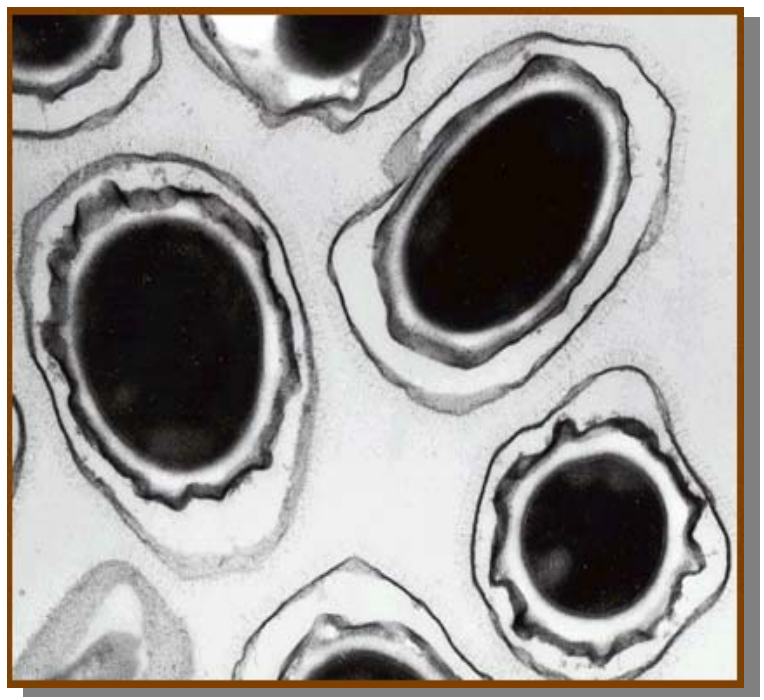
USING UV SPECTRA AND GEL ELECTROPHORESIS TO DISCERN THE CONSITUENT CARBOHYDRATE COMPONENT OF SPORE SURFACES

by David Paquiot, Olga Tarasenko, Pierre Alusta, and Kalle Levon

Aim

- The knowledge of the carbohydrate composition of the oligosaccharide component of the exterior of bacterial spores is a necessary prerequisite for the construction of specific biosensors that will serve to detect the presence of pathogens in a given environment.

- This information will also help elucidate how phenomenon such as bacterial recognition takes place in living systems.



