

# Bacillus pumilus Endospores: A Possible Model for Bacillus anthracis

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## ABSTRACT

The growing threat of bioterrorism, particularly the use of aerosolized *Bacillus anthracis* endospores, has necessitated the study and utilization of non-virulent, endospore-forming model organisms of *B. anthracis* for developing and assessing methods of sensitive, accurate bioweapon detection. *Bacillus anthracis* simulants should possess endospore dimensions comparable to *B. anthracis* and demonstrate antibody reactivity similar to other reported model organisms including *B. atrophaeus* and *B. thuringiensis*. The present study was conducted to analyze *B. pumilus* endospores (ATCC# 700814) as a possible new model for *B. anthracis*, and to compare endospores of *B. atrophaeus*, *B. thuringiensis*, and *B. pumilus*. Methods involved the preparation, enumeration, electron microscopic evaluation, and detection of these endospore-forming organisms using secondary fluorescently-labeled antibody. Scanning electron microscopy revealed that endospores of each *Bacillus* sp. had dimensions similar to those reported for *B. anthracis*, indicating they are acceptable size-based simulants. Antibody binding analyses showed polyclonal *B. atrophaeus* antibodies bound all three organisms, monoclonal *B. atrophaeus* antibodies cross-reacted with *B. pumilus*, and monoclonal *B. thuringiensis* antibodies bound only *B. thuringiensis*. The limit of fluorescence-based detection was approximately 30 endospores for each of the simulants. Results of this study demonstrate that *B. pumilus* endospores have similar dimensions and antibody reactivity with other recognized *B. anthracis* simulants, suggesting *B. pumilus* may be an appropriate non-pathogenic model for *B. anthracis*.

## INTRODUCTION

The delivery of *Bacillus anthracis*-contaminated envelopes to public officials in 2001 caused the U.S. to realize the depth of the threat of bioterrorism towards civilians, emphasizing the need for the study and timely identification of widely destructive biological agents. Endospores are dormant survival bodies produced by some bacteria, including *B. anthracis*, which survive deleterious changes in temperature and pH, nutritional deprivation, and irradiation, but can germinate into vegetative cells when in favorable environments.

Infection with *B. anthracis* may be acquired as cutaneous, gastrointestinal, and inhalational forms, with inhalational anthrax posing the greatest threat. Following inhalation, *B. anthracis* can have up to a 60 day incubation period. Initial nonspecific symptoms present such as high fever, cyanosis, shock, dyspnea, and mediastinal widening (1). With the widely varying incubation period and early nonspecific symptoms, anthrax can easily remain undiagnosed. If left untreated, inhalational anthrax has an 80% mortality rate (2).

It is imperative that technologies be developed for specific, rapid detection of biological agents so that these pathogens may be combated with appropriate antibiotics in a timely manner. Such investigations may be more readily undertaken by the use of less harmful models to protect the health of researchers. Accepted size-based simulants for *Bacillus anthracis* include *B. atrophaeus* and *B. thuringiensis* (3).

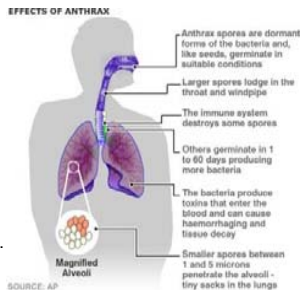


Fig. 1: Effects of anthrax

This study was conducted to:

- 1) evaluate *Bacillus pumilus* as new model for *Bacillus anthracis* in terms of dimensions via scanning electron microscopy
- 2) test and compare antibody-antigen binding of *Bacillus pumilus* endospores with those of other *Bacillus* spp.

## MATERIALS AND METHODS

### Production of Endospores

- *Bacillus pumilus* (ATCC# 700814) was grown on sporulation medium AK agar #2 at 30°C.
- Endospores were removed on select days.
- Percent sporulation was estimated by spore stain (Schaeffer-Fulton), shown to the right.
- Samples were washed, then heated to destroy vegetative cells.
- Dilutions grown on Tryptic Soy agar provided endospore counts.

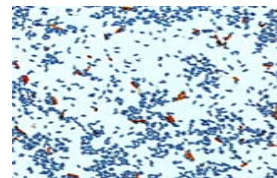


Fig. 2: Spore stain of *B. pumilus*. The pink bodies are vegetative cells, spores are blue.

