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Spore Size Comparison Between Several Bacillus Species

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Introduction

- **GOALS** - Better understand properties of simulants vs. agents. Provide data to help choose appropriate simulants and surrogates for specific tasks.
- **METHOD** - Compare properties of simulants to those of the agents.
- **FACILITY**
Microbiology
Team
BSL3
Laboratory



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Properties (Non-Medical)

- **Spore Size – length, width, aspect ratio**
- **Spore Density**
- **Effect of decon agents**
- **Fluorescence spectra**
- **Effect of UV radiation**



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Spore Size

- Important to design and development of samplers and detectors
- Published spore sizes - spores produced under different conditions for each species without extensive comparisons, size distributions or ranges.
- Systematic comparison of the size of *B.anthraxis* spores to size of other *Bacillus* spores (simulants/surrogates) - all spores produced under the same conditions.



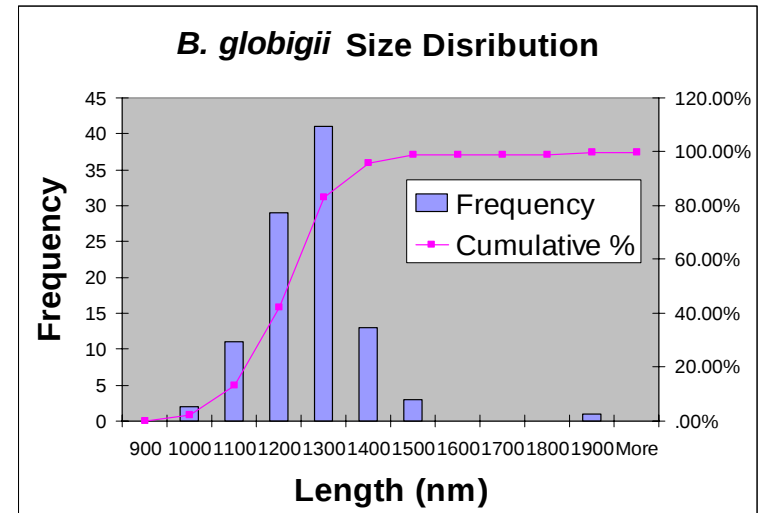
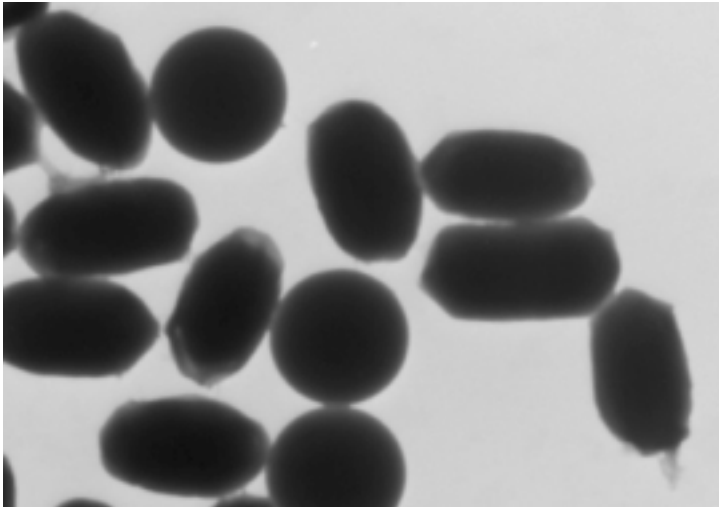
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Methods

- Prepared spores of *B.anthraxis* and other *Bacillus* species, including some common simulants/surrogates.
- Spores were fixed, negatively stained and imaged by TEM.
- Measurements of 100 spores (length, width, aspect ratio).
- Produced distributions.



B. globigii SB512 (*B. atrophaeus*)



- **Length**

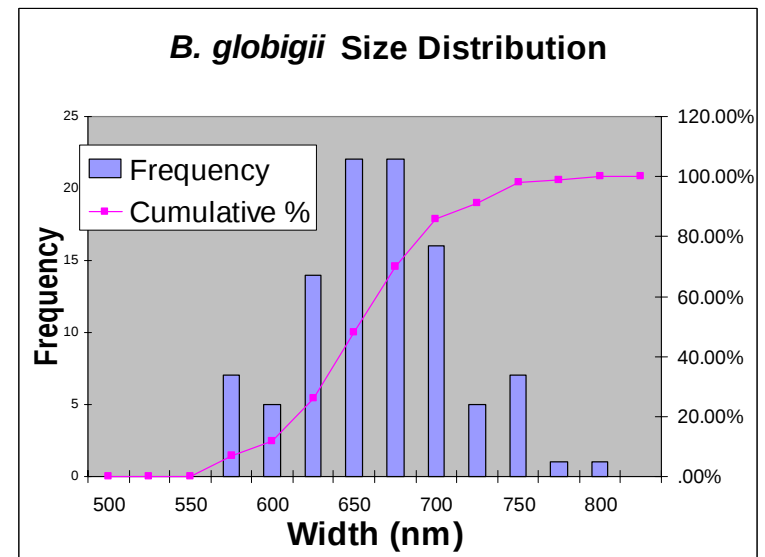
Mean $1.22 \pm 0.12 \mu\text{m}$

Range $0.91\text{-}1.90 \mu\text{m}$

- **Width**

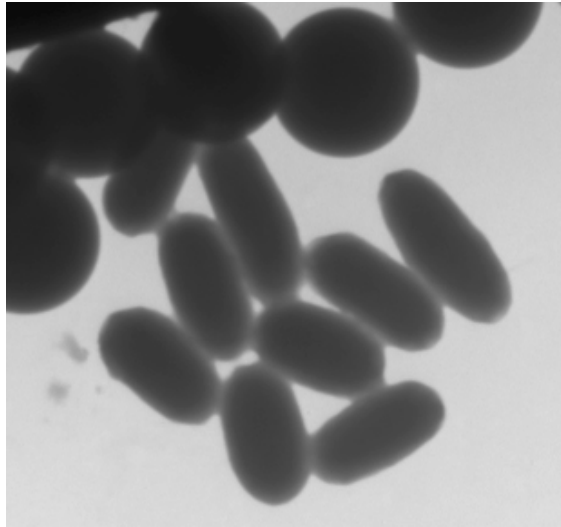
Mean $0.65 \pm 0.05 \mu\text{m}$

Range $0.56\text{-}0.80 \mu\text{m}$



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B. subtilis 1031



- **Length**

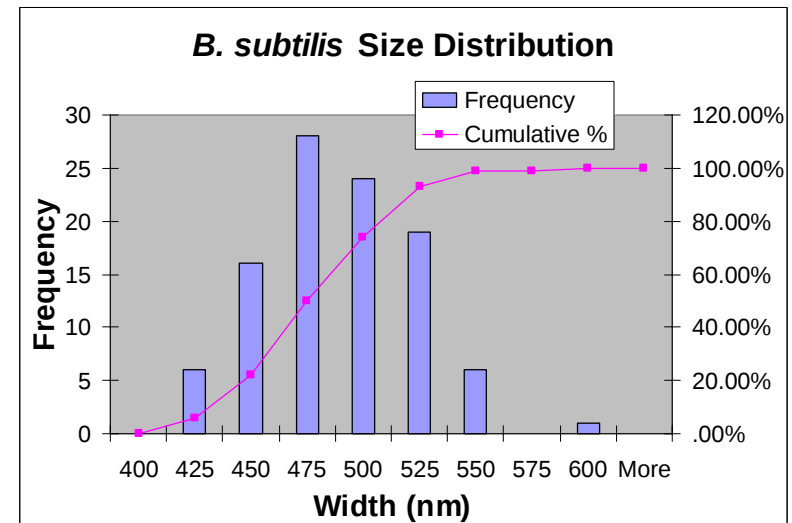
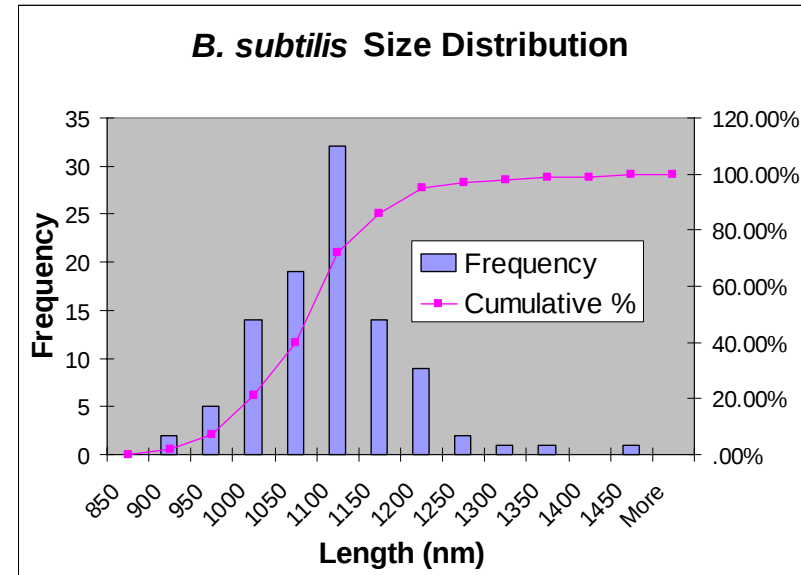
Mean $1.07 \pm 0.09 \mu\text{m}$

