A Model Method for the Rapid Identification of Biological Warfare Agents

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Anthrax in Biological Warfare

- *Bacillus anthracis* spores
  - Highly resistant to environmental effects- heat, drying, and sunlight (Eitzen, 1997).
  - Cheap to produce- $50 per kilogram.
  - Easily grown in quantity.
  - Stable agent for dissemination (National Academy, Washington D.C., 1999).

Bacillus Spores

- *B. subtilis* – Difference in Morphology (Mizuki et al., 1998; Driks, 2002).
  - No appendages
- *B. cereus* – Phylogenetically and Morphologically similar.
  - Appendages
  - Similarity verified through VNTR analysis and BOX-PCR genomic fingerprinting (Kim et al., 2002).

Detection Technologies

- Metabolic assays (National Academy, Washintgon, D.C., 1999).
- Nucleic acid-based probes (Lytwyn et al., 2000; Hamels et al., 2001).
- Molecular typing (Boyd et al., 1996).
- Unfeasible for real-world use-
  - Slow
  - Unstable
  - Non-specific

Glycoconjugate Polymers

- Study of Carbohydrate Interactions (Bovin et al., 1993).
- High affinity and specificity of interactions achieved by polyvalency.
- Predetermined Properties
  - Type of Ligands attached
- Medicinal Use

Purpose

- The purpose of this project was to develop a rapid method of identifying *B. anthracis* spores by analyzing the monosaccharide compositions of closely related *Bacillus* spores and determining their binding specificities with glycoconjugate polymers.

Methodology

- Bacterial Spore Samples
  - *B. cereus* ATCC 11778
  - *B. subtilis* ATCC 9372
- Multivalent Fluoresceinated Polymers
  - Disaccharide-PAA-flu
  - ELISA Glycoconjugate assay
- Isolation of Spore Outer Layer/Appendages
- Monosaccharide Compositions of Spores
  - FACE Monosaccharide Composition Kit

Results- FACE Electrophoresis

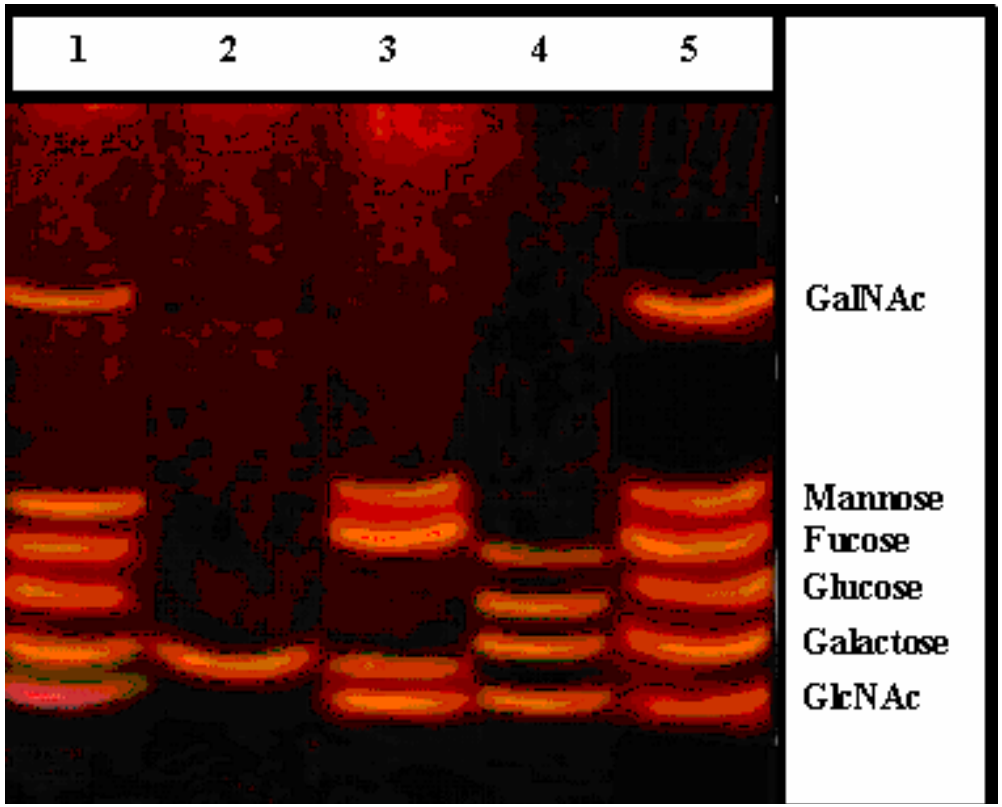


Figure 1. Monosaccharides composition of (1-5) spores' inner shell: Lane 1- neutral *B. cereus*; Lane 2- amino *B. cereus*; Lane 3- neutral *B. subtilis*; Lane 4- amino *B. subtilis;* Lane 5- AMAC–labeled monosaccharides standard.