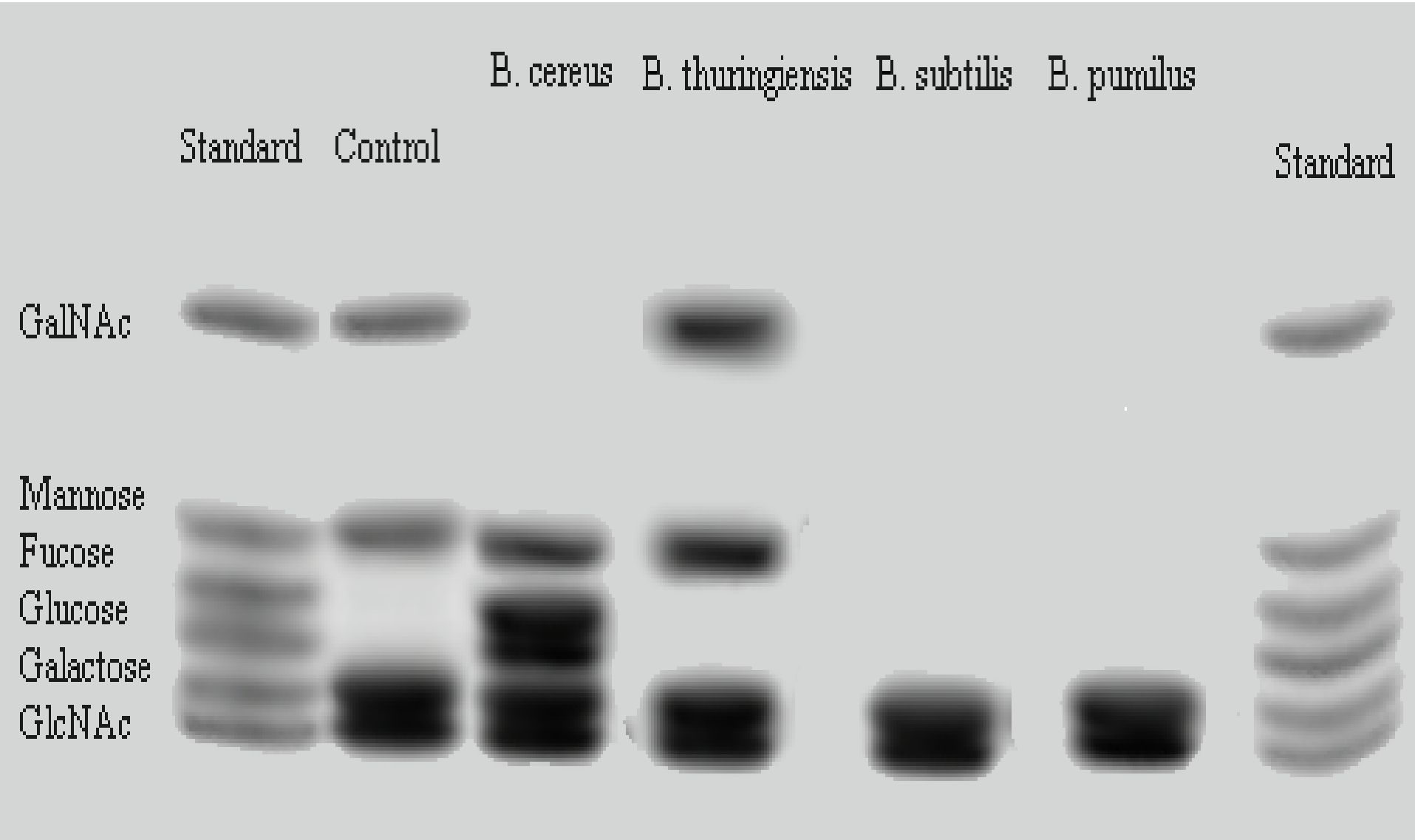
Methods

- FACE electrophoresis of *B. subtilis, B. pumilus, B. cereus,* and *B. thuringiensis* spores
  - neutral carbohydrates
  - amine carbohydrates
- UV spectroscopy of *B. subtilis, B. pumilus, B. cereus,* and *B. thuringiensis* spores
- UV spectroscopy of commercial available carbohydrates

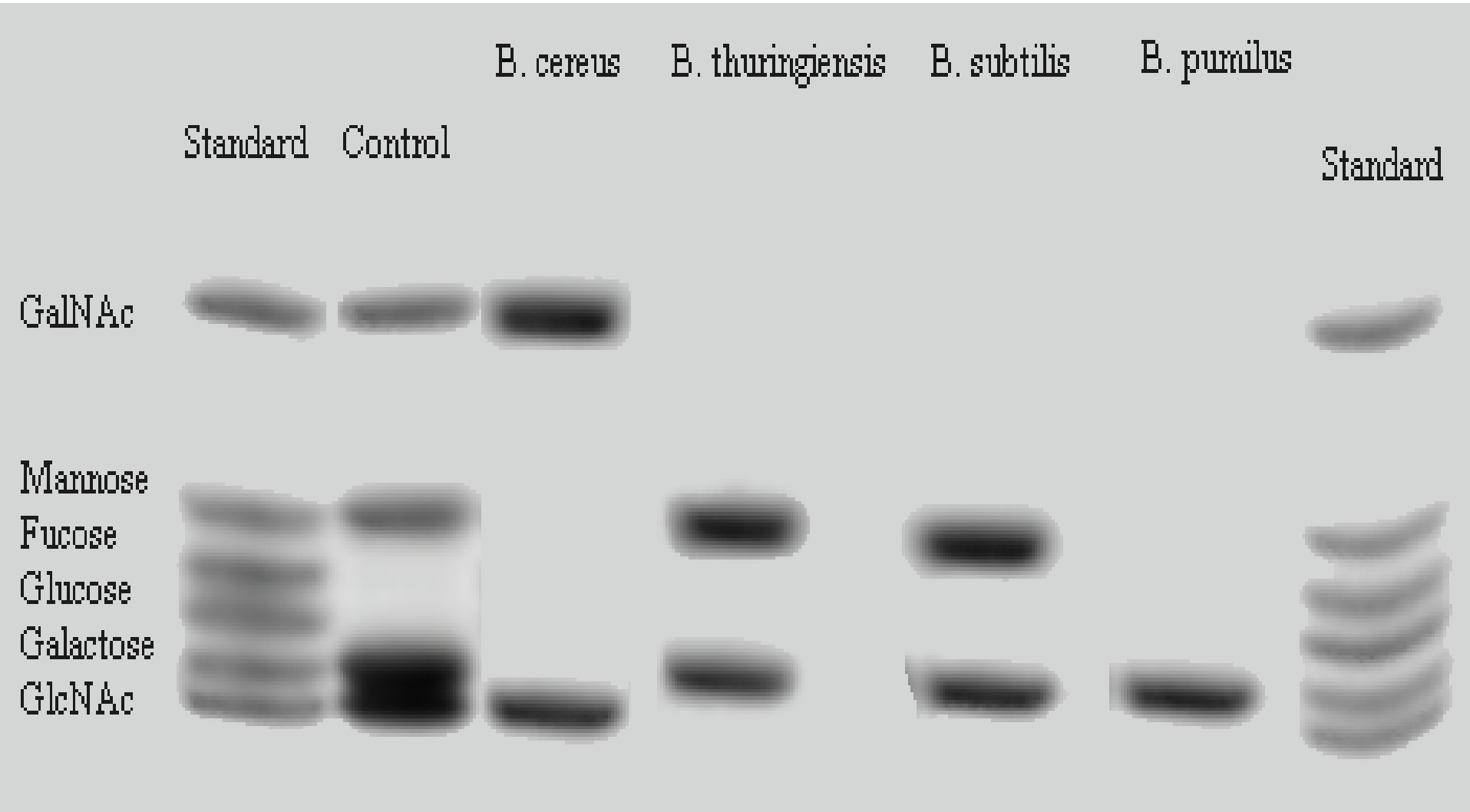
FACE Gel Electrophoresis

- Isolation of amine and neutral carbohydrates from intact spores by following fluoropore labeling procedure
- Polyacrilamide gel electrophoresis
- Gel electrophoresis analysis
- Conclusion
  - From this information we made an initial deduction of what carbohydrates were present on the various spores