Range $0.87\text{-}1.41 \mu\text{m}$

- **Width**

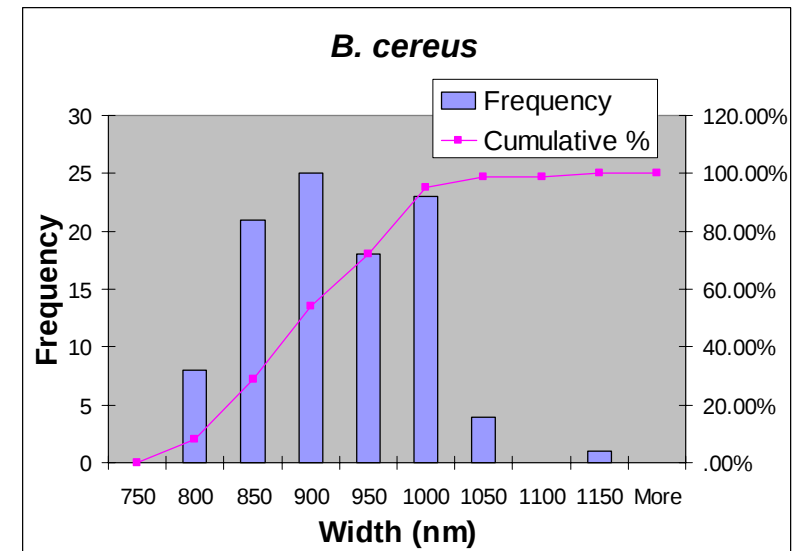
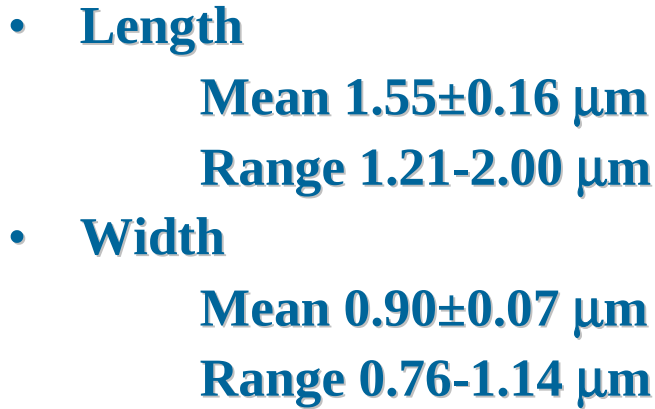
Mean $0.48 \pm 0.03 \mu\text{m}$

Range $0.41\text{-}0.58 \mu\text{m}$

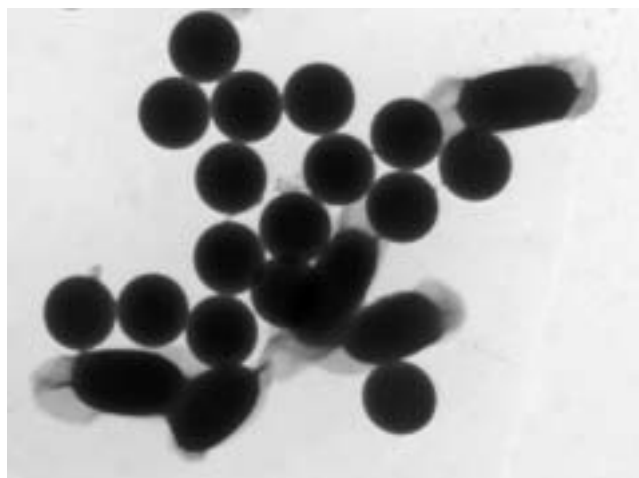


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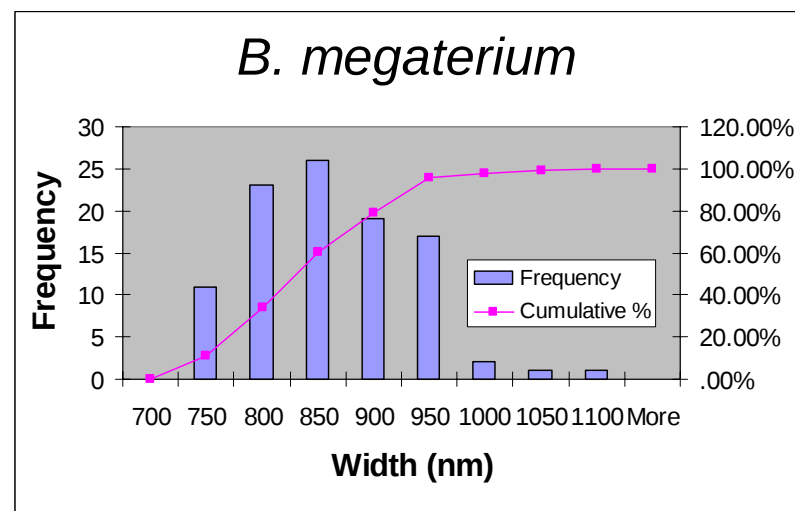
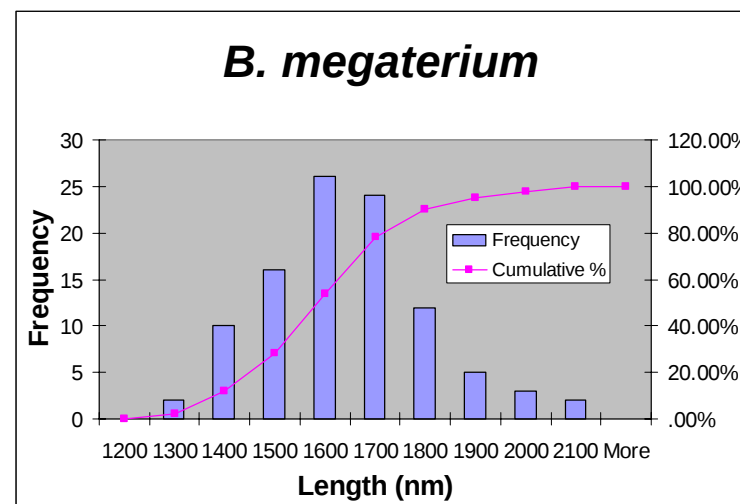
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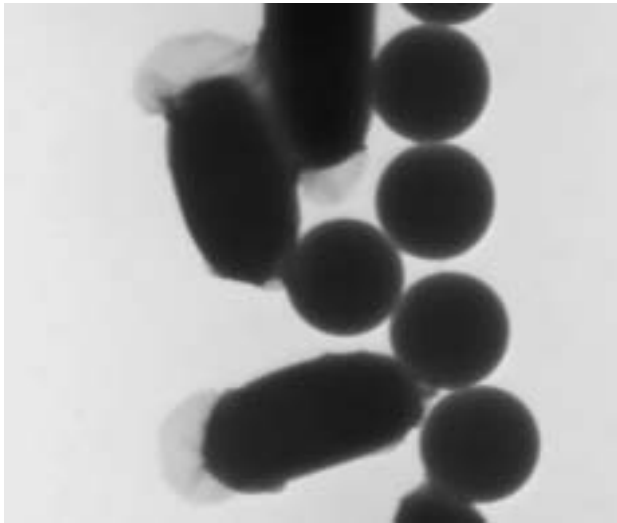
B. megaterium WW-15-4900



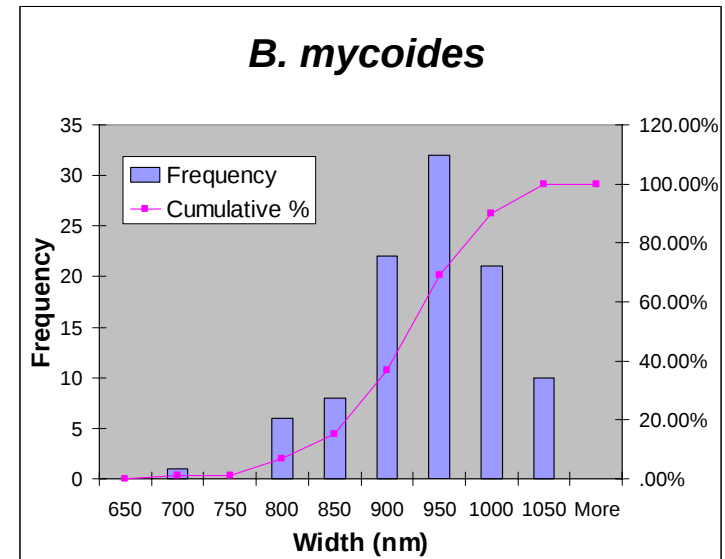
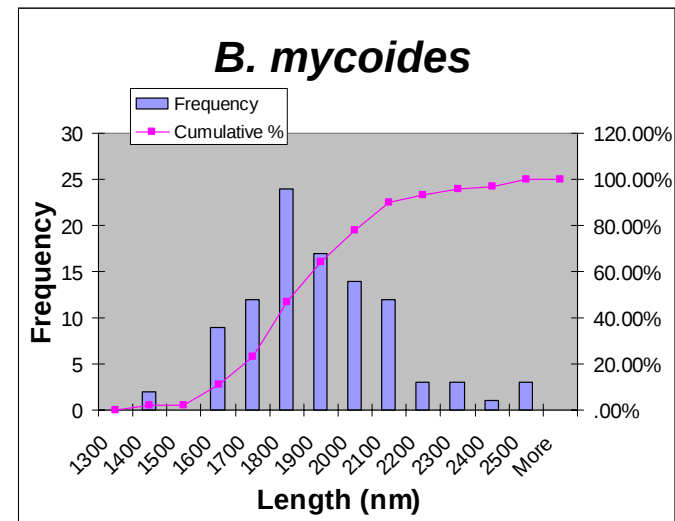
- **Length**
Mean $1.60 \pm 0.16 \mu\text{m}$
Range 1.28-2.04 μm
- **Width**
Mean $0.84 \pm 0.07 \mu\text{m}$
Range 0.70-1.05 μm



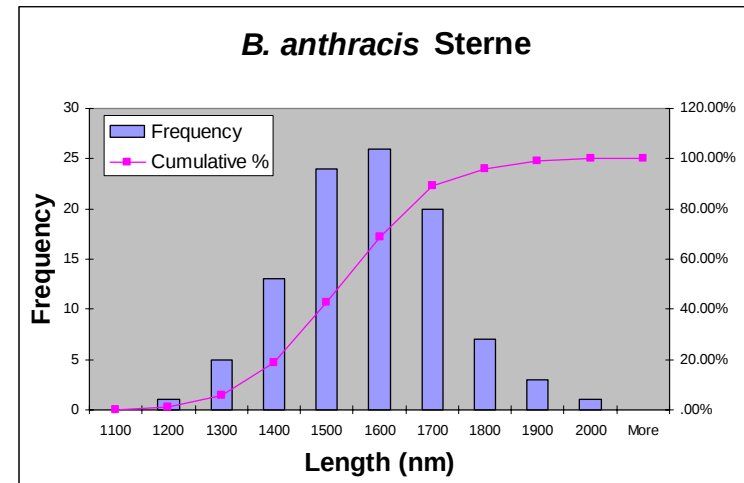
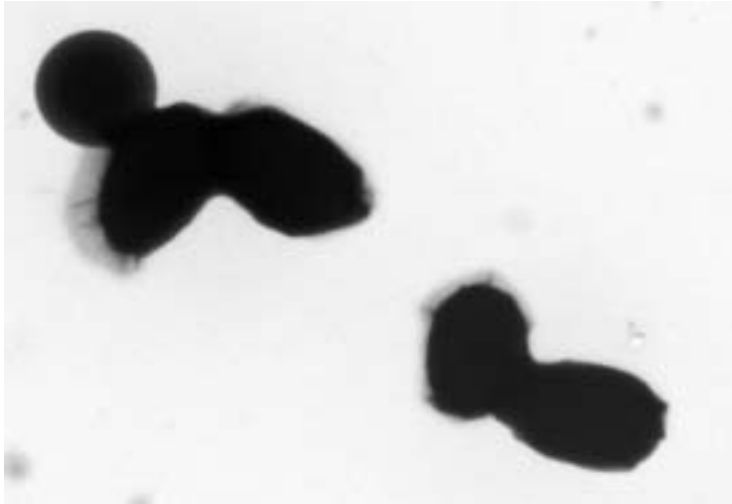
B. mycoides ATCC 10206



- **Length**
Mean $1.85 \pm 0.21 \mu\text{m}$
Range $1.33\text{-}2.44 \mu\text{m}$
- **Width**
Mean $0.91 \pm 0.07 \mu\text{m}$
Range $0.65\text{-}1.04 \mu\text{m}$



B. anthracis (Sterne)



- **Length**

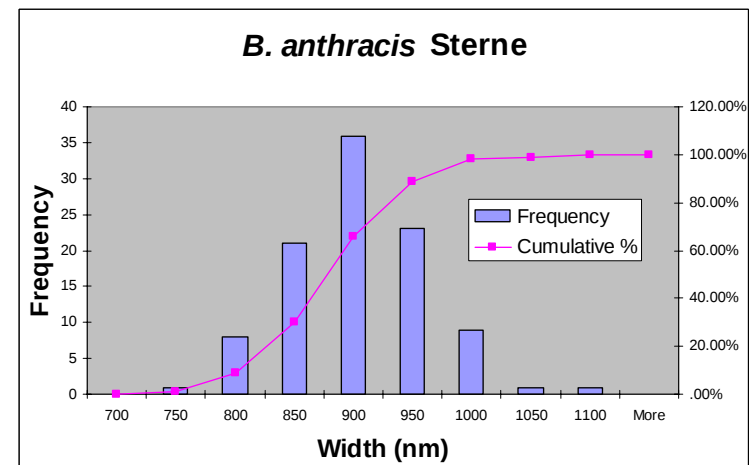
Mean $1.53 \pm 0.15 \mu\text{m}$

Range 1.19-1.92 μm

- **Width**

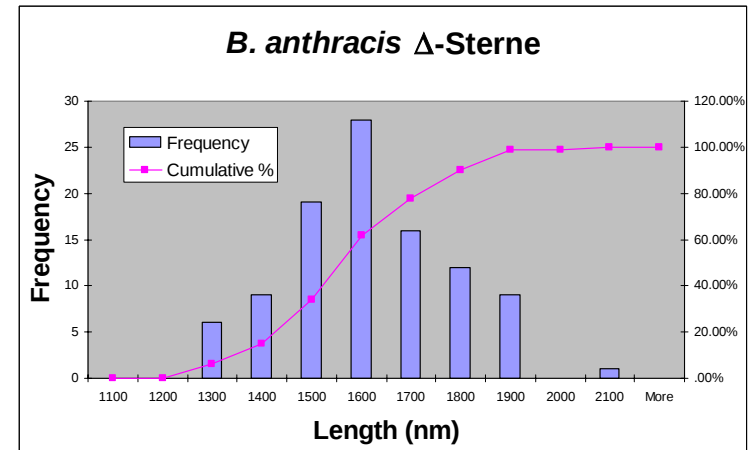
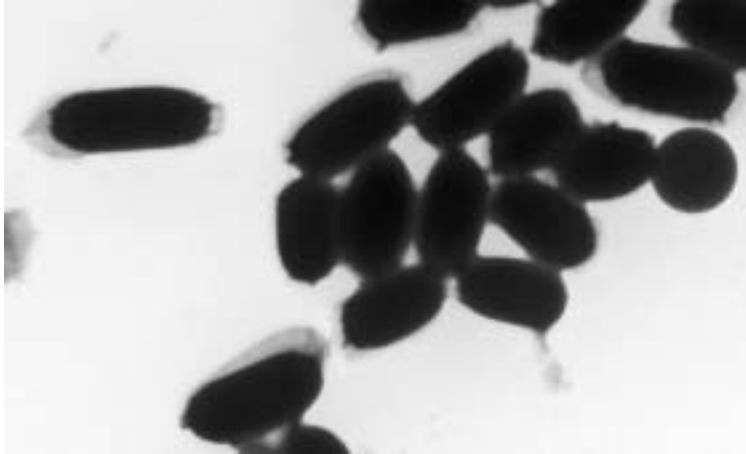
Mean $0.88 \pm 0.06 \mu\text{m}$

Range 0.71-1.09 μm



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B. anthracis (Δ -Sterne)



- Length

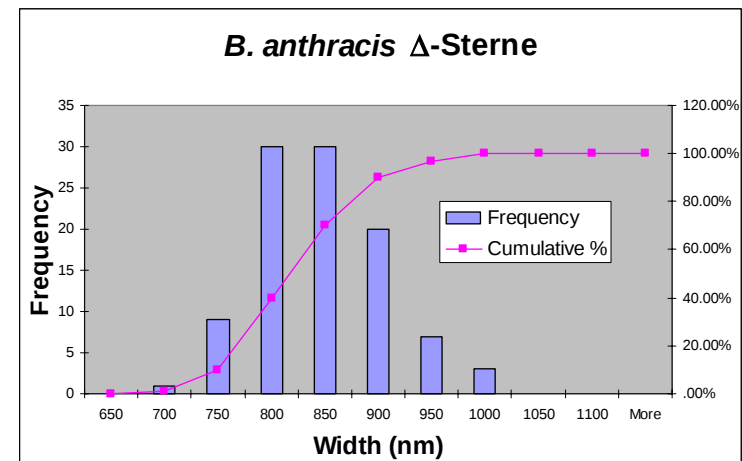
Mean $1.56 \pm 0.16 \mu\text{m}$

Range 1.23-2.05 μm

- Width

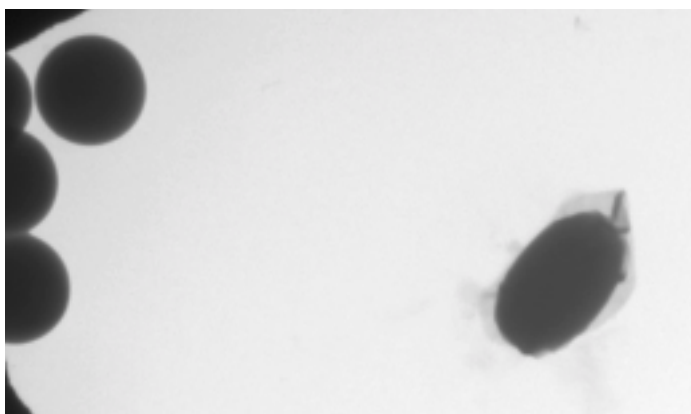
Mean $0.82 \pm 0.06 \mu\text{m}$

Range 0.68-0.98 μm



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B. anthracis (Albia)



