

Bacterial Spores – Challenges and Future Directions for Biodefense, Argonne National Laboratory, Argonne, IL, November 14-16, 2004.

Optimization of Germination and Growth of *Bacillus anthracis* for Multi-step Detection

Jun Hang, Appavu Sundaram, Peixuan Zhu, Daniel R. Shelton*, Jeffrey S. Karns*, Platte Amstutz, Cha-Mei Tang[†]

Creatv MicroTech, Inc., Potomac, MD 20854 & USDA/ARS, Environmental Microbial Safety Laboratory, Beltsville, MD 20705*

Bacillus anthracis is a biological warfare and terrorism agent. *B. anthracis* spores (the infectious agent) are very persistent and can be “weaponized” for delivery via air, food or water. Creatv MicroTech is currently developing biosensor instruments and assays for rapid detection of *B. anthracis* spores. One of the goals of this work is to optimize conditions for rapid spore germination and vegetative cell growth. In selected germinant solutions, almost 100% of spores germinated within ten minutes. Among five culture broths tested (at three temperatures) BHI at 37°C allowed for the most rapid growth of vegetative cells. Three supplements, 3-amino-L-tyrosine, luminol and bicarbonate, significantly accelerated cell growth in TSB; however, only minor enhancement was seen in BHI. Therefore, BHI is a preferred culture broth for *B. anthracis*. In addition, *B. anthracis* spores germinated rapidly in BHI, obviating the need for additional germinants. The effects of heat shock on spore germination and cells growth was also tested. No differences were observed in growth rates between heat-treated and non-heated spores, although heating at 80°C for more than 5 minutes inhibited growth. To evaluate *B. anthracis* growth, a quantitative real-time PCR technique was developed to provide more accurate evaluation of growth rate than conventional colony counting method.

[†]Correspondence to: cmtang@creatvmicrotech.com