Gel Electrophoresis Image of Neutral Carbohydrates



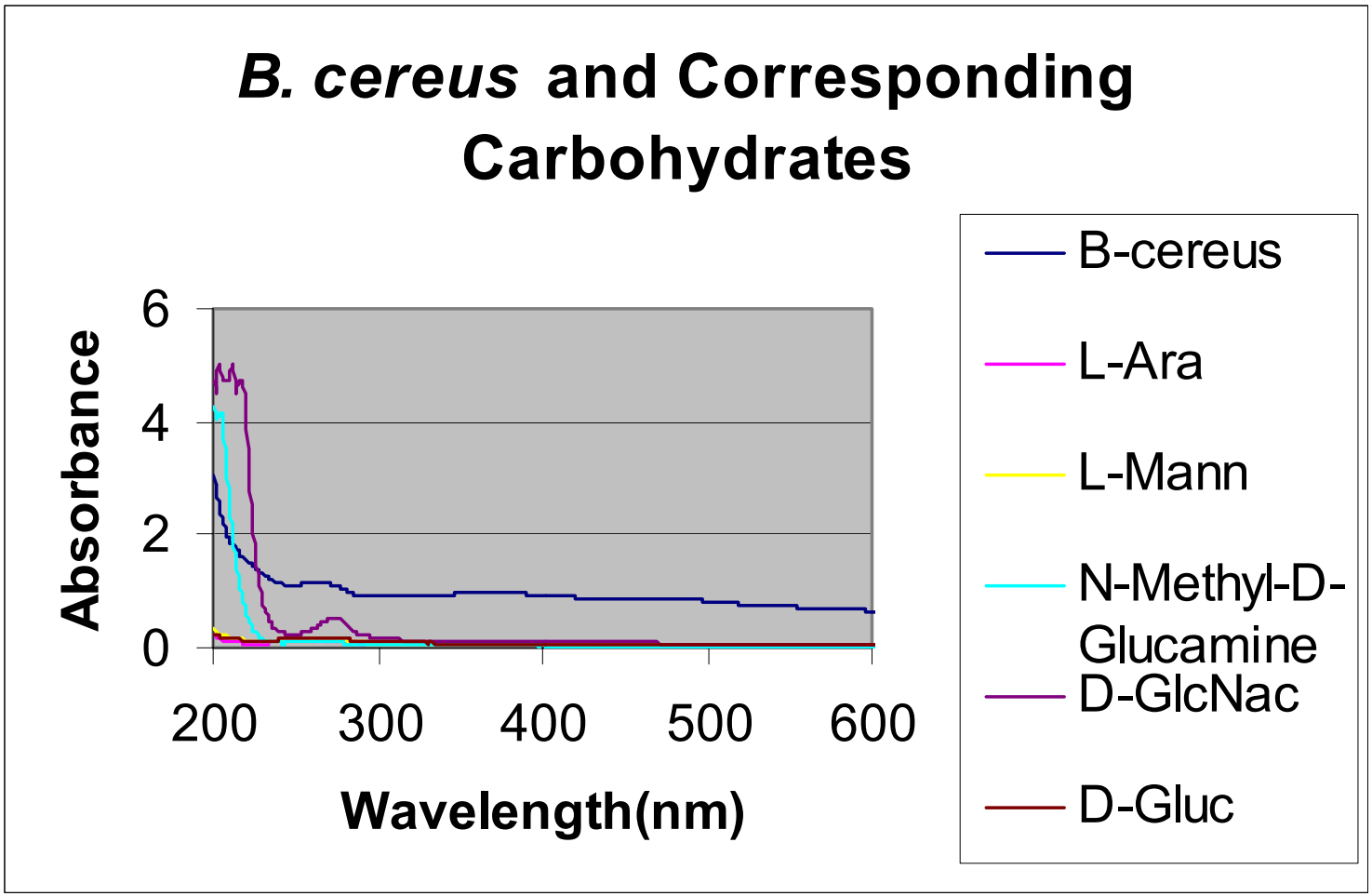
Gel Electrophoresis Image of Amine Carbohydrates



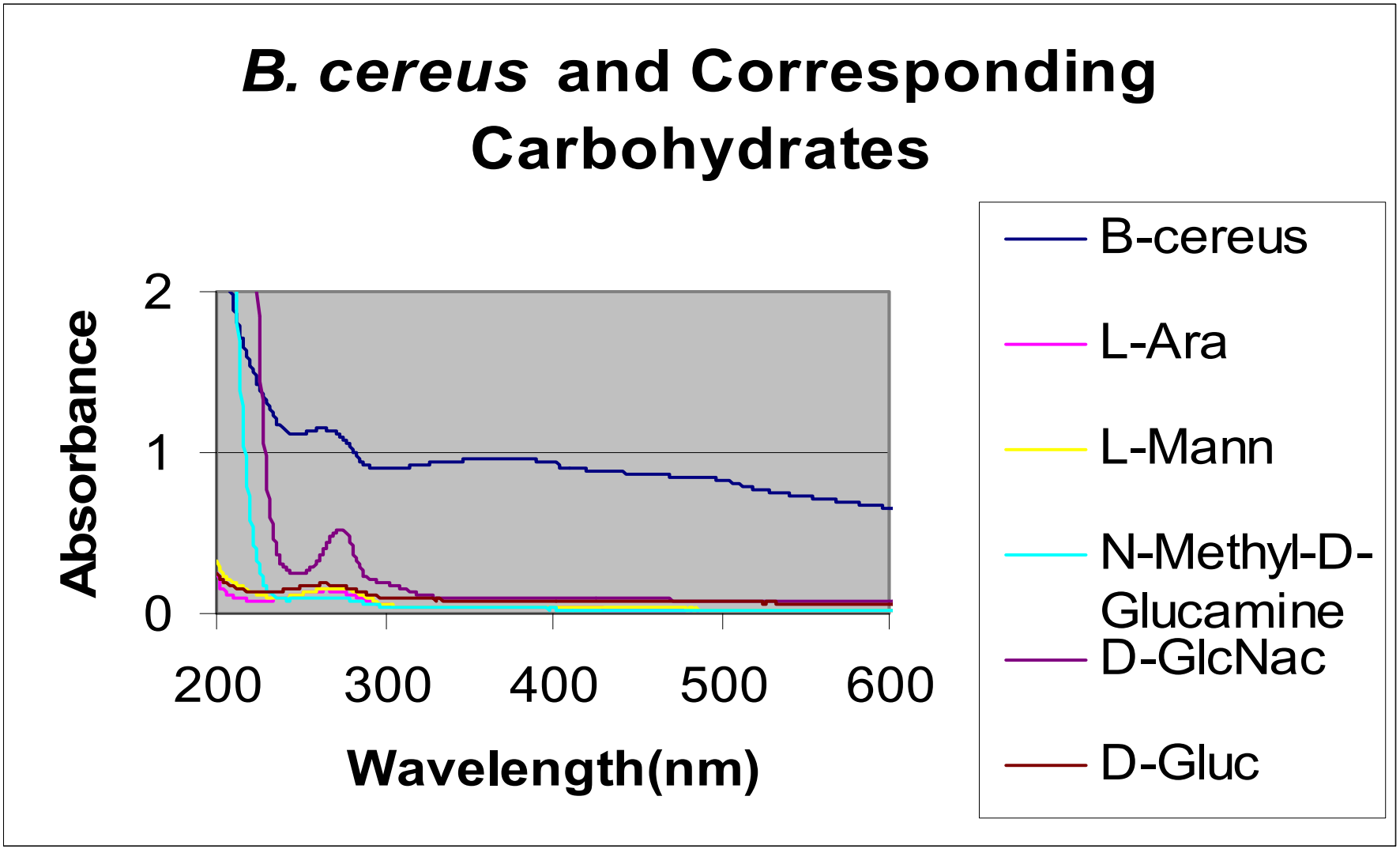
UV Spectroscopy

- UV spectrometry was the next method. It provided us with a means of comparisons with the gel electrophoresis
- With the knowledge that the characteristic peaks of carbohydrates are between 200nm and 400nm, we decided to run a battery of spectra only on carbohydrates
- Afterward we compared the UV spectra of the intact spores against that of commercially carbohydrates
- We found a correspondence between some carbohydrates and the intact spore spectra between 200nm and 400nm
- These are listed in the following graphs

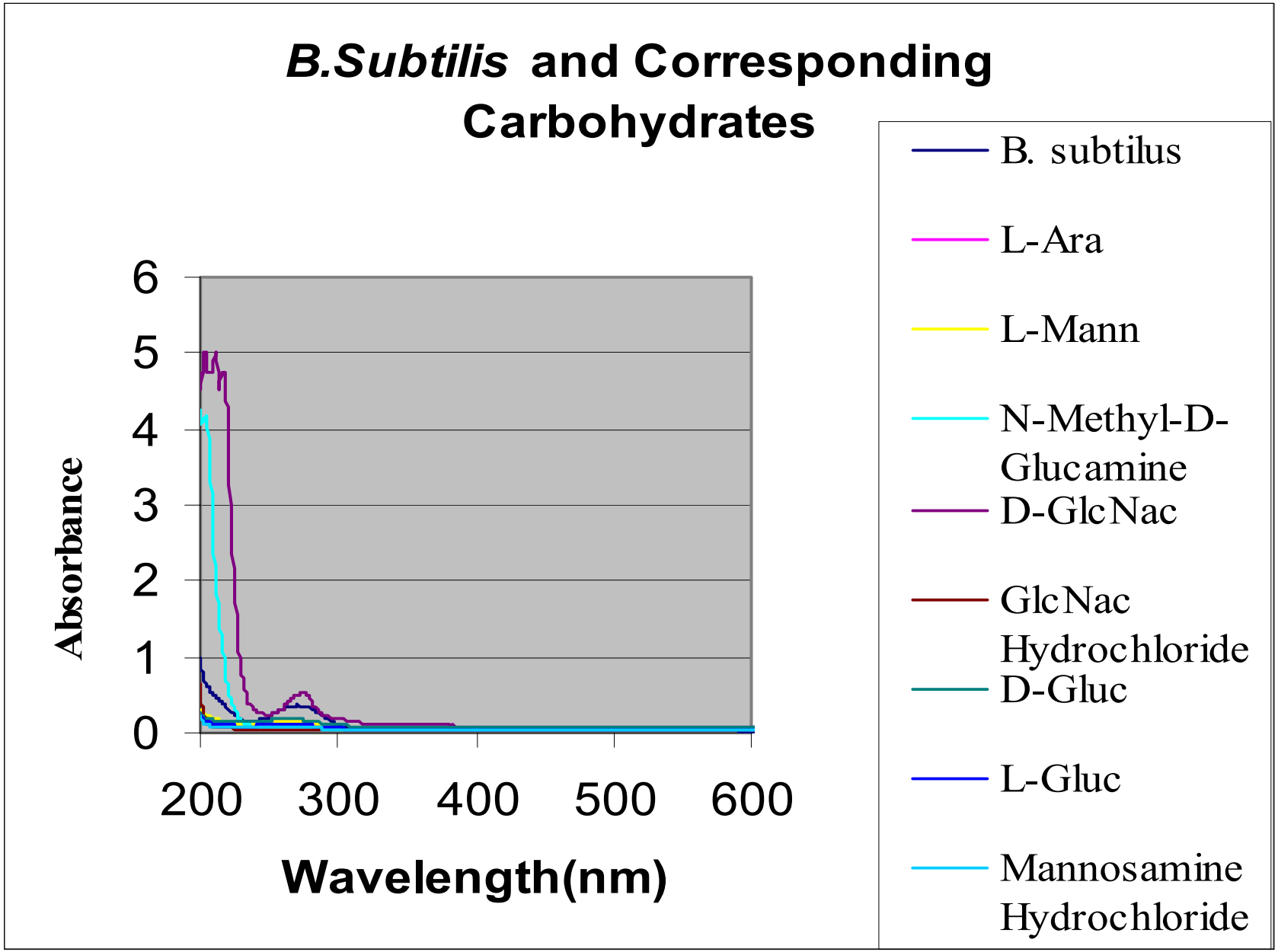
B. cereus and corresponding carbohydrates



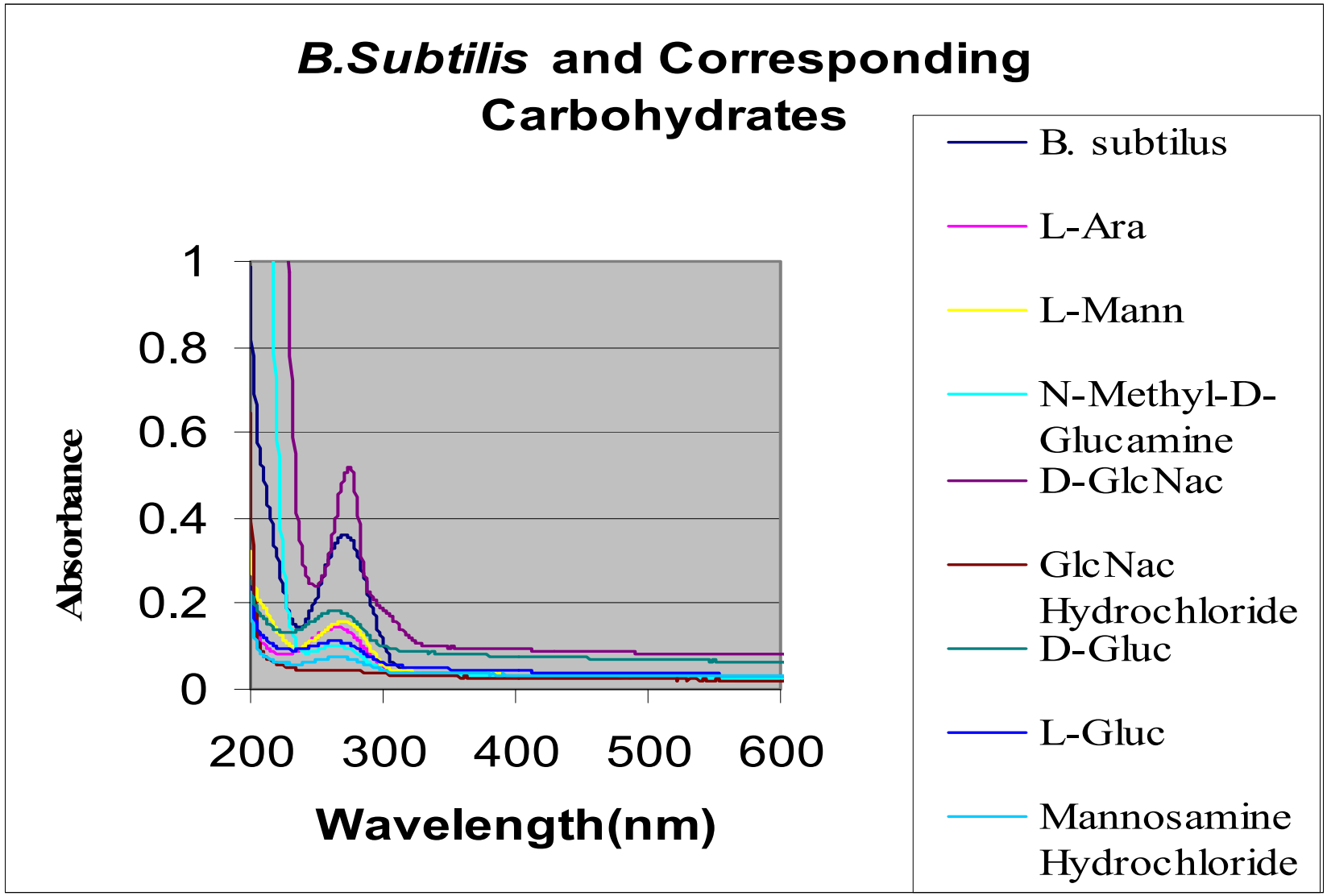
B. cereus and corresponding carbohydrates II