Neutral Monosaccharide Composition of Inner Shells of *Bacillus* Spores

	Monosaccharides					
Spores	GalNAc	Mannose	Fucose	Glucose	Galactose	GlcNAc
B. cereus	+	+	+	+	+	+
B. subtilis	--	+	+	--	+	+

Amine Monosaccharide Composition of Inner Shells of *Bacillus* Spores

	Monosaccharides					
Spores	GalNAc	Mannose	Fucose	Glucose	Galactose	GlcNAc
B. cereus	--	--	--	--	+	--
B. subtilis	--	--	+	+	+	+

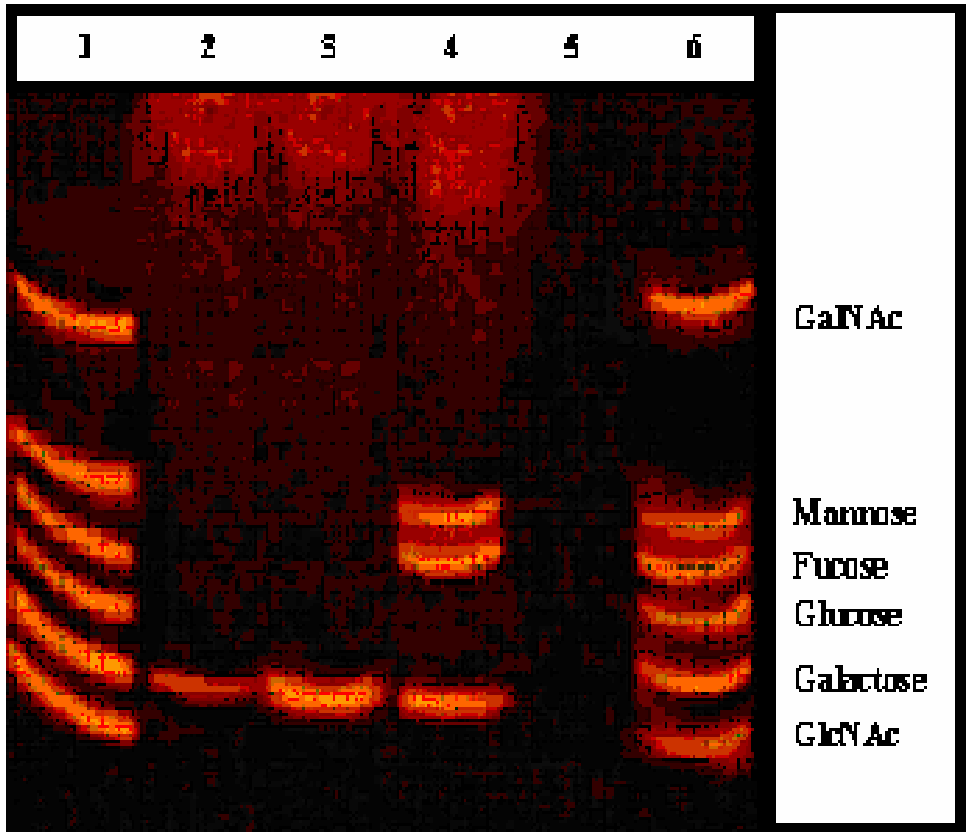


Figure 2. Monosaccharides composition of appendages of *B. cereus* and outer layer of *B. subtilis:* Lane 1, 6- AMAC–labeled monosaccharides standard; Lane 2 neutral *B. cereus*; Lane 3- amino *B. cereus*; Lane 4- neutral *B. subtilis*; Lane 5- amino *B. subtilis.*