- **Length**

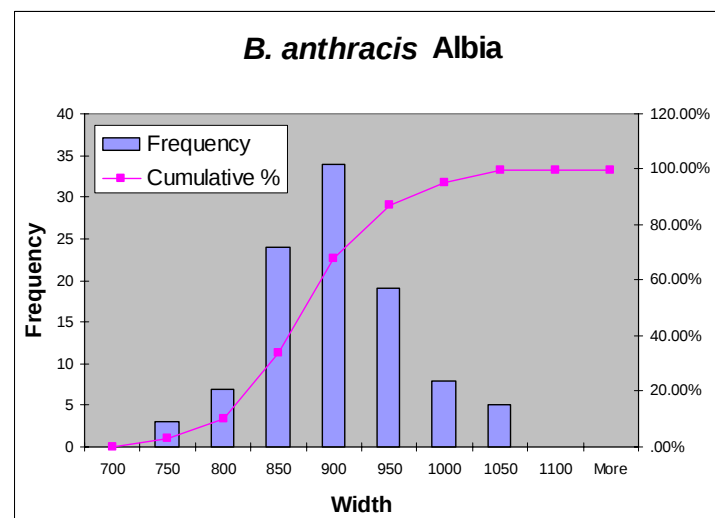
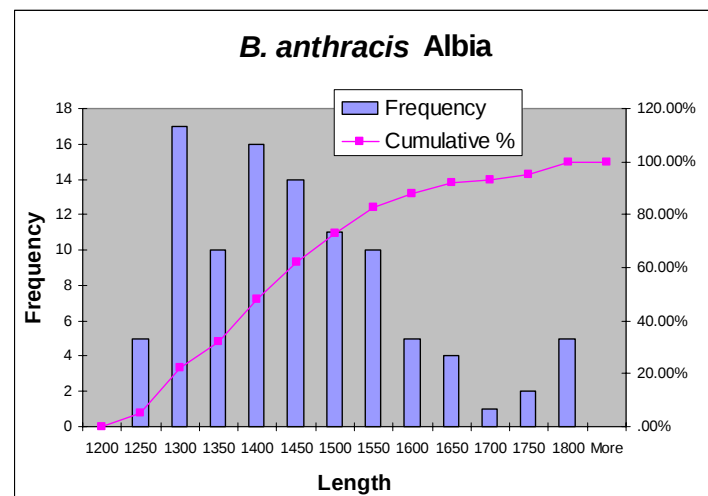
Mean $1.43 \pm 0.14 \mu\text{m}$

Range 1.22-1.79 μm

- **Width**

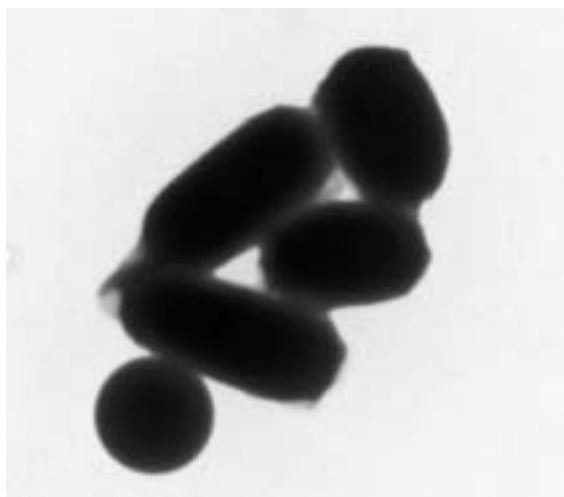
Mean $0.87 \pm 0.07 \mu\text{m}$

Range 0.71-1.03 μm



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B. anthracis (NCTC 1087)



- **Length**

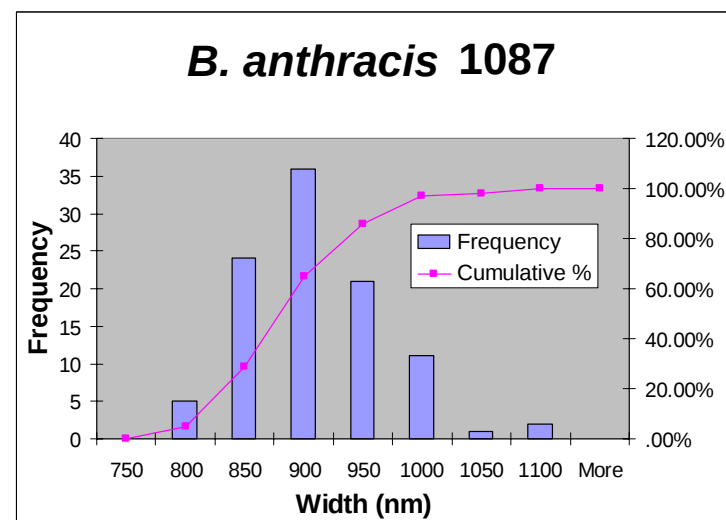
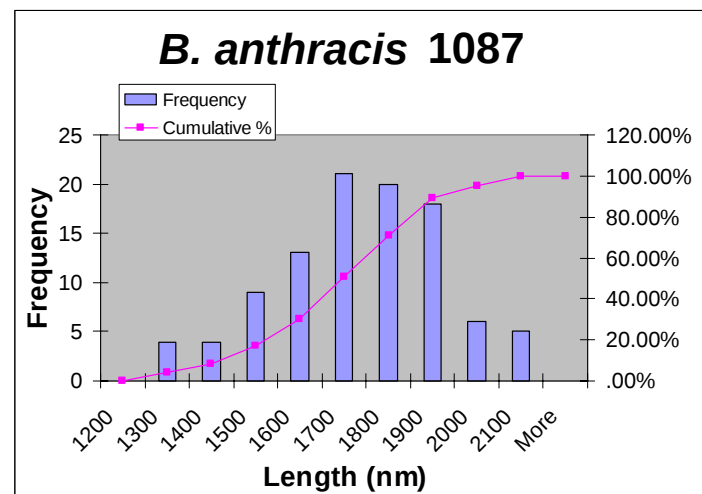
Mean $1.68 \pm 0.19 \mu\text{m}$

Range 1.23-2.08 μm

- **Width**

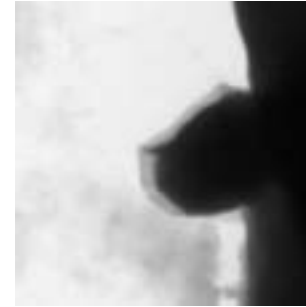
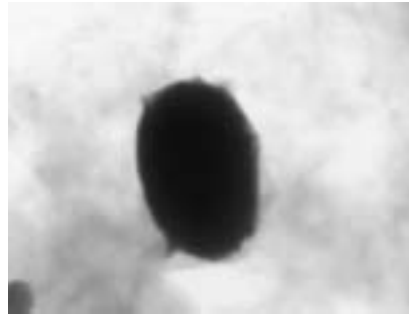
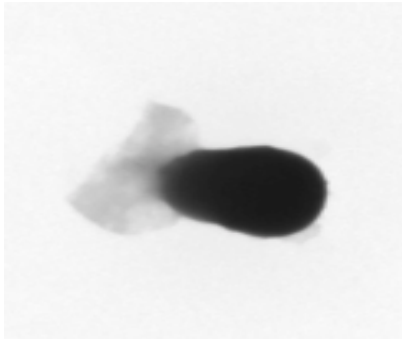
Mean $0.89 \pm 0.06 \mu\text{m}$

Range 0.76-1.09 μm

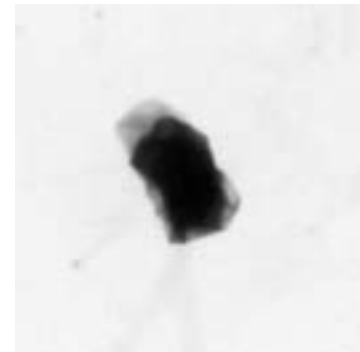


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***B. anthracis* (NCTC 1928)**

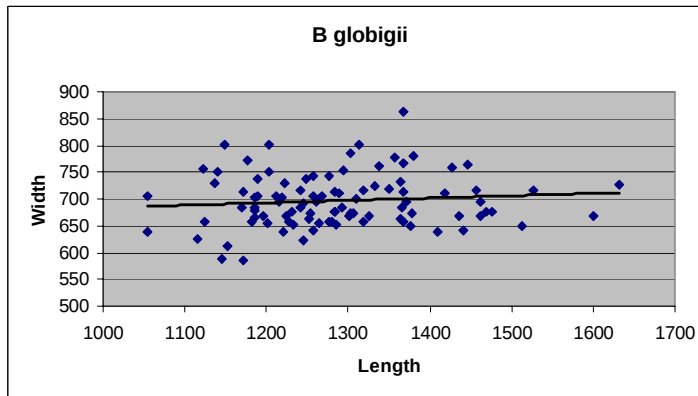


***B. anthracis* (LA1)**



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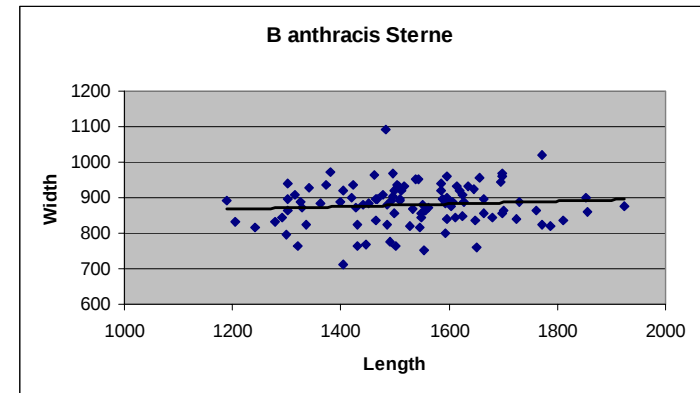
Aspect Ratio (L/W) of Bacillus spores



