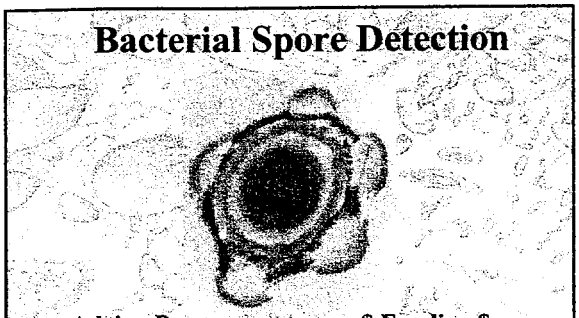
Bacterial Spore Detection



Adrian Ponce
James P. Kirby
Erik G. Shipton
Michael Kempf

$ Funding $
Planetary Protection (JPL)
Caltech President's Fund
Astrobiology Institute

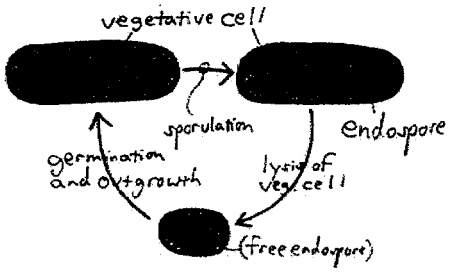
Disease Causing Endospores

In addition to NASA, the EPA, FDA, USDA and DOD are interested in determining the amount of harmful bacteria in the air.

Diseases:
- **Anthrax** (*Bacillus anthracis*)
- **Tetanus** (*Clostridium tetani*)
- **Botulism** (*Clostridium botulinum*)
- **Gas Gangrene** (*Clostridium* sp.)

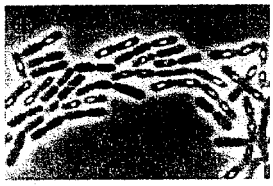
Biotech Applications:
Caterpillar insecticide (*Bacillus thuringiensis*)

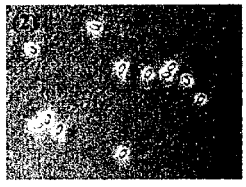
Production of Endospores



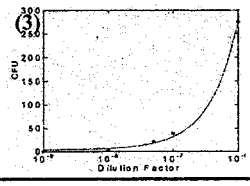
Endospores have increased resistance to:
(1) Temperature, (2) Dessication, (3) Radiation

Endospore Preparation

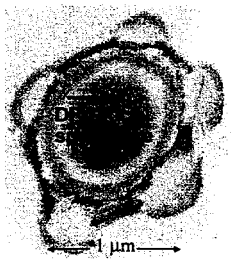


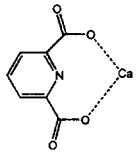


1) Sporulation
2) Purification
3) Concentration Determination



Real-Time Spore Detection based on Tb-DPA Luminescence



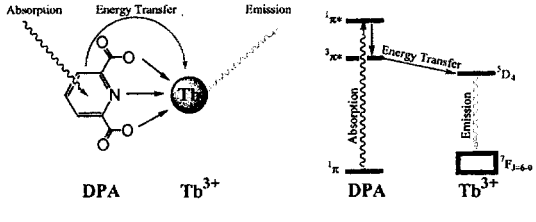


10^{-15} moles DPA

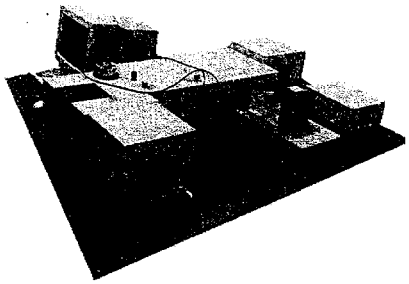
[DPA] ~ 1 M (inside spore)

~ 1 pM (1 spore in 1 ml)

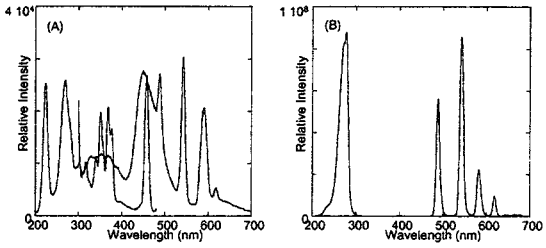
Tb-DPA Complex Photophysical Properties



Fluorimeter—Spex Fluorolog τ322

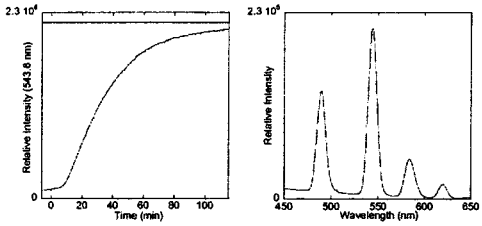


Excitation and Emission Spectra

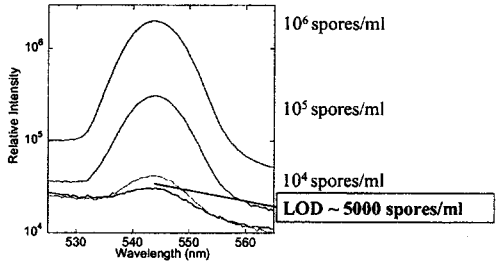


Tb **Tb-DPA**

Germination of Endospores



Spore Detection Limit



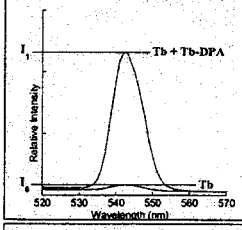
Quantifying Luminescence Turn-On Factor

$$\text{Tb} + \text{DPA} \xrightleftharpoons{K_{11}} \text{Tb-DPA}$$

$$K_{11} = \frac{[TbDPA]}{[Tb][DPA]}$$

$$[DPA]_{tot} = [DPA] + [TbDPA]$$

$$[Tb]_{tot} = [Tb] + [TbDPA]$$



$$TOF = \frac{I_1}{I_0} = \frac{I_{TbDPA} + I_{Tb}}{I_{Tb}^0}$$

$$TOF = \frac{\varepsilon_1\phi_1[TbDPA] + \varepsilon_0\phi_0[Tb]}{\varepsilon_0\phi_0[Tb]_{tot}}$$

$$TOF = \frac{\varepsilon_1\phi_1}{\varepsilon_0\phi_0} f_{11}^{Tb} + \left(1 - f_{11}^{Tb}\right)$$

$$TOF = f_{11}^{Tb}\left[\frac{\varepsilon_1\phi_1}{\varepsilon_0\phi_0} - 1\right] + 1$$

$$f_{11} = \frac{[TbDPA]}{[Tb]_{tot}} = \frac{K_{11}[DPA]}{1 + K_{11}[DPA]} = \frac{K_{11}\left\{-1 + K_{11}[DPA]_{tot} - K_{11}[Tb]_{tot} + \sqrt{(1 - K_{11}[DPA]_{tot} + K_{11}[Tb]_{tot})^2 + 4K_{11}[DPA]_{tot}}\right\}/2K_{11}}{1 + K_{11}\left\{-1 + K_{11}[DPA]_{tot} - K_{11}[Tb]_{tot} + \sqrt{(1 - K_{11}[DPA]_{tot} + K_{11}[Tb]_{tot})^2 + 4K_{11}[DPA]_{tot}}\right\}/2K_{11}}$$

$f_{11}(K_{11}, [DPA]_{tot}, [Tb]_{tot})$ - All in terms of experimentally obtainable parameters