### Analysis of Endospores

- A sample from the optimal day of sporulation (day 8) was analyzed with a scanning electron microscope.
- SEM micrographs were evaluated with NIH-provided Image J software to determine endospore dimensions.

### Detection of Endospores

- *Bacillus* spp. spores were applied to slides as microarrays and various primary and corresponding secondary antibodies were added.
- Fluorescence was determined with a confocal laser scanner and antibody-antigen binding patterns were analyzed.

## RESULTS

### Analysis of Endospore Dimensions

	mean length	mean width
<i>Bacillus atrophaeus</i>	1.39 ± 0.04 μm	0.77 ± 0.01 μm
<i>Bacillus pumilus</i>	1.23 ± 0.03 μm	0.58 ± 0.01 μm
<i>Bacillus thuringiensis</i>	1.61 ± 0.18 μm	0.80 ± 0.07 μm

Table 1: Endospore dimensions of *Bacillus* species.

Previous studies reported that the spore diameter for *B. anthracis* is between 0.81 ± 0.08 μm and 0.86 ± 0.08 μm. The length of these spores fall into two categories: those with spore length of 1.26 ± 0.13 μm or shorter, and those with spore length between 1.49 and 1.67 μm (4).

### Detection of Endospores

#### Microarray Composition

- Row 1 = *Bacillus atrophaeus*
- Row 2 = *Bacillus pumilus*
- Row 3 = *Bacillus thuringiensis*
- Row 4 = *Clostridium sporogenes*
- Row 5 = Goat IgG, Cy5 label (+ control)

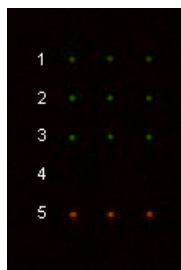
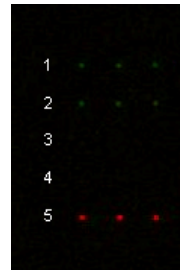


Fig. 5: Fluorescent detection of *Bacillus* spp. endospores. At left, polyclonal anti-*B. atrophaeus*-Cy 3 (green label) was added. At right, monoclonal anti-*B. atrophaeus*-Cy 3 was added. Cross reactivity of both antibodies occurred with *B. pumilus* (row 2).



	Polyclonal anti- <i>B. atrophaeus</i>	Monoclonal anti- <i>B. atrophaeus</i>	Monoclonal anti- <i>B. thuringiensis</i>	Polyclonal anti- <i>B. anthracis</i>	Monoclonal anti- <i>B. anthracis</i>
<i>B. atrophaeus</i>	+	+	-	+	-
<i>B. pumilus</i>	+	+	-	+	-
<i>B. thuringiensis</i>	+	-	+	+	-
<i>C. sporogenes</i>	-	-	-	-	-

Table 2: Antibody binding with endospores. (+) indicates binding.

Polyclonal anti-*B. atrophaeus* bound with all three *Bacillus* spp. endospores while monoclonal anti-*B. atrophaeus* bound to *B. atrophaeus* and *B. pumilus*. Monoclonal anti-*B. thuringiensis* only bound with *B. thuringiensis*. Additionally, polyclonal anti-*B. anthracis* bound all *Bacillus* spp. and monoclonal anti-*B. anthracis* failed to bind with any endospores. No binding occurred with the negative control *Clostridium sporogenes*.

## DISCUSSION

Based on similarities in endospore dimensions, *B. pumilus* seems to be an acceptable non-pathogenic, size-based simulant for *B. anthracis*. The monoclonal anti-*B. atrophaeus* bound *B. atrophaeus* and *B. pumilus* only, suggesting they may have similar endospore coat compositions. Because *B. atrophaeus* and *B. thuringiensis* are reported to be acceptable simulants for *B. anthracis*, and the monoclonal anti-*B. atrophaeus* only cross reacted with *B. pumilus*, it appears that *B. pumilus* would be a comparable, if not better, simulant than *B. thuringiensis*. While cross-reactivity among *Bacillus* species is reportedly common (5), the use of a monoclonal *B. anthracis*-specific antibody suggests preliminary detection can be achieved. These studies show that *B. pumilus* could be used as a new model organism for *B. anthracis*, and may be used in developing and testing anthrax detection technologies. This fluorescent antibody-based detection process can be performed in just a few hours, which would be beneficial for rapid presumptive identification. Consequently, treatment of the organism during a possible terrorist attack could be begun at an early stage following exposure, resulting in decreased morbidity and mortality.

## LITERATURE CITED

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