B. globigii

Mean 1.85 +/- 0.19

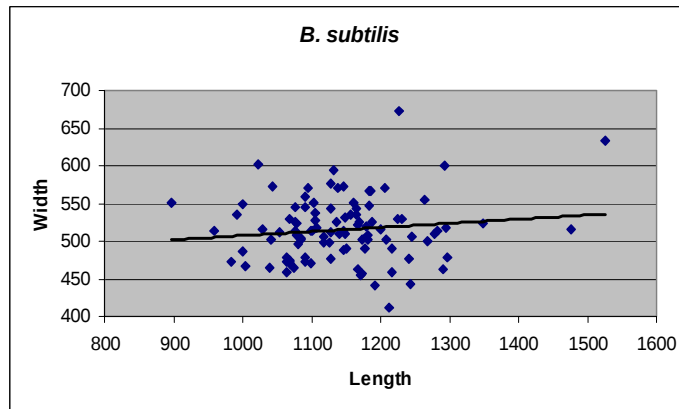
Range 1.43-2.39



B. a. Sterne

Mean 1.75 +/- 0.20

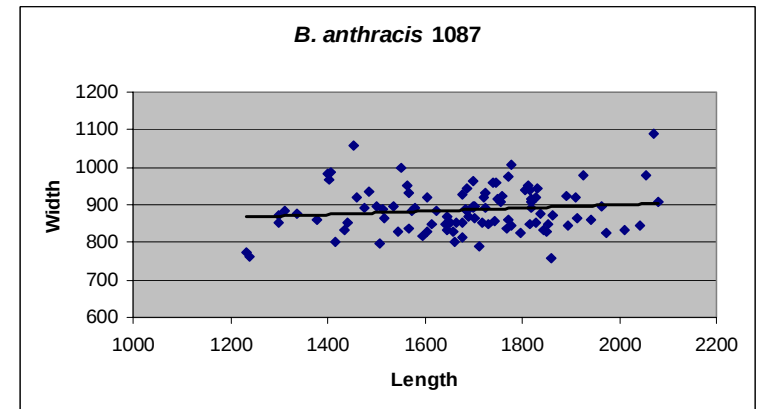
Range 1.33-2.20



B. subtilis

Mean 2.23 +/- 0.25

Range 1.63-2.95



B. a. NCTC 1087

Mean 1.91 +/- 0.23

Range 1.37-2.45



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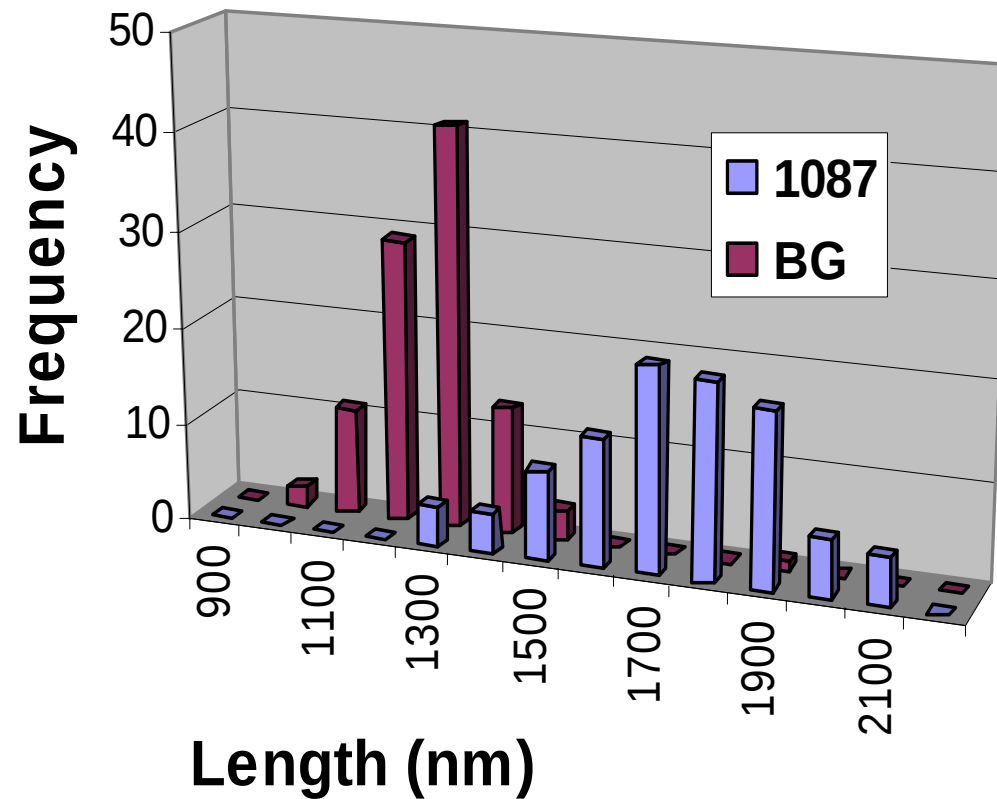
Comparison of *B. anthracis* with other *Bacillus* species

	Length (μm)	Width (μm)
<i>B. anthracis</i> Albia	1.43 +/- 0.14	0.87 +/- 0.07
<i>B. anthracis</i> 1087	1.68 +/- 0.19	0.89 +/- 0.06
<i>B. anthracis</i> Sterne	1.53 +/- 0.15	0.88 +/- 0.06
<i>B. anthracis</i> D-Sterne	1.56 +/- 0.16	0.82 +/- 0.06
<i>B. globigii</i>	1.22 +/- 0.12	0.65 +/- 0.05
<i>B. subtilis</i>	1.07 +/- 0.09	0.48 +/- 0.03
<i>B. cereus</i>	1.55 +/- 0.16	0.90 +/- 0.07
<i>B. megaterium</i>	1.60 +/- 0.16	0.84 +/- 0.07
<i>B. mycoides</i>	1.85 +/- 0.21	0.91 +/- 0.07



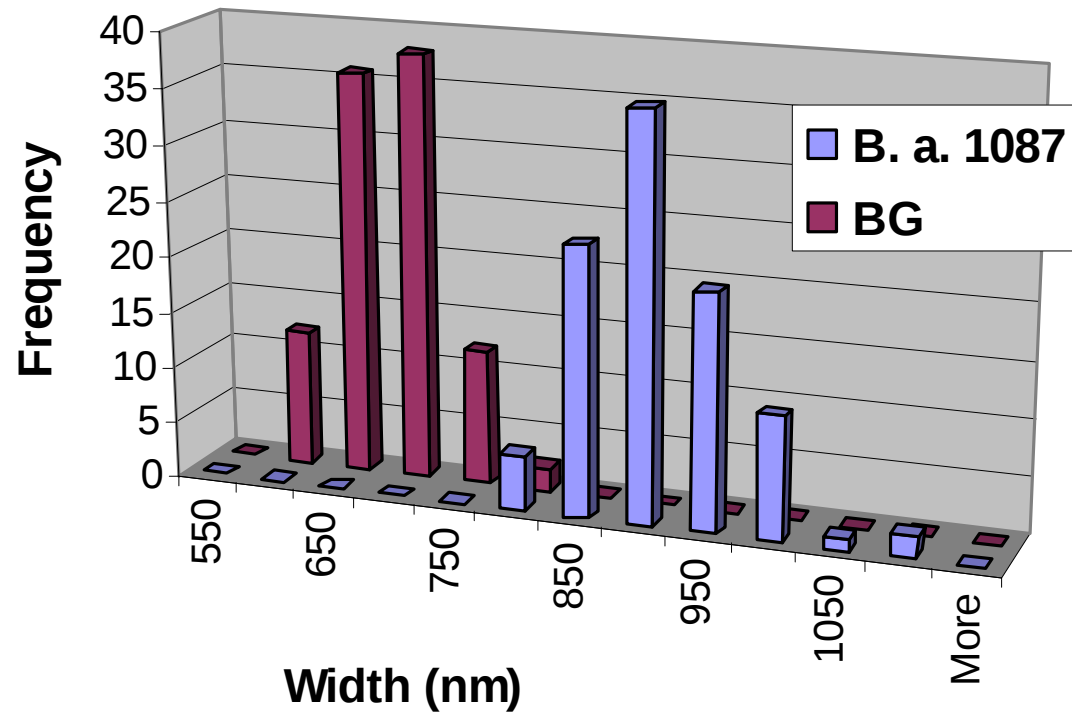
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B.a. 1087 vs. *B. g.* (Length)