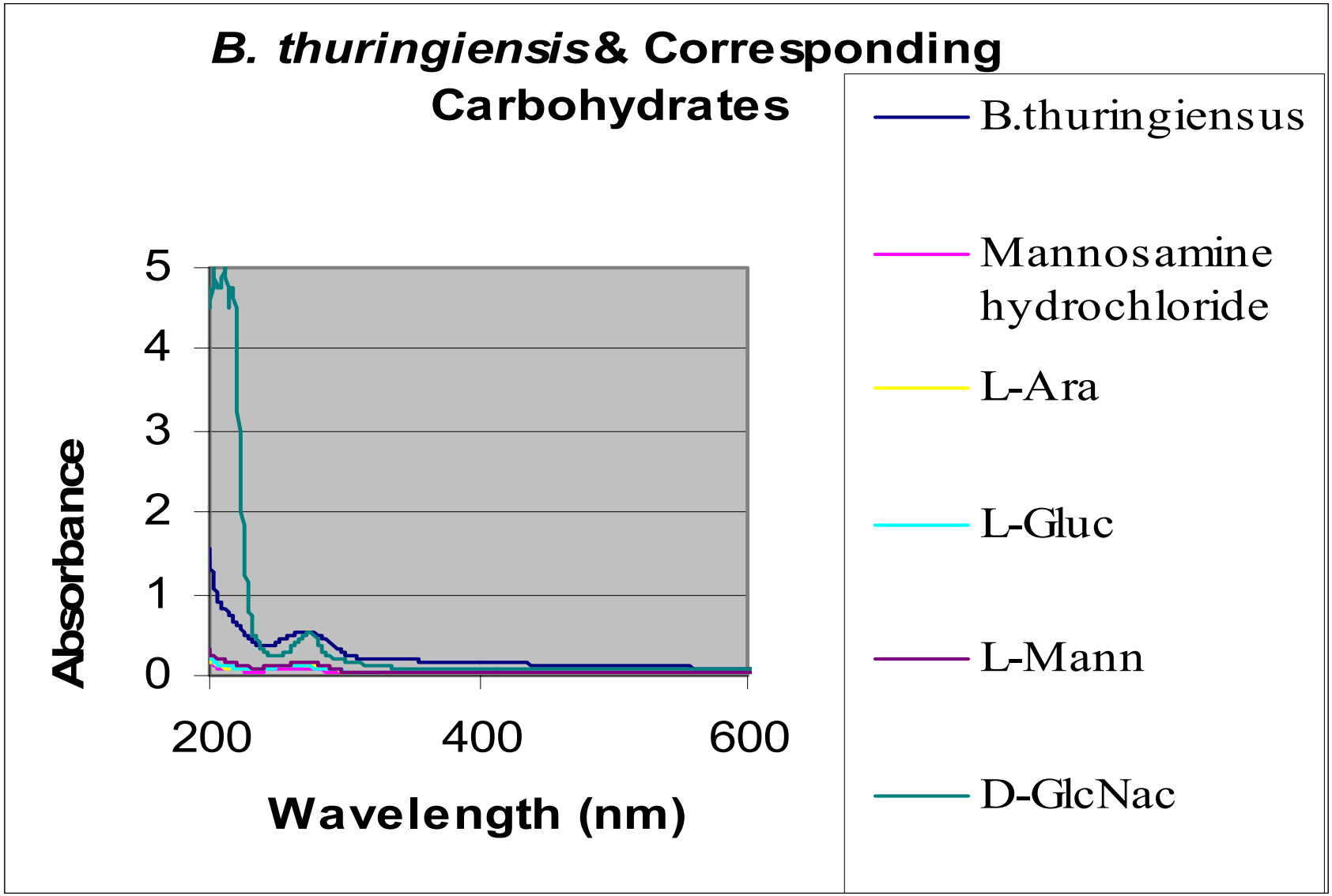
B. subtilis and Corresponding Carbohydrates



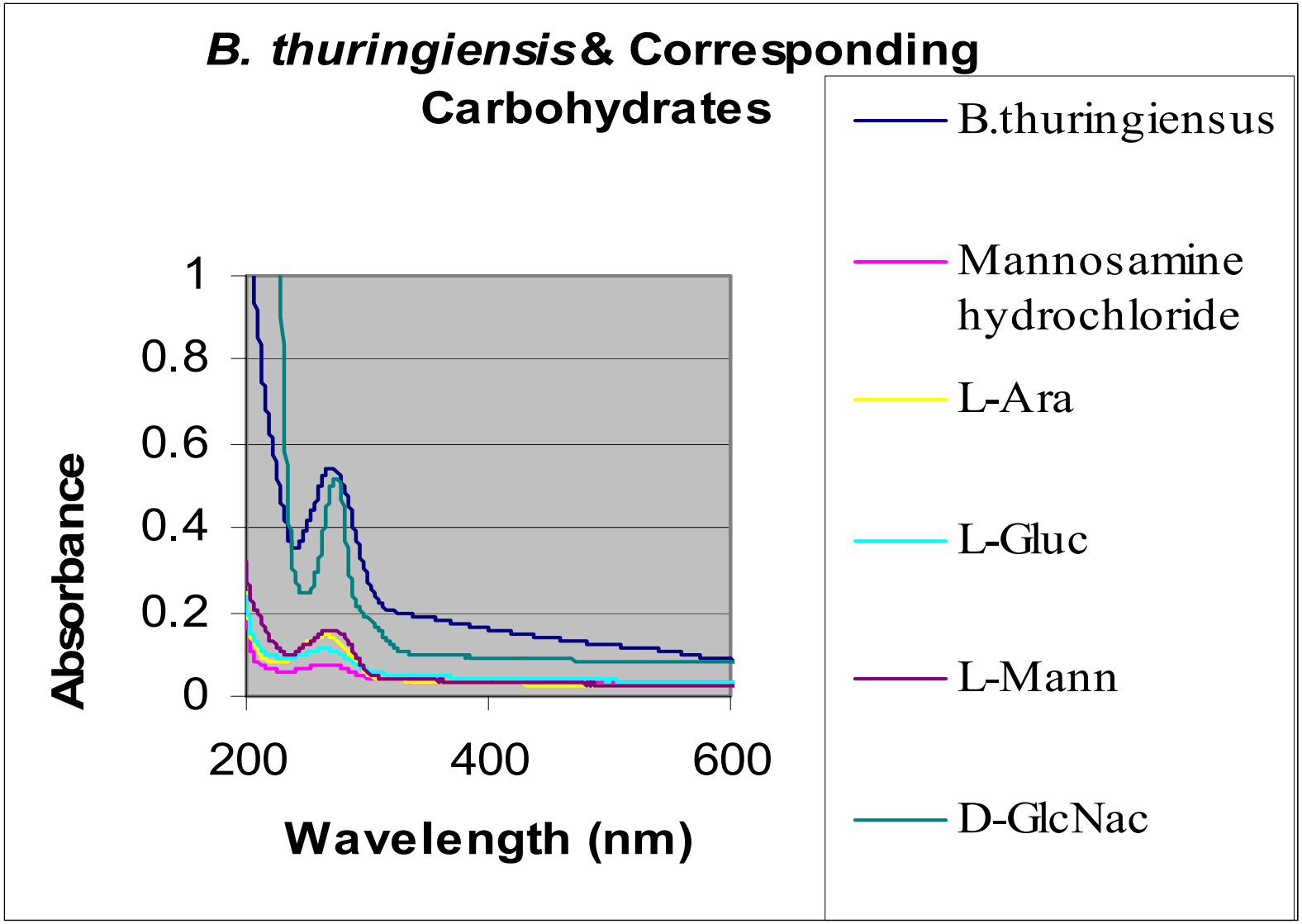
B. subtilis and Corresponding Carbohydrates



