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# Real-Time Mass Spectrometry of Individual Airborne Bacteria

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## ABSTRACT

A method for the real-time detection of individual bacteria is described. Airborne bacteria and bacterial spores are directly sampled by laser ablation in an ion trap mass spectrometer with an atmospheric pressure inlet system. Either positive or negative ion mass spectra can be obtained. Ions of a particular value of m/z can be further characterized by tandem mass spectrometry in the ion trap. Spectra averaged from several hundred individual bacteria of the same species appear to differ somewhat from spectra of bacteria of other species and to be readily distinguishable from spectra of nonbiological particles.

## INTRODUCTION

We are developing a method for real-time analysis of airborne microparticles based on laser ablation in an ion trap mass spectrometer.[1,2] Experiments with aerosolized bacteria show some promise for discrimination of bacteria from particles of nonbiological origin. While there are large fluctuations from cell to cell, making identification of individual organisms tenuous, spectra averaged over several hundred cells differ slightly from species to species. The present approach might be used to provide an advanced warning of substantial changes in the background level of airborne bacteria or bacterial spores, triggering a more specific but labor and time-intensive identification process.

The experimental approach is diagrammed in Fig. 1. Airborne particles enter the apparatus through an atmospheric orifice and are isolated from the surrounding air by skimmers. The particles are individually detected as they pass through two CW laser beams. The two timing pulses are used to trigger a pulsed excimer laser that samples and ionizes the particle within the electrodes of an ion trap mass spectrometer. After the laser pulse, the stored ions are mass analyzed by

## DISCLAIMER

conventional ion trap methods.[3]

The time for a particle to pass through the two CW laser beams is a function of the aerodynamic size of the particle, as shown in Fig. 2. A calibration curve is obtained from microspheres of known size. For particles of micrometer dimensions, we can obtain a reliable estimate of particle size in this way. The digitized transit time for each particle can be stored together with its mass spectrum. Knowledge of the particle size provides important additional information for particle classification.

A large number of ions can be produced from a single microorganism - enough to fill the ion trap. An example of a single particle mass spectrum is shown in Fig. 3. This is a positive-ion mass spectrum of a single *Bacillus subtilis* cell, obtained with a laser pulse of approximately 5 mJ at 308 nm wavelength, focused into a spot of 0.5 mm diameter. The mass scan was initiated at 50 Da to avoid detector overload from the lighter ions, primarