell recovery.
- Gel analysis identified proteins that were either more or less intense in Cm treated cells.