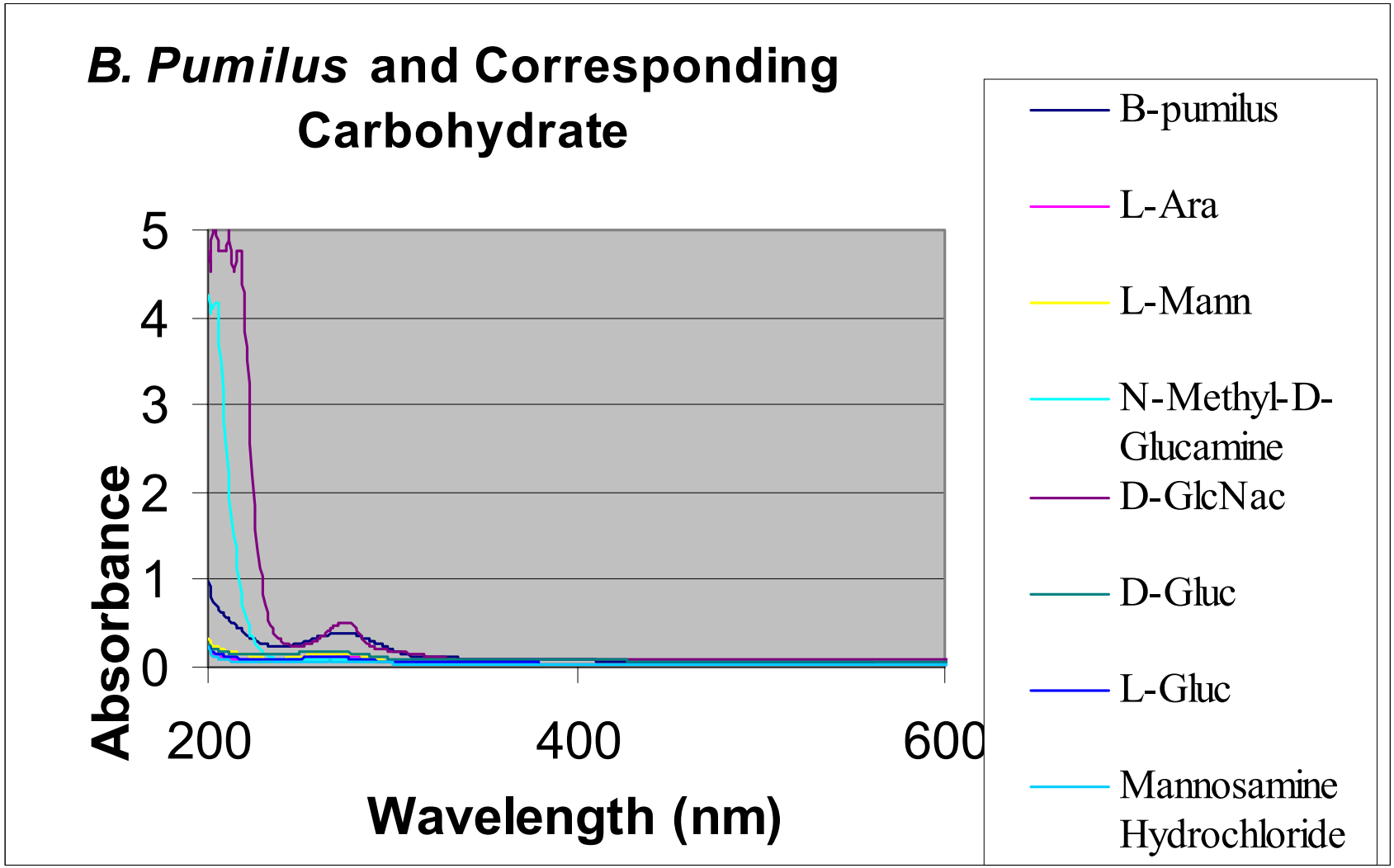
B. thuringiensis and corresponding carbohydrates



