

Real-time PCR and PCR-tandem mass spectrometry for biodetection

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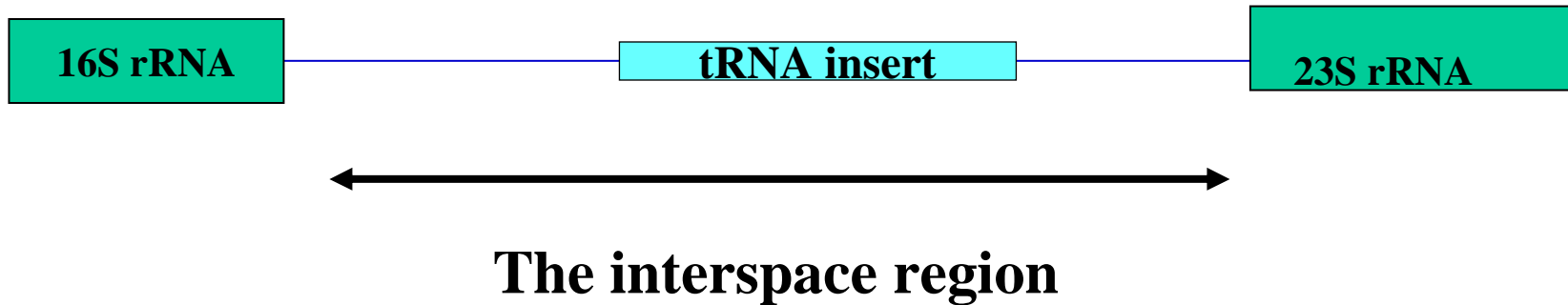
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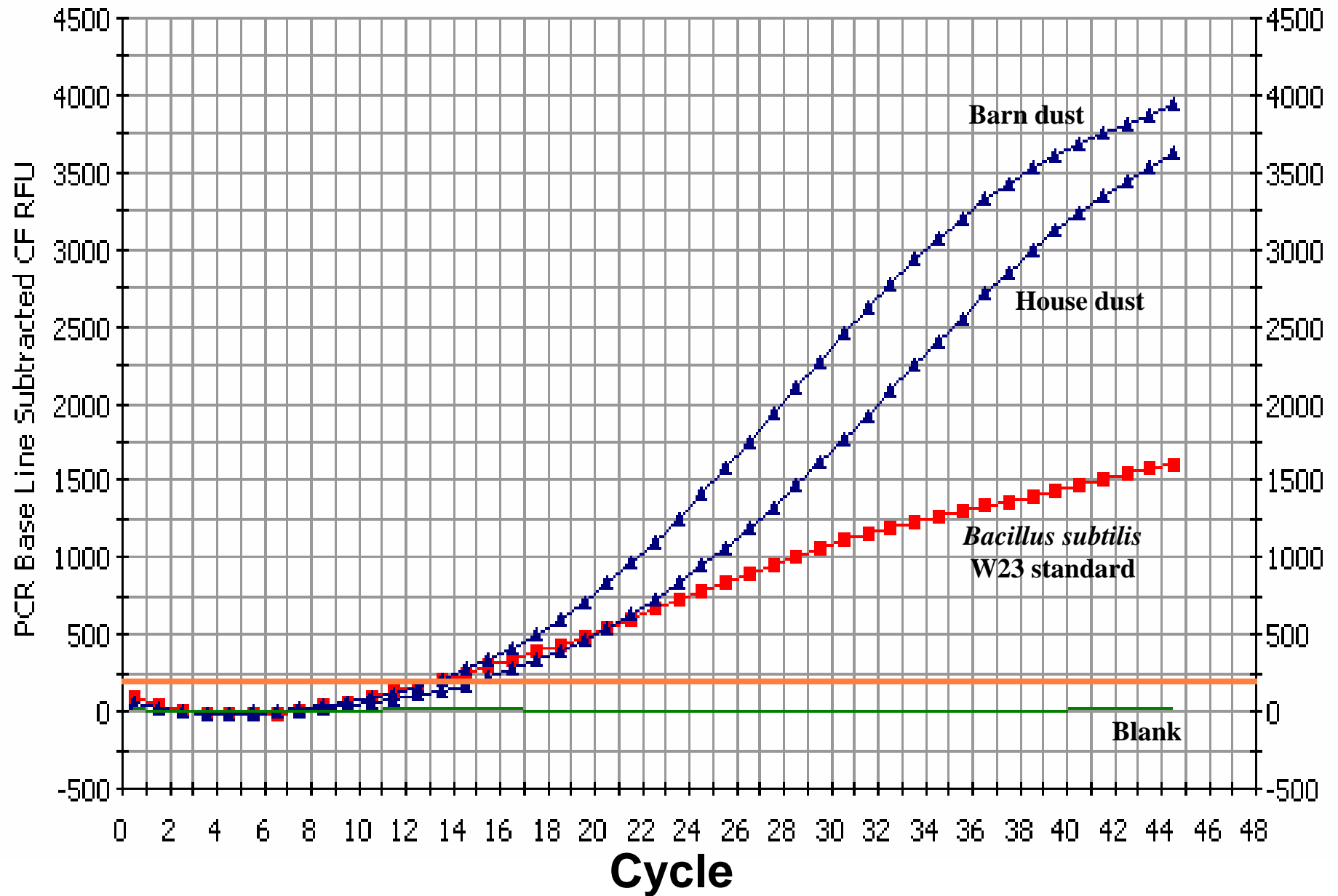
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Categorizing bacilli