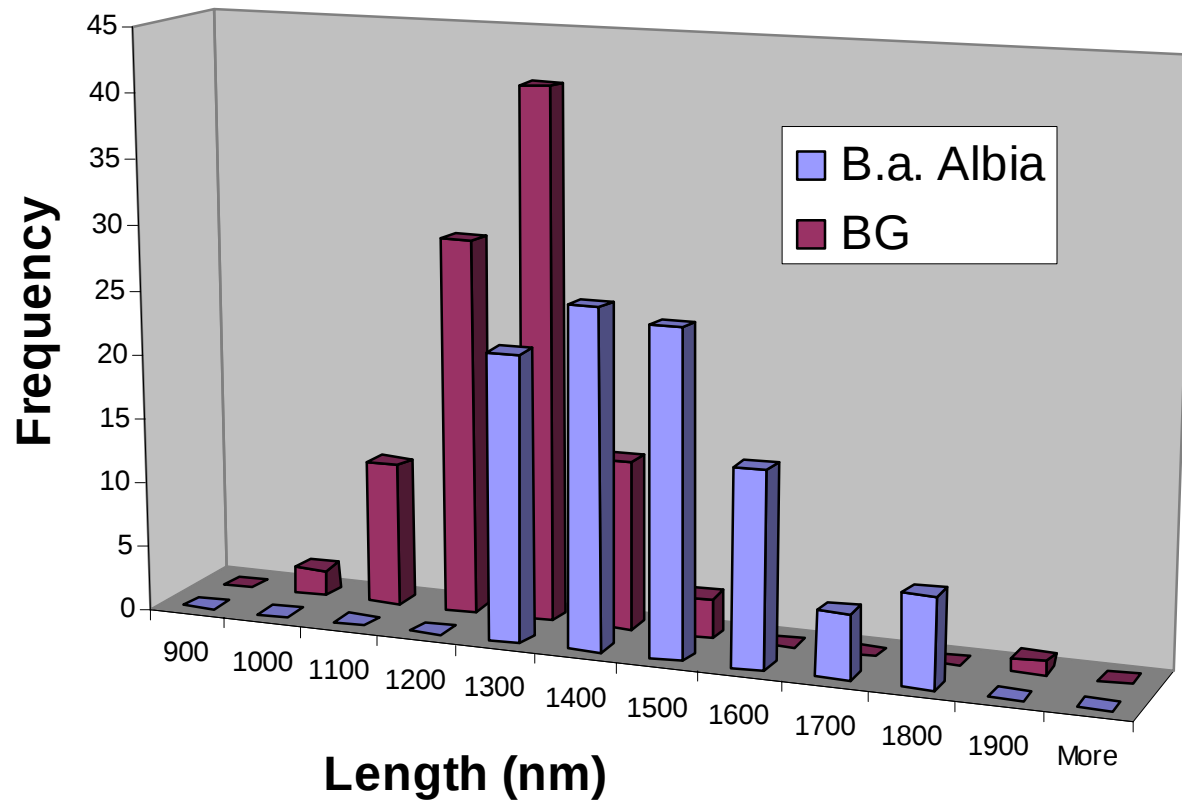
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B.a. 1087 vs. *B. g.* (Width)



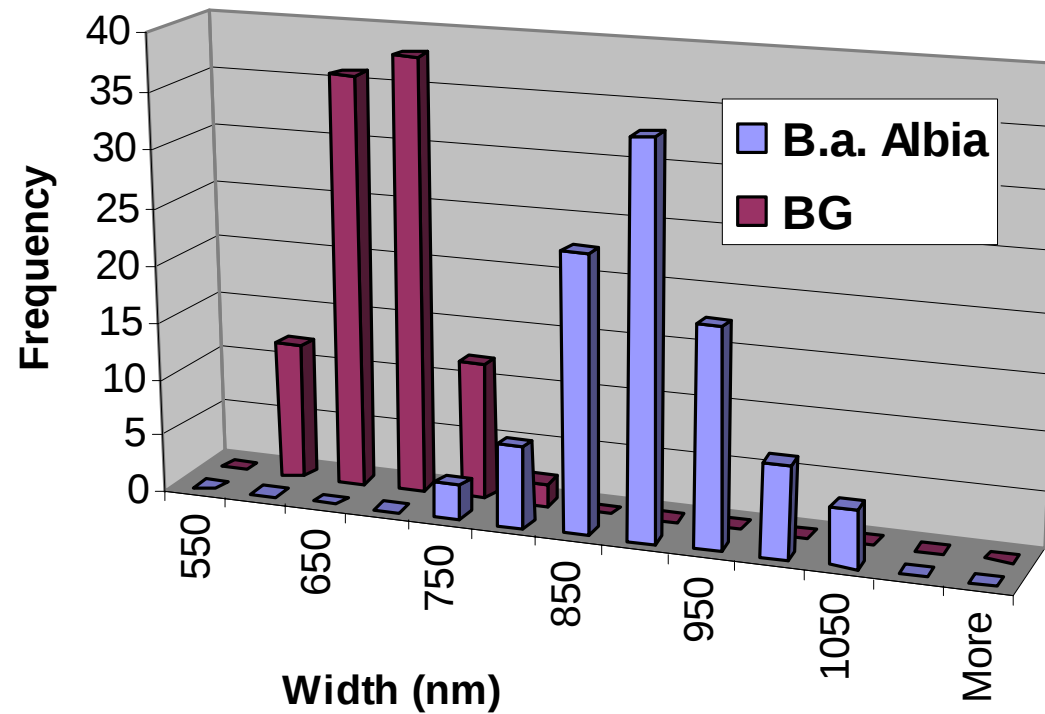
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B.a. Albia vs. *B. g.* (Length)



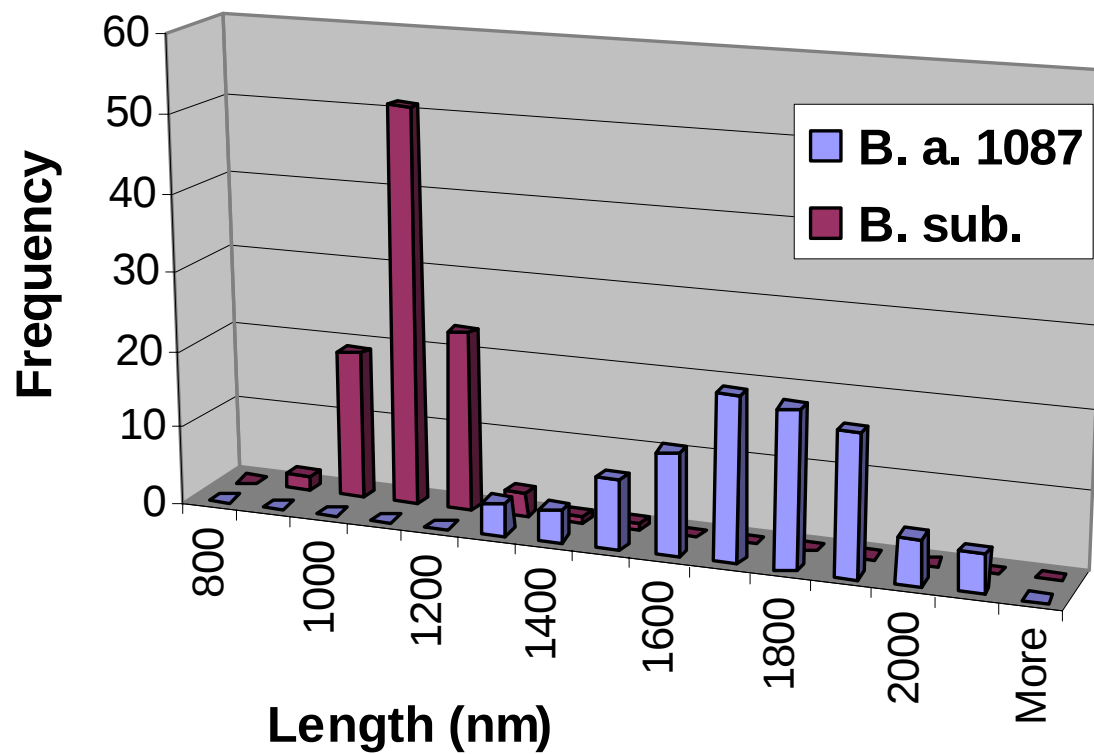
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B.a. Albia vs. B. g. (Width)