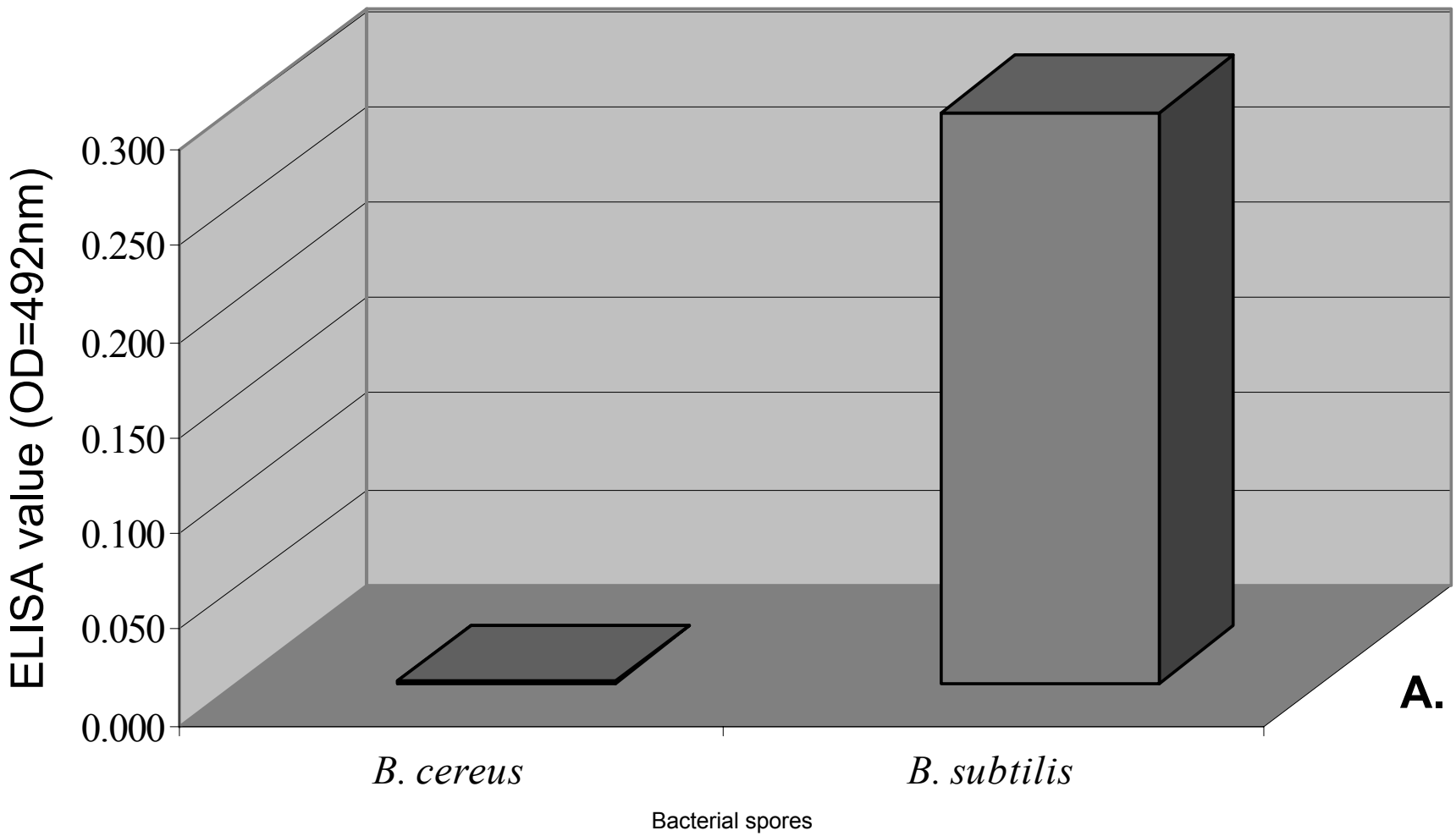
Amine Monosaccharide Composition of Inner Shells of *Bacillus* Spores

	Monosaccharides					
Spores	GalNAc	Mannose	Fucose	Glucose	Galactose	GlcNAc
B. cereus	--	--	--	--	+	--
B. subtilis	--	--	+	+	+	+