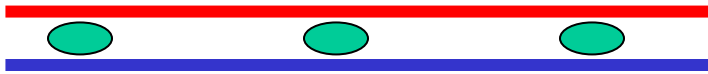


Real-time PCR (16s rRNA) - environmental samples

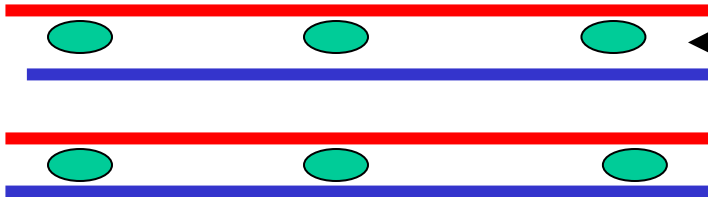


Real-time PCR

ds DNA

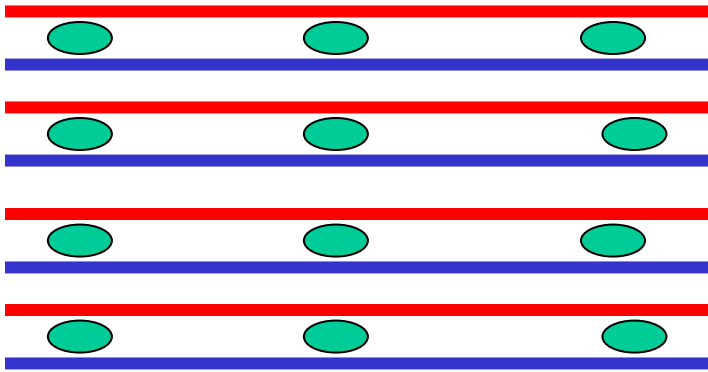


↓ Cycle one



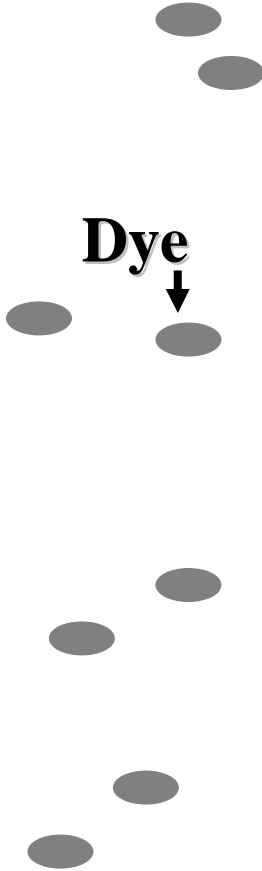
Dye

↓ Cycle two



↓ Cycle 30

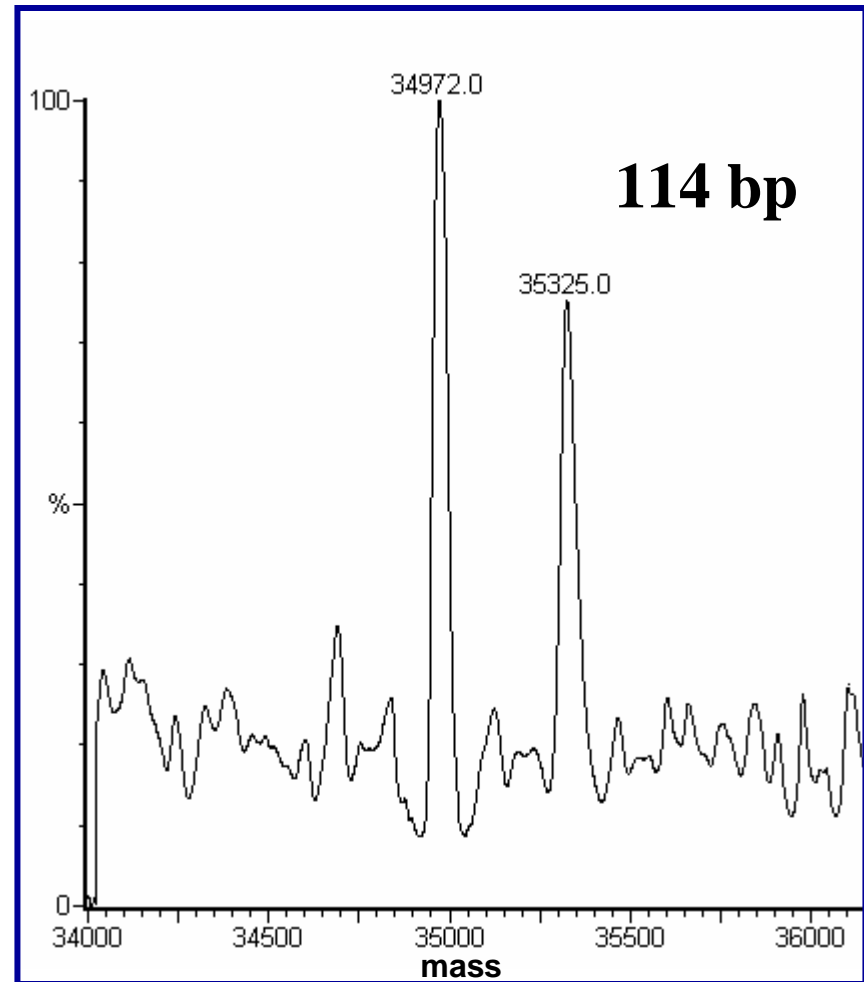
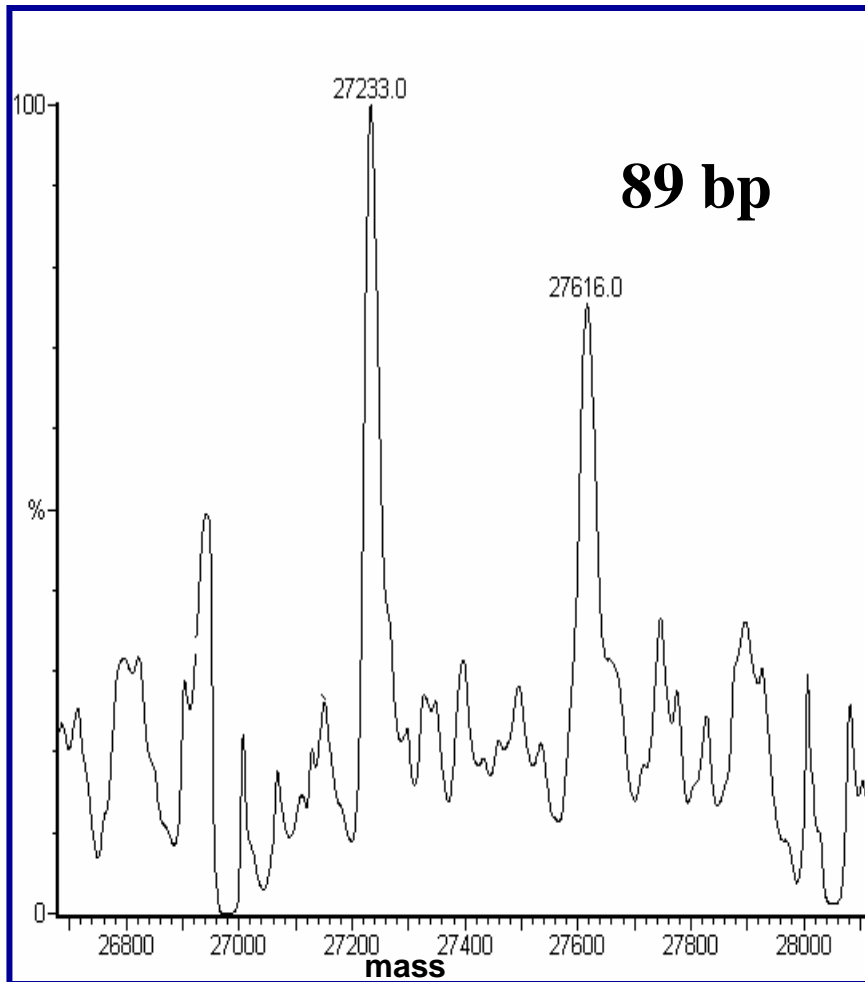
$$2^{30}$$



Electrospray mass spectrometry analysis of PCR products (rRNA interspace region)

Predicted Mass for *B. anthracis* (ANR-1)
Non-Coding Strand: 27618.94
Coding Strand: 27237.79

Predicted Mass for *B. subtilis* (W23)
Non-Coding Strand: 35329.01
Coding Strand: 34972.75



16S ribosomal RNA sequence

11 operons

Bacillus anthracis:

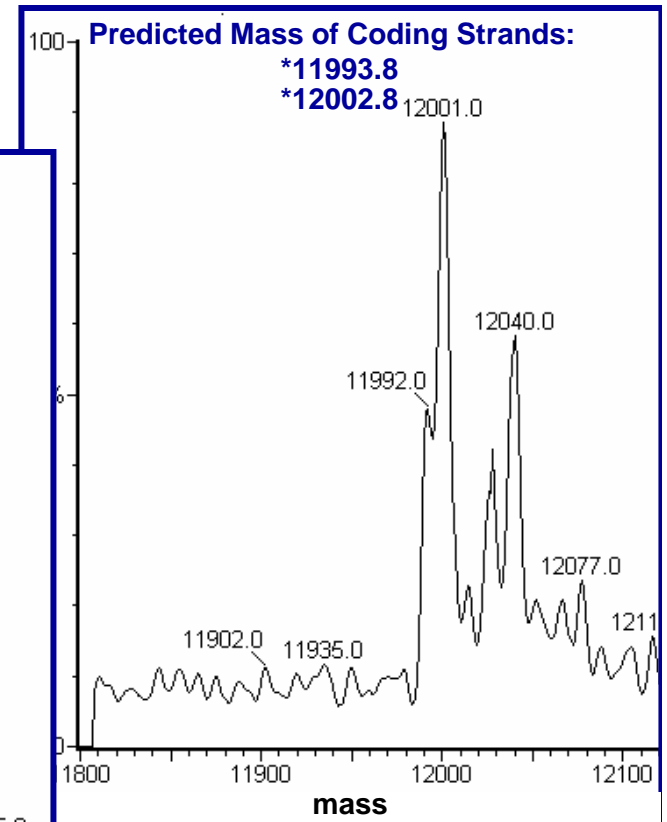
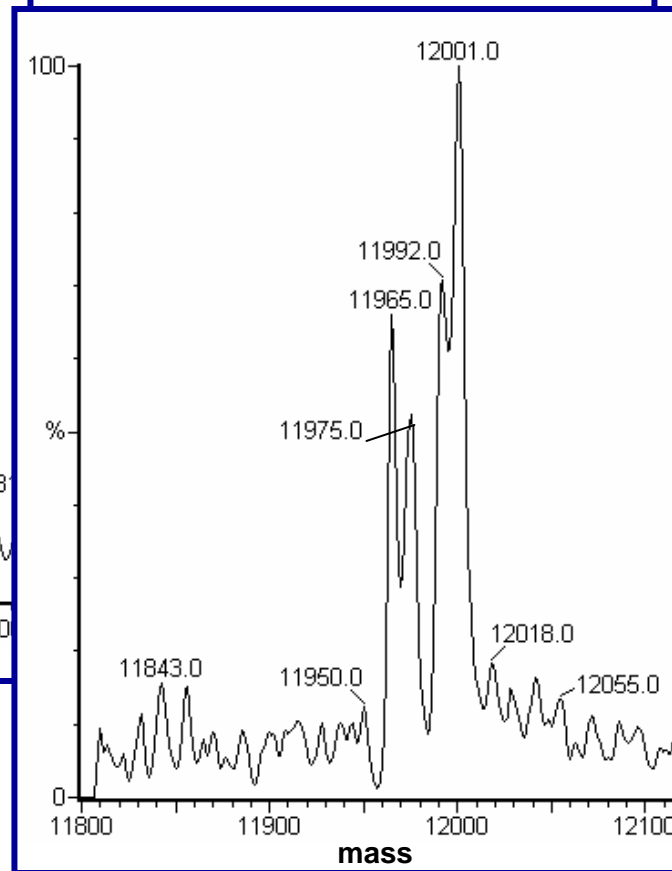
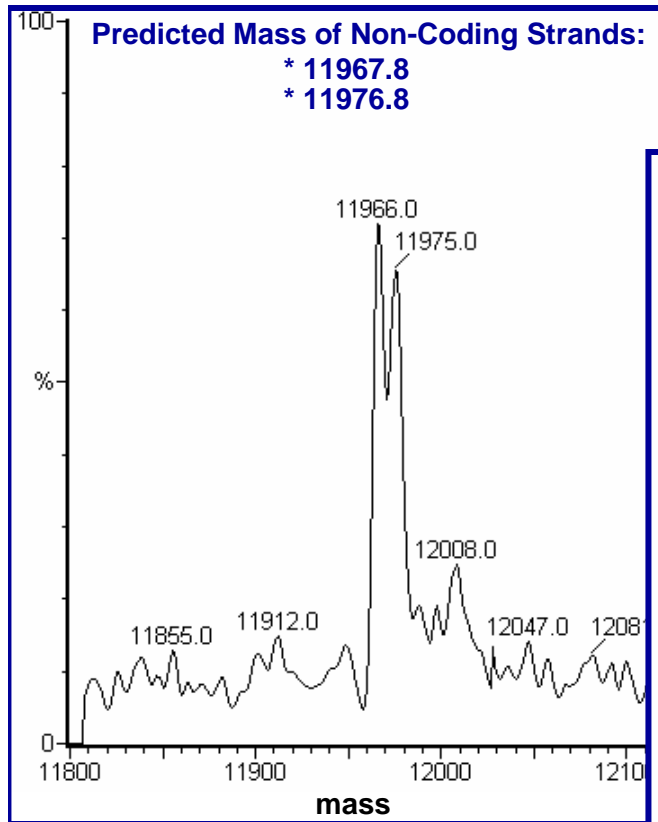
6 operons contain A, 5 have T (position 1146)