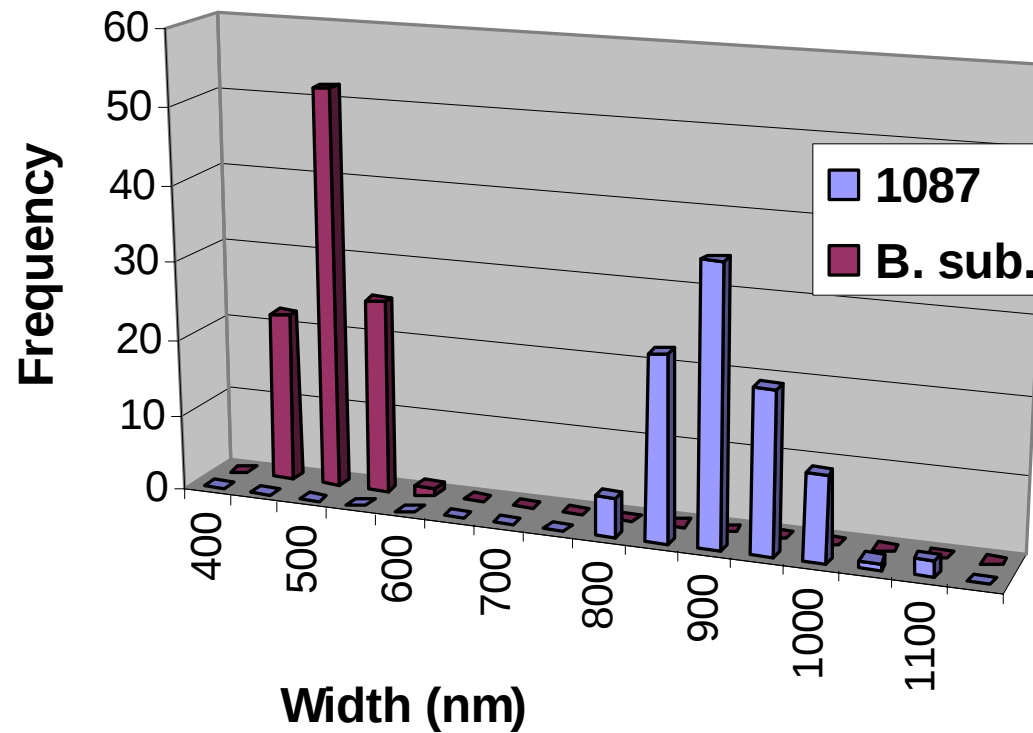


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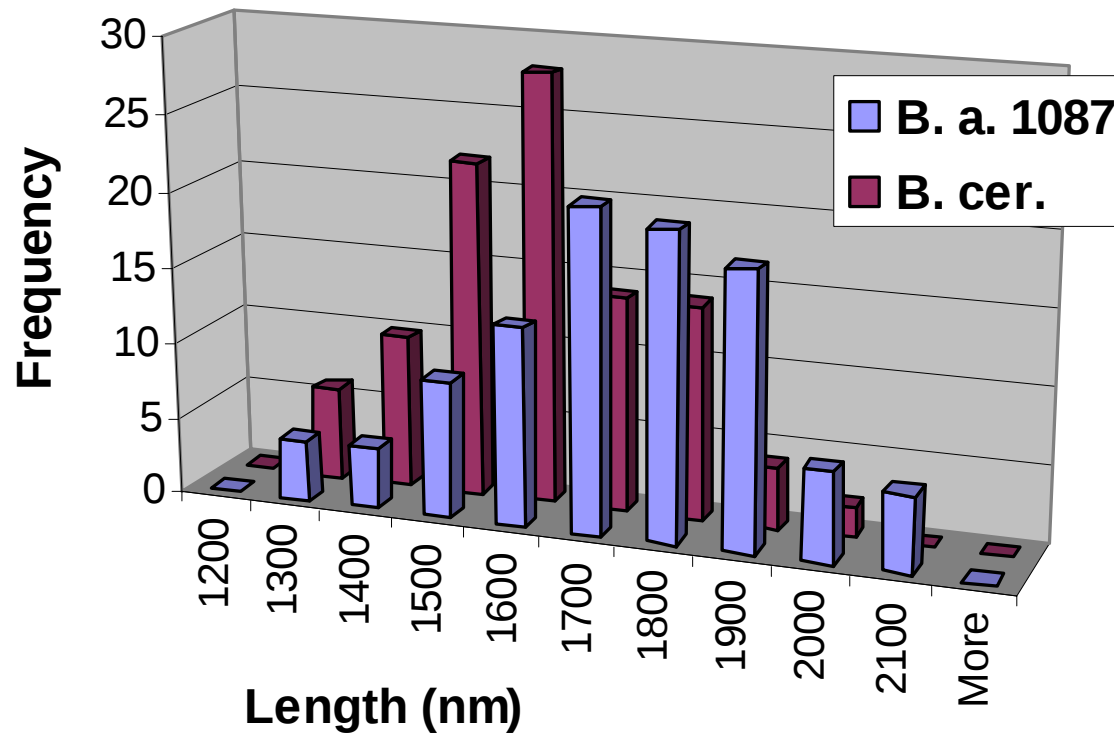
B.a. 1087 vs. *B. subtilis* (Length)



B.a. 1087 vs. *B. subtilis* (Width)

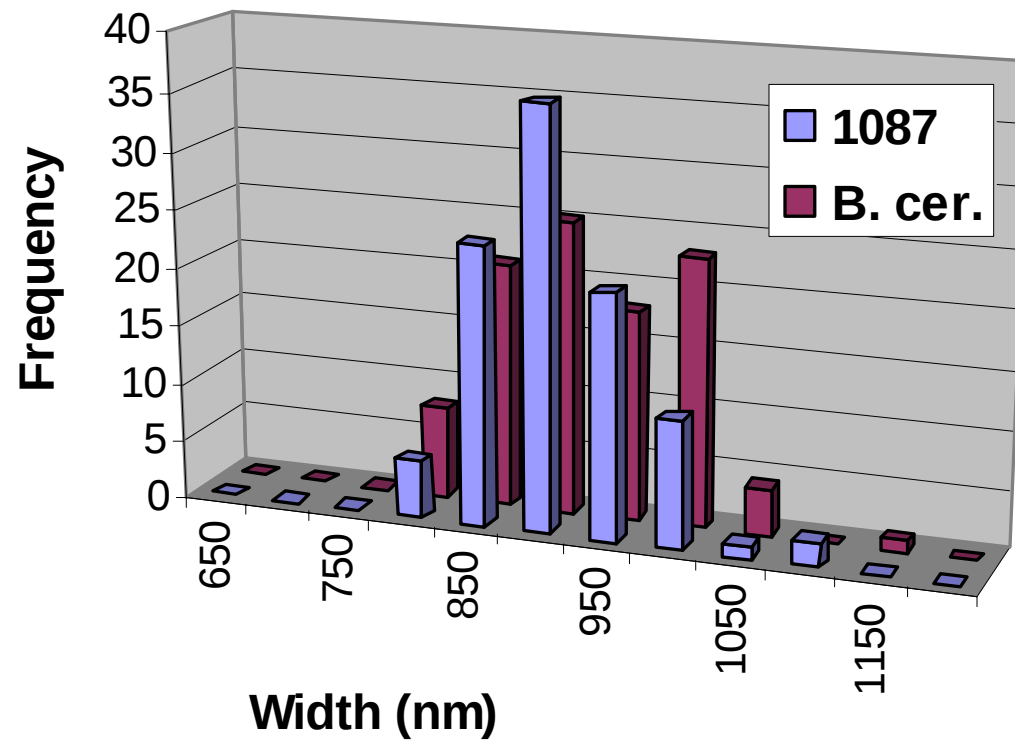


B.a. 1087 vs. *B. cereus* (Length)

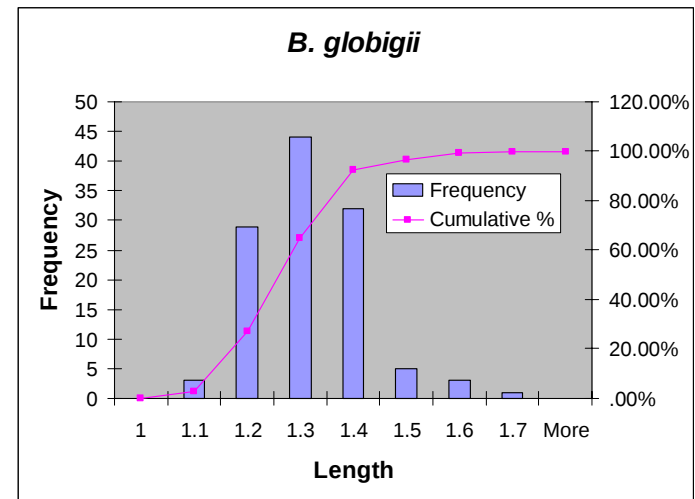
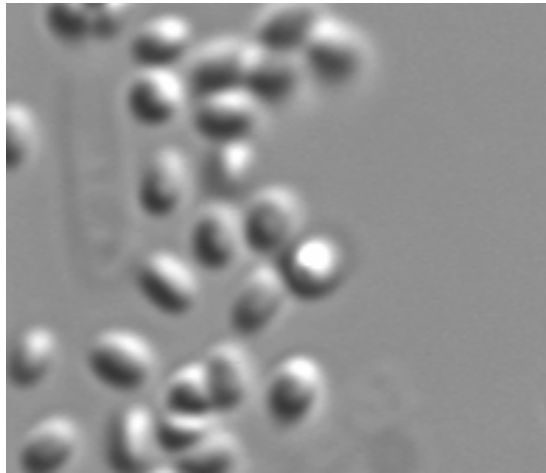


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B.a. 1087 vs. *B. cereus* (Width)