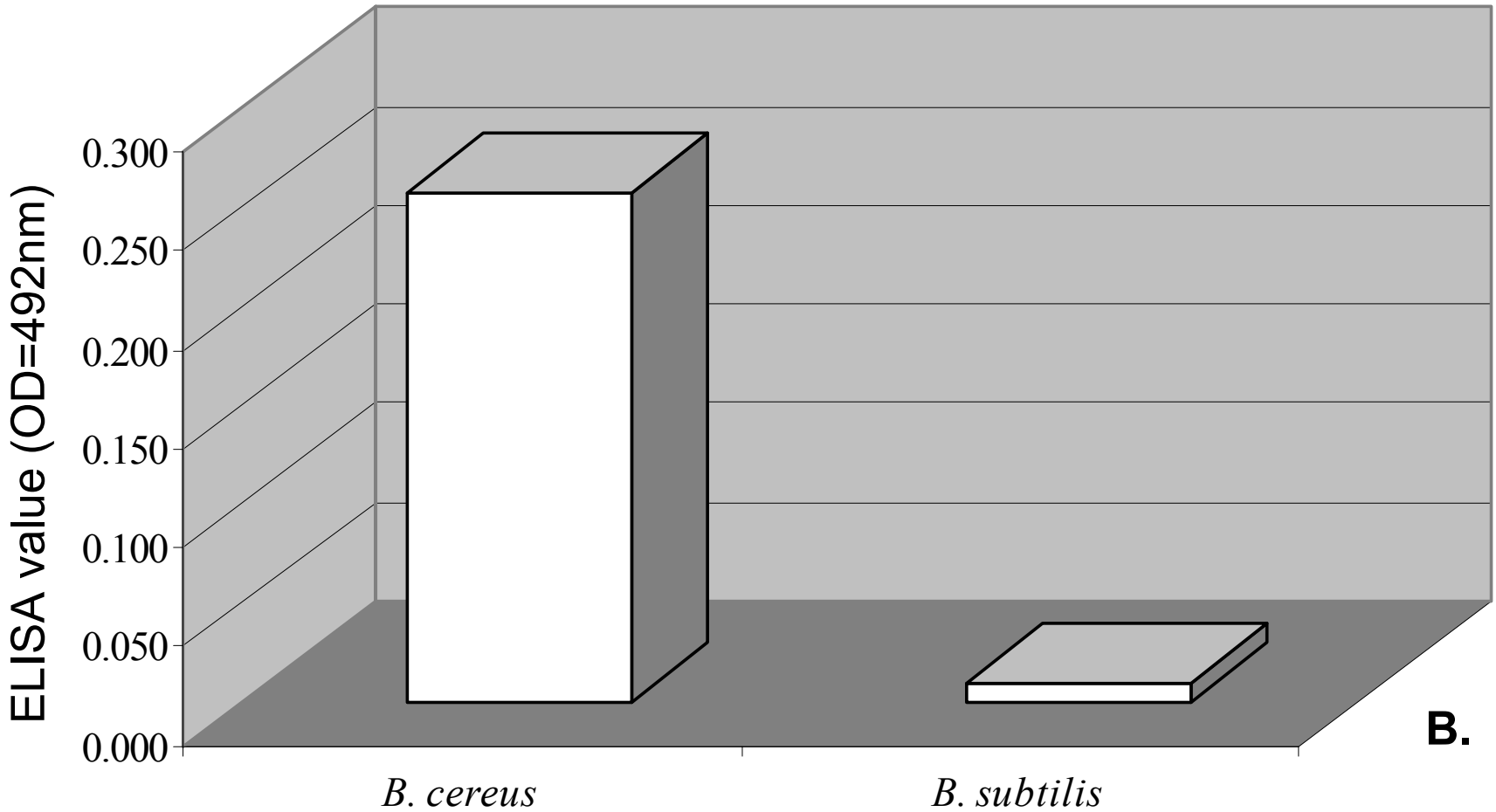
Amine Monosaccharide Composition of Inner Shells of *Bacillus* Spores

	Monosaccharides					
Spores	GalNAc	Mannose	Fucose	Glucose	Galactose	GlcNAc
B. cereus	--	--	--	--	+	--
B. subtilis	--	--	+	+	+	+

Results- ELISA (Polymer A)



Results- ELISA (Polymer B)



B.

Discussion

- Unique monosaccharides present in *Bacillus* spores.
- Monosaccharide composition of outer layer/appendages and inner shells are different.
- Monosaccharides serve as specific receptors.

Conclusion

- Glycoconjugate polymers may be used to rapidly identify *Bacillus* spores due to their ability to bind to glycan receptors present on the cell surfaces of the spores.
- This method may also be applied to the detection of *B. anthracis* spores due to their similarity with *B. cereus* spores.

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