B. cereus/B. thuringiensis:

All contain A

Sacchi et al. *Emerg. Infect. Dis.* 8: 1117-1123. : 2002

Molecular Weight of Two Non-Coding and Two Coding Single-Stranded 39-mers



Real-time PCR

Dust collection

DNA purification

Amplification

Detection

Under one hr

Under 2 hr

Under 7 min

Under one min

PCR-MS and MS-MS

Dust collection

DNA purification

Amplification

Detection

Clean-
up



Mass spectral
Data Base

PCR-Quadrupole-ESI-MS (56 bp)

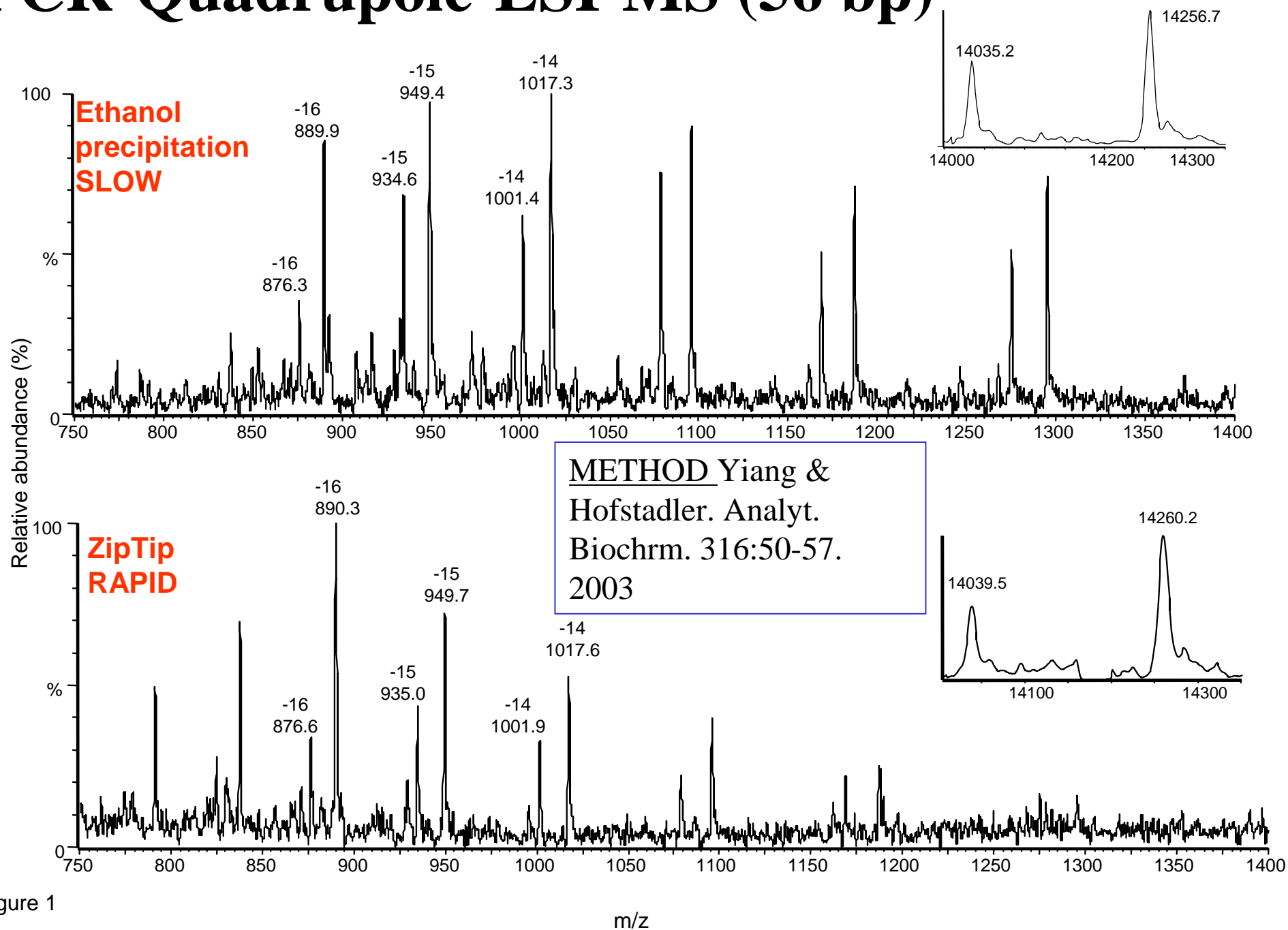
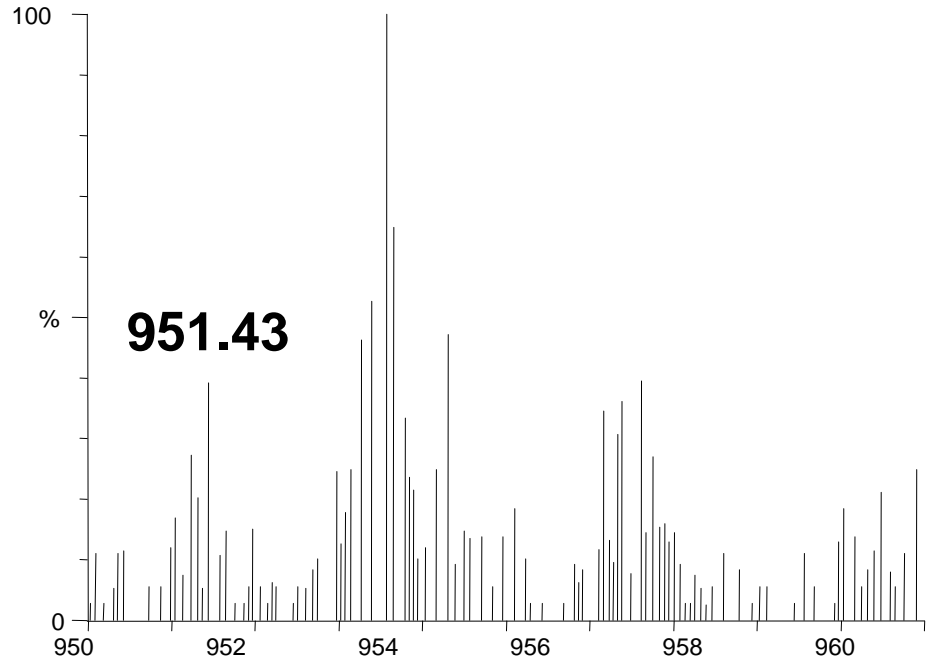
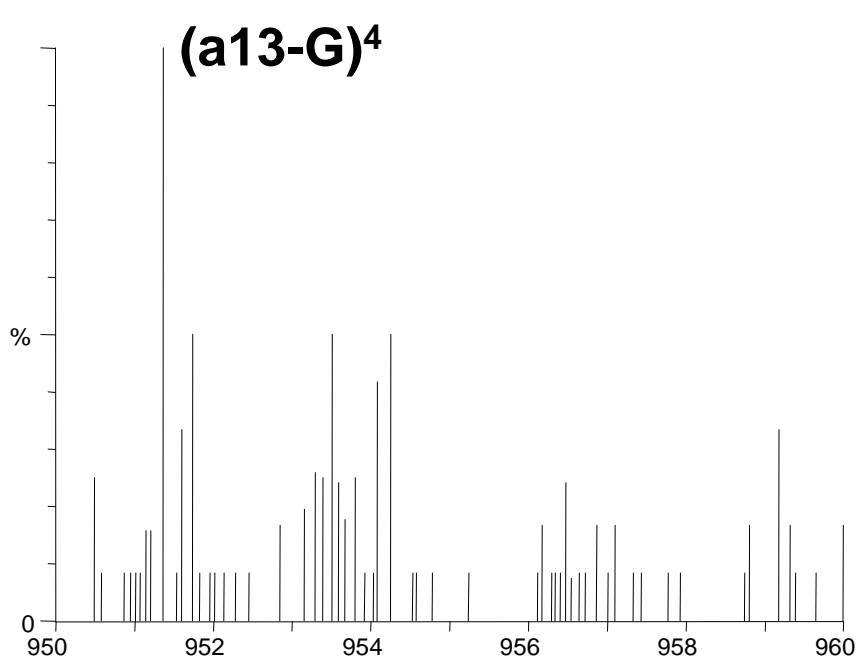
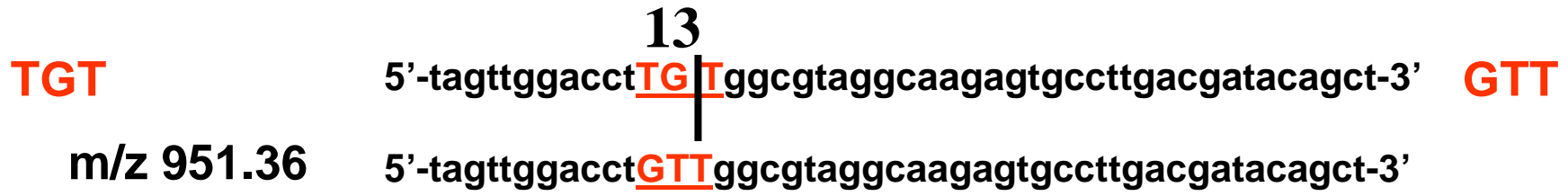


