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Measuring Total and Germinable Spore Populations

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Abstract

It has been shown that bacterial endospores can be enumerated using a microscopy based assay that images the luminescent halos from terbium ions bound to dipicolinic acid, a spore specific chemical marker released upon spore germination. Further development of the instrument has simplified it towards automation while at the same time improving image quality. Enumeration of total spore populations has also been developed allowing measurement of the percentage of viable spores in any population by comparing the germinable/culturable spores to the total. Percentage viability will allow a more quantitative comparison of the ability of spores to survive across a wide range of extreme environments.

1. Introduction/Previous Work

1.1 Detection of Bacterial endospores

Bacterial endospore (spore) formers are frequently considered the most resilient form of life, and in their dormant state have been shown to withstand extremes of temperature, pressure, radiation, and humidity [1]. Spores can be detected by measuring the release of dipicolinic acid (DPA), a spore specific chemical marker, during germination. Terbium ions (Tb^{3+}) doped into the germinant media bind the DPA and the complex luminesces brightly under UV excitation [2]. This lab has applied this method in a rapid (~1hr) first generation microscopy based instrument that can directly enumerate germinating spores by imaging the luminescent halo forming around each spore [8]. A direct correlation between culturable counts and germinable counts was found for different inactivation methods (heat and UV), demonstrating the usefulness of germinability as a complementary viability measurement to culturability.

1.2 Environmental Sample Results

Spores have been found across a wide range of extreme conditions [1, 3]. Detecting viable microbial life in samples from extreme environments can be challenging using culturing methods alone [5]. This lab has used the Tb^{3+}-DPA microscopy assay for detecting germinable spores in a variety of samples from extreme environments including the Atacama desert and Greenland ice core [6, 7].

The percentage of viable spores is an even more interesting parameter to look at than simply the number of viable (germinable and culturable) spores recovered, but to do this total spore counts are needed for each sample as well. Percentage viability allows comparison of the effects different extreme environments have on spores by controlling for differences in total spore populations in different settings. An environmental study of this nature would measure combined effects and complement lab based studies where each particular stress can be studied individually [1, 4]. Development of the capability to measure total spore counts, and therefore percent viability, using Tb^{3+}-DPA is discussed below.

2. Results and Continued Work

2.1 Instrument development

The first generation microscope instrument has been improved and condensed towards the development on an automated instrument. Figure 1 shows an image of the instrument hardware now. The microscope has been replaced with a simple relay lens that provides 1:1 imaging on to the CCD. The much greater light collection efficiency of this lens makes imaging possible without magnification. In addition the relatively large depth of field (1 – 2 mm) and the ability to view a 8.7 × 6.5 mm all at once make finding and focusing on the region of interest in a sample much simpler, directly enabling the goal of full automation.

Figure 1 also shows the new excitation light source made up of 10 UV (280 nm) LEDs positioned in a ring around the collection lens. The monochromatic nature of the LEDs provides a much lower background excitation source helping to improve signal to noise from the spores. It also

allows for greater Tb^{3+} concentrations leading to stronger spore signal. Lastly the ring geometry of the light source provides much better fill of the imaged area, simplifying image post processing and insuring that sufficient power to detect spores reaches the whole image area. Figure 2A. shows an example of an image taken with the new setup, direct enumeration of the 262 spores germinated (out of ~ 650 deposited).

2.2 Total spore enumeration

The key measurement for percentage viability (germinability or culturability) is the total spore, (viable and nonviable) measurement. Using the Tb^{3+}-DPA instrument developed for detection of spore germination it is also possible to measure the total spore population. Figure 2B shows a sample exposed to Tb^{3+} after autoclaving. The spores observed look just like their germinated counterparts in Figure 2A showing that DPA released by lysis still permits individual spore imaging. Currently ~50% of total spores are detected after lysis, but optimization of the kill exposure will allow detection of > 90%.

2.3 Preliminary % viability results

Using the combination of the total spore detection with germinability and culturability analyses, permits measurement of the percentage viability for spores in environmental samples. Preliminary results from terrestrial analog sites including Mt. Kilimanjaro and Lake Vida will be presented.

3. Figures

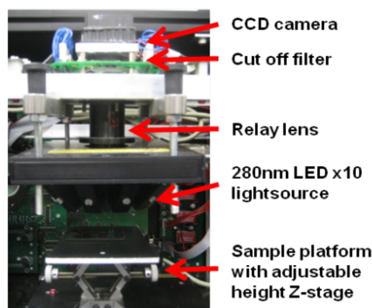

Figure 1. Improved experimental setup for enumeration of spores by detection Tb^{3+}-DPA luminescence.

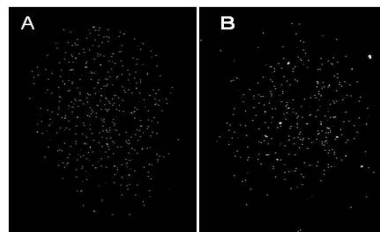

Figure 2. (A) Image of germinated pure spore suspension droplet. (B) Total spore measurement image of pure spores after autoclaving.

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