B. globigii SB512 (*B. atrophaeus*)



- Length

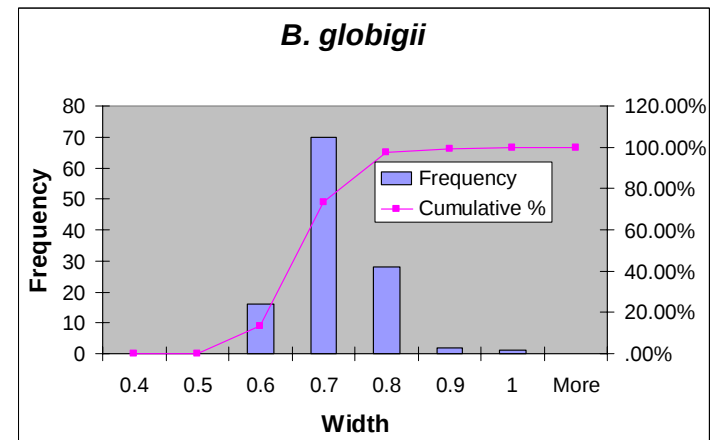
Mean $1.27 \pm 0.10 \mu\text{m}$

Range 1.04-1.61 μm

- Width

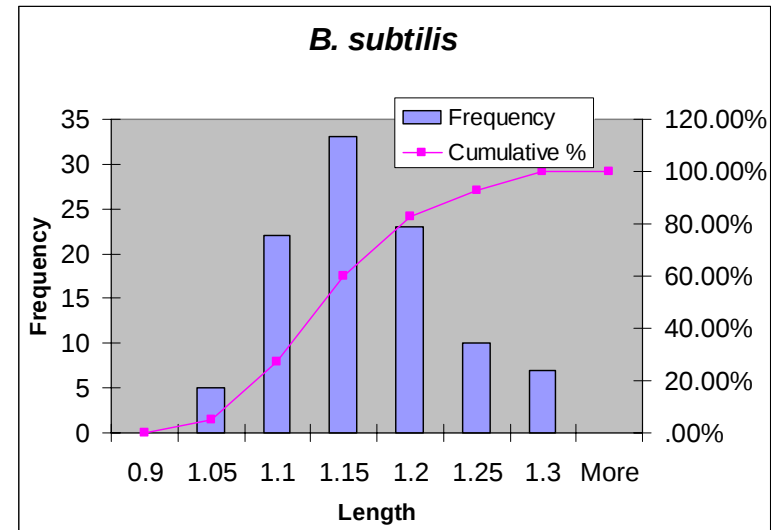
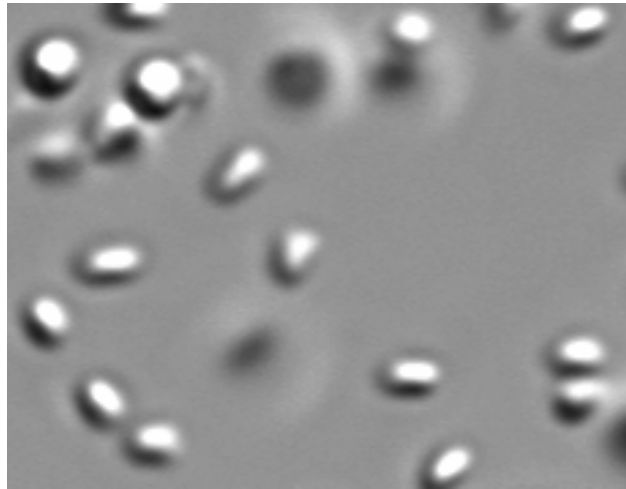
Mean $0.67 \pm 0.07 \mu\text{m}$

Range 0.50-0.94 μm



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B. subtilis 1031



- **Length**

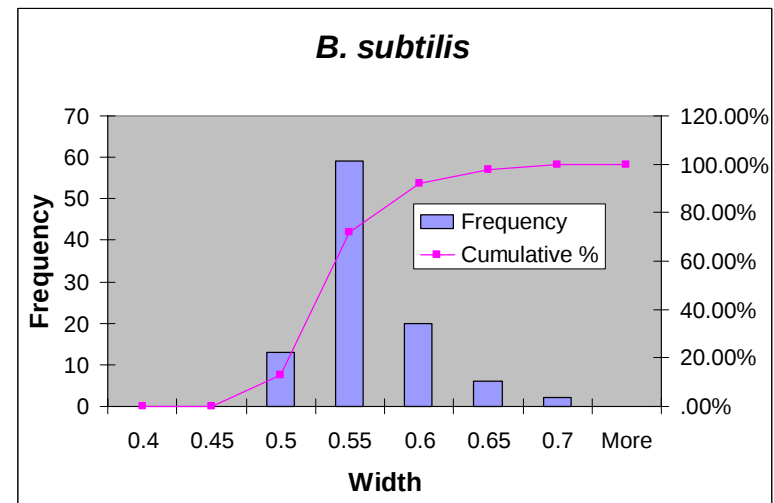
Mean $1.14 \pm 0.07 \mu\text{m}$

Range 1.04-1.28 μm

- **Width**

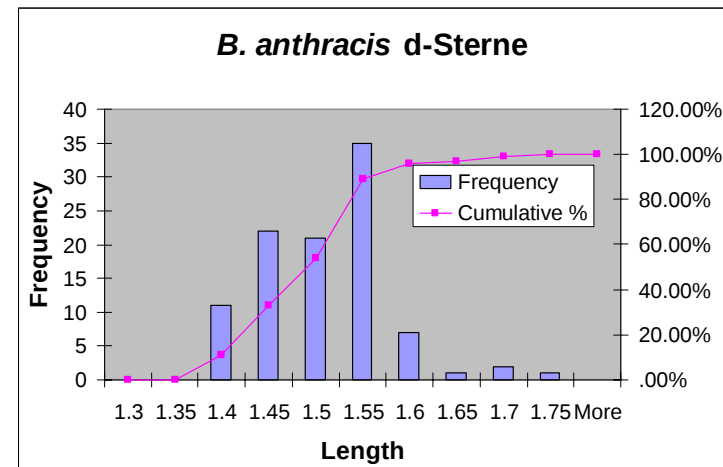
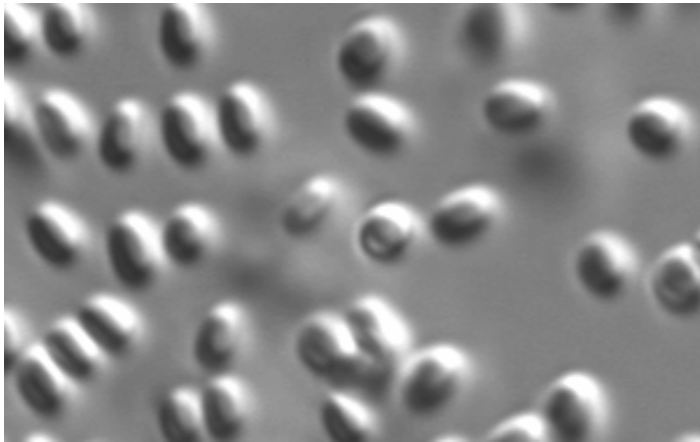
Mean $0.54 \pm 0.04 \mu\text{m}$

Range 0.46-0.69 μm



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B. anthracis (Δ -Sterne)



- **Length**

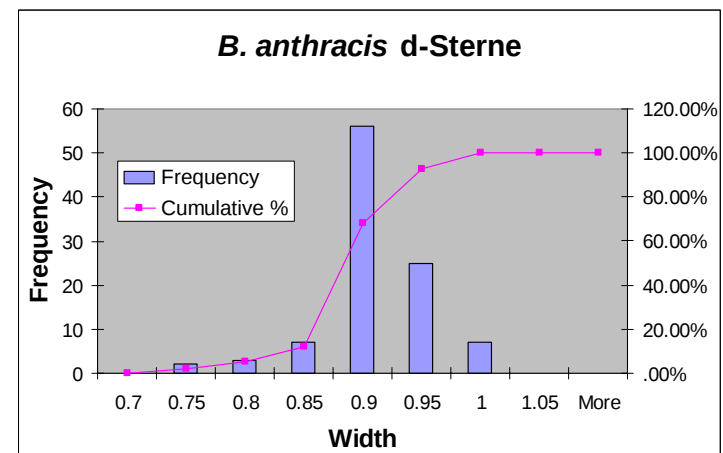
Mean $1.48 \pm 0.07 \mu\text{m}$

Range 1.37-1.71 μm

- **Width**

Mean $0.89 \pm 0.05 \mu\text{m}$

Range 0.75-1.00 μm



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Comparison of Measurements by TEM vs. OM

		Length	Width
<i>B. globigii</i>	TEM	1.22 +/- 0.12	0.65 +/- 0.05
	OM	1.27 +/- 0.10	0.67 +/- 0.07
<i>B. subtilis</i>	TEM	1.07 +/- 0.09	0.48 +/- 0.03
	OM	1.14 +/- 0.07	0.54 +/- 0.04
<i>B. anthracis</i> (d-Sterne)	TEM	1.56 +/- 0.16	0.82 +/- 0.06
	OM	1.48 +/- 0.07	0.89 +/- 0.05



CONCLUSIONS

- Systematic comparison of spore properties of several *Bacillus* species.
- Compared spore size properties of 6 species of *Bacillus* by TEM.
- *B. anthracis* spores are significantly larger than spores of the most common simulants, *B. g.* and *B. subtilis*.
- *B. anthracis* spores are similar in size to *B. cereus* and *B. megaterium*.
- Spores of pathogenic and non-pathogenic *B. anthracis* are similar in size.



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Acknowledgements

MICROBIOLOGY TEAM

Leslie Williams

Warren Gardner

UNIVERSITY OF PENNSYLVANIA – BIOMEDICAL IMAGING CORE LABORATORY

Neelima Shah

Ray Meade



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