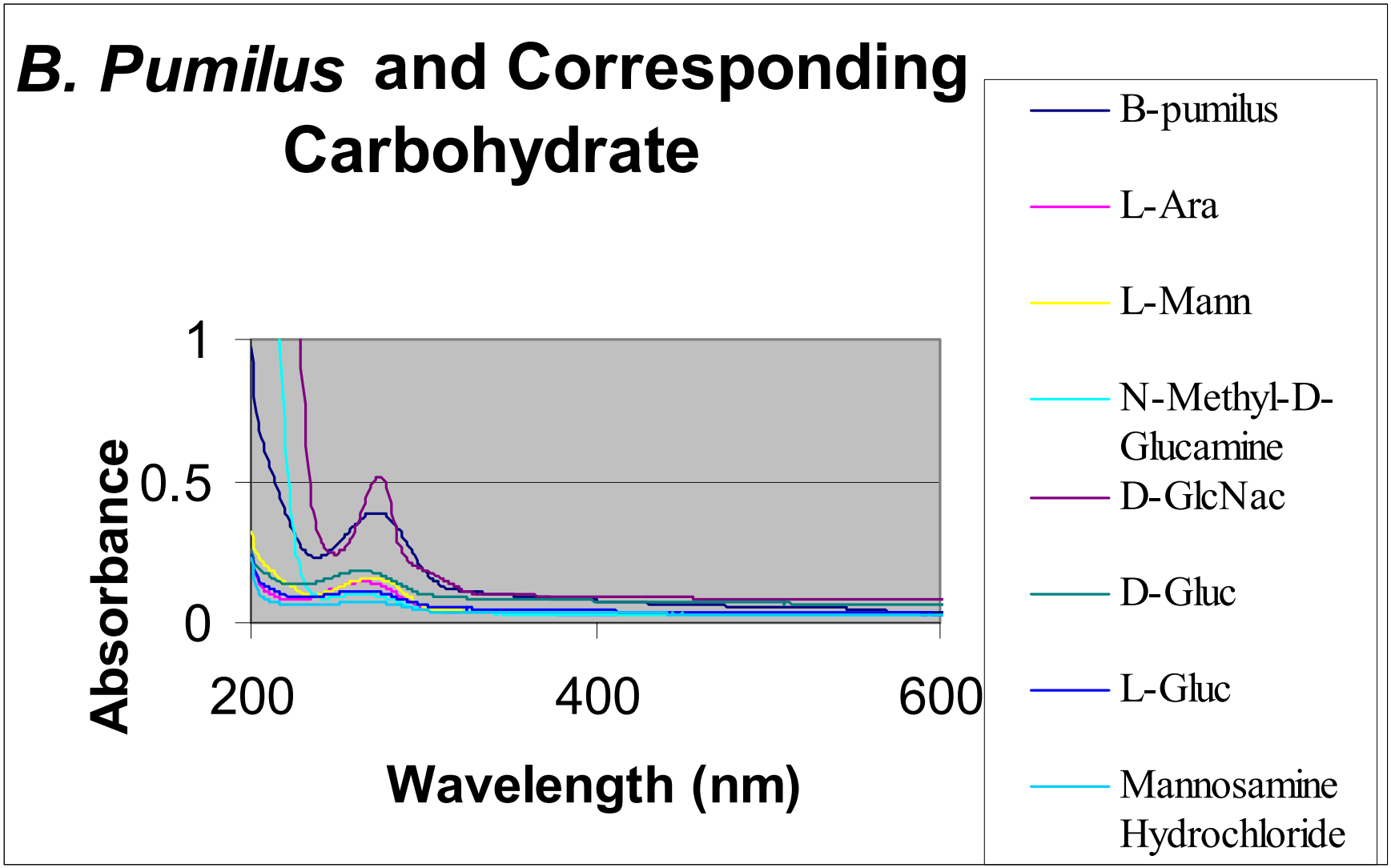
B. thuringiensis and corresponding carbohydrates



B. pumilus and corresponding carbohydrates



B. pumilus and corresponding carbohydrates



Resulting Carbohydrates from both FACE Electrophoresis and UV Spectral Analysis

- ***B. cereus***
  - Mannose, Glucose, GlcNAc, GalNac, L-arabinose, L-Mannose, N-Methyl-D-Glucamine, D-GlcNAc, D-Glucose
- ***B. thuringiensis***
  - GalNAc, Mannose, Galactose, GlcNAc, L-Glucose, D-GlcNAc, L-Mannose, L-Arabinose, Mannosamine Hydrochloride
- ***B. pumilus***
  - GlcNAc, Galactose, L-Arabinose, L-Mannose, N-Methyl-D-Glucamine, D-GLcNAc, L-Glucose, D-Glucose, Mannosamine hydrochloride
- ***B. subtilis***
  - Mannose, Glucose, GalNAc, GlcNAc, L-arabinose, L-Mannose, N-Methyl-D-Glucamine, D-GlcNAc, D-Glucose

Conclusions

- We found that UV analysis of intact spores done in conjunction with gel electrophoresis showed the carbohydrate content of the exterior of bacterial spore

- However these methods did not tell us the sequences that these carbohydrate are found as well as which monosaccharides will interact with carbohydrate -FITC- polymers and these questions will comprise the next part of our study