Figure 1

100 SpecDiff program differentiates pairs of MS MS spectra of 46 bp PCR products by identifying discriminating peaks (**Q** **DF**)



David Tabb, Oakridge

Direct Protein detection (in comparison to PCR)

- Potentially faster
- Avoids unstable biological reagents
- Sensitivity for environmental analysis
 - remains to be proven
- Targets need to be defined

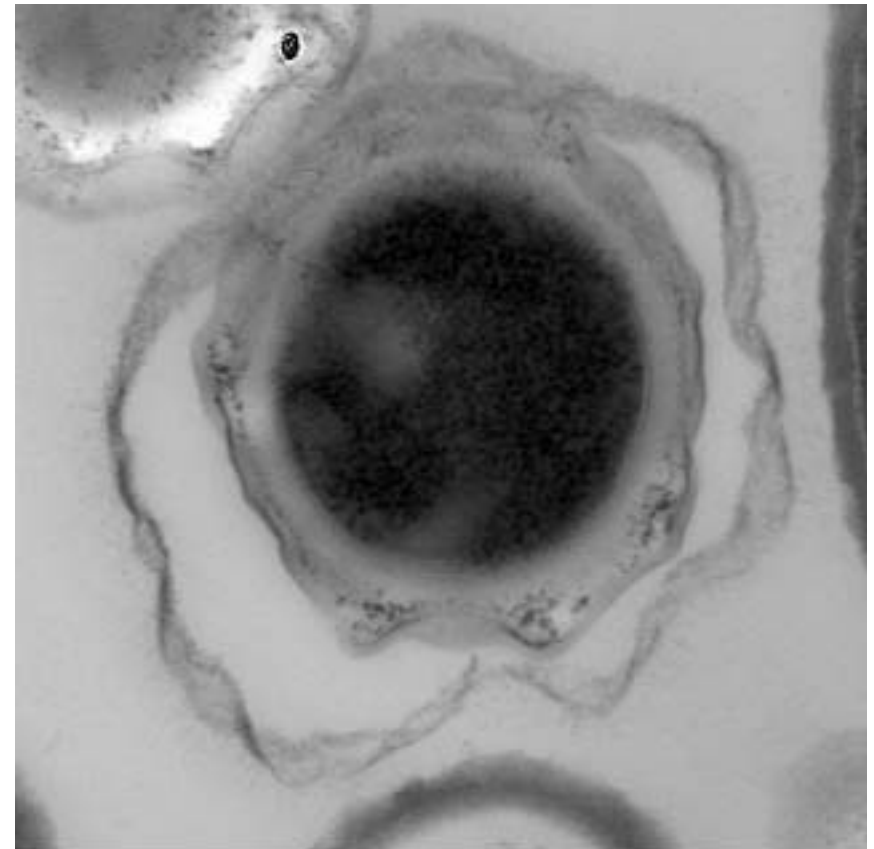
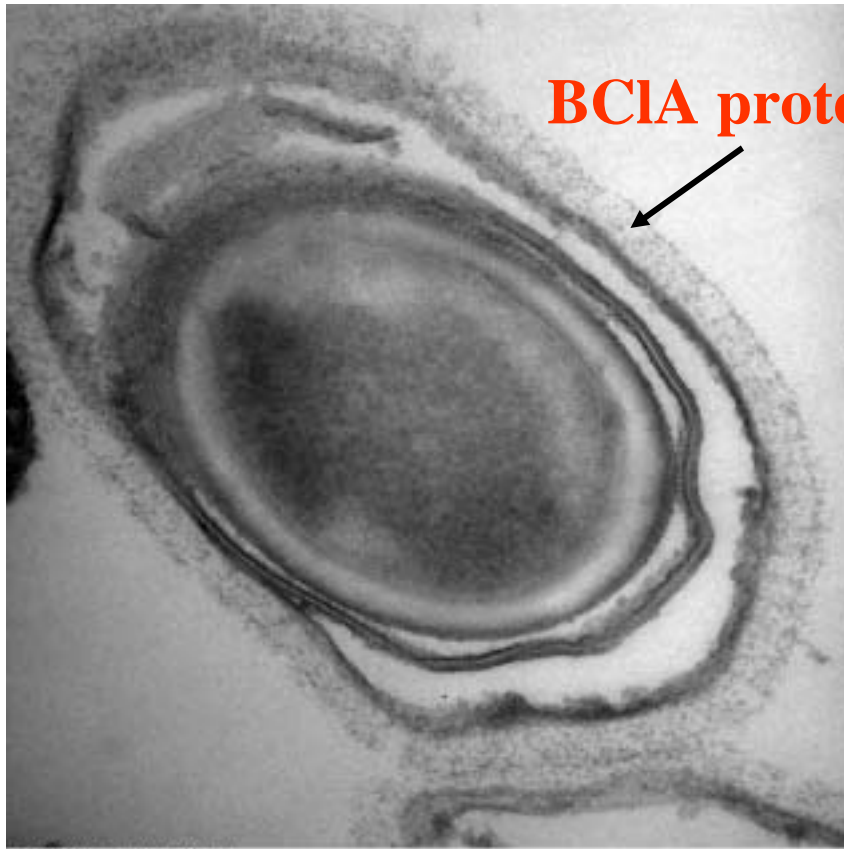
Carbohydrates and glycoproteins of *B.anthraxis*

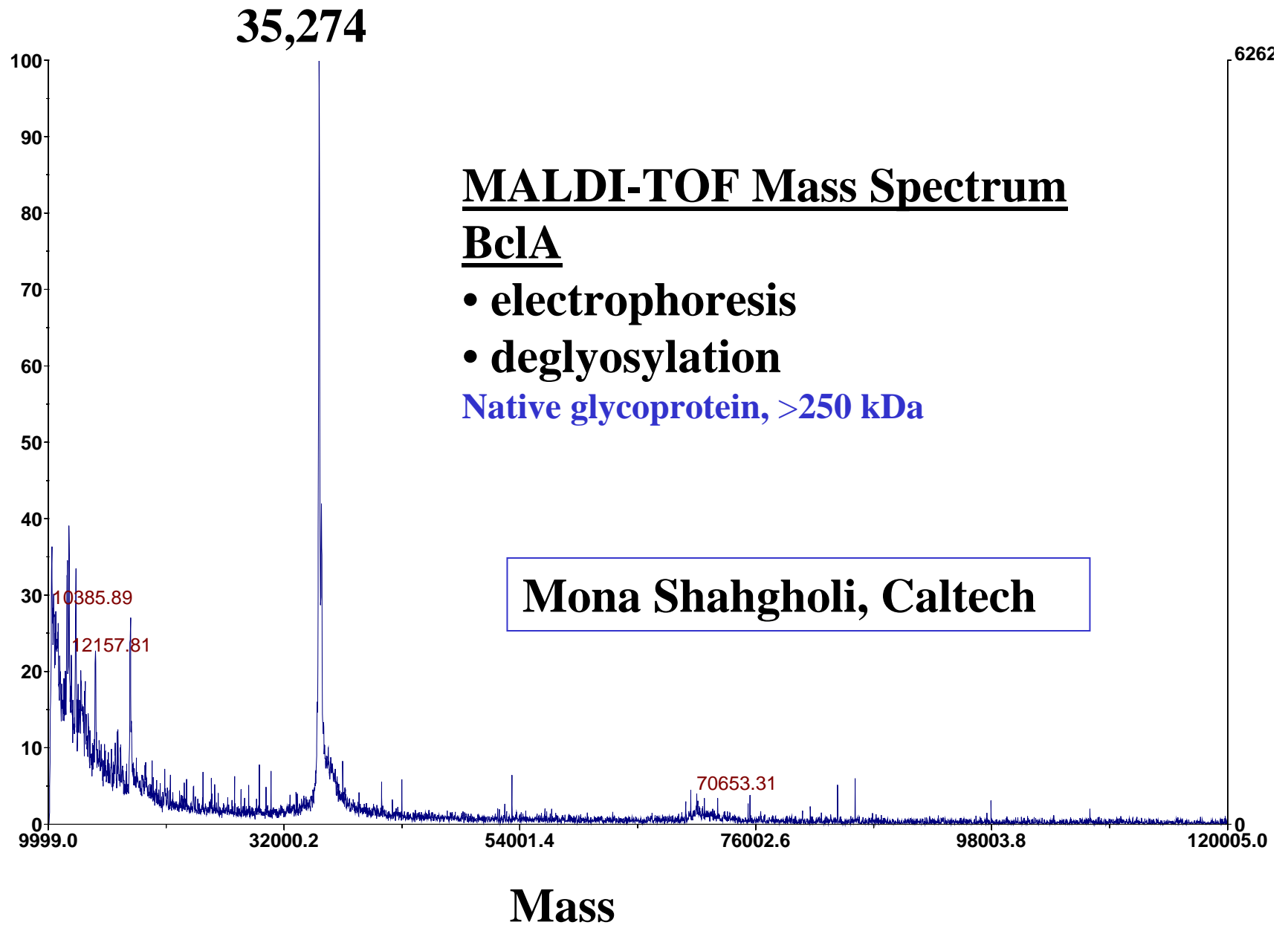
- **Spore-specific sugars**
 - Fox et al., J. Clin. Microbiol. 31:887-894. 1993
 - Carbohydrate profile for *B. anthracis* distinct from *B. thuringiensis/B. cereus*
- **Exosporium glycoprotein, BclA**
 - Sylvestre et. al. Mol. Microbiol. 45:169-178. 2002

B. anthracis spore

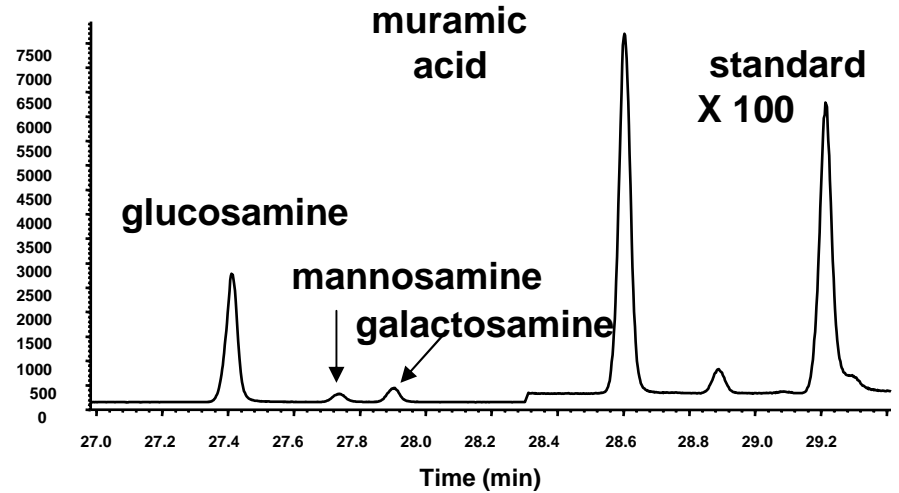
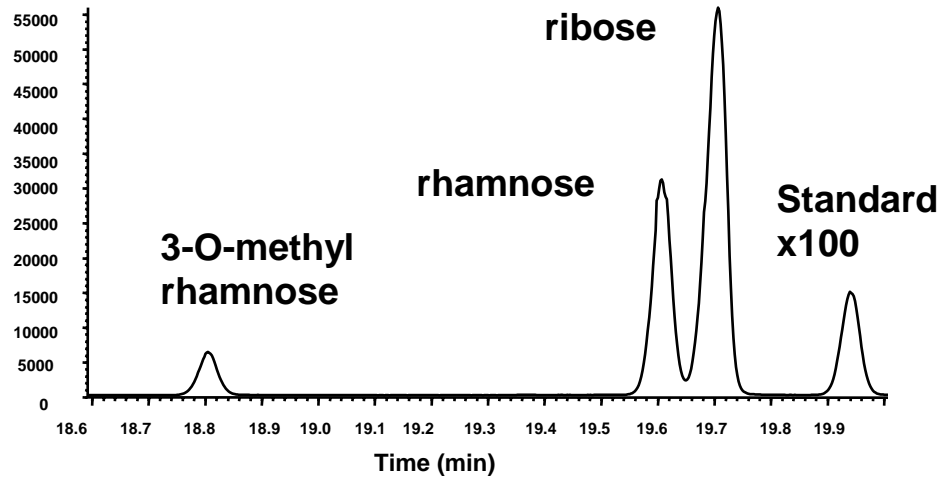
Stained for glycoprotein

No glycoprotein stain



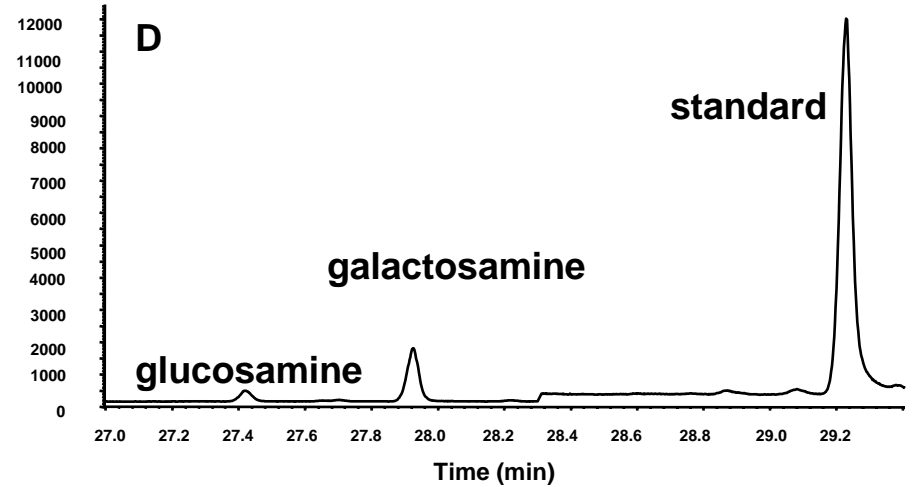
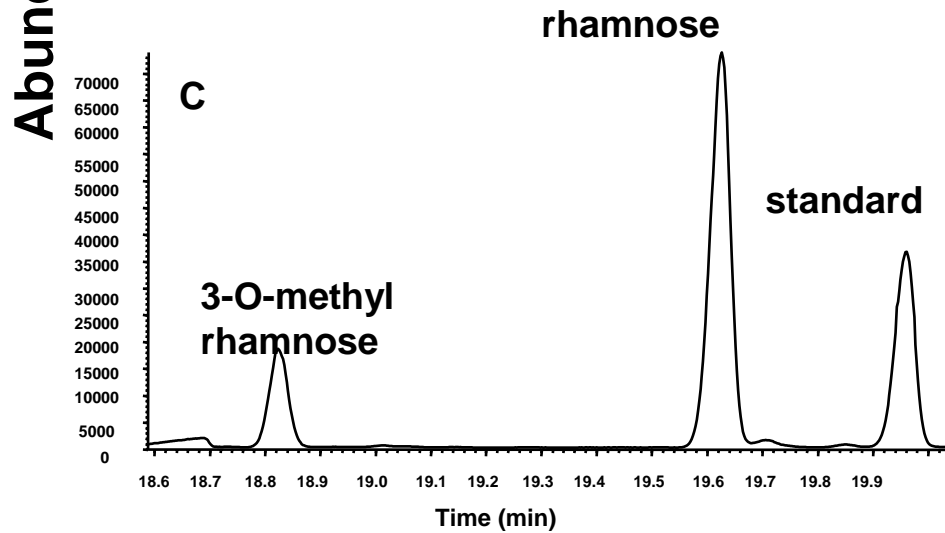


Spore



Abundance

Isolated BCIA



Conclusions

- **Real-time PCR**
 - the current gold standard
 - complex enzymatic reaction
- **PCR -MS-MS**
 - structure information
 - improved specificity
- **Protein analysis (e.g. ESI or MALDI MS-MS)**
 - potential simplicity
 - sensitivity to be proven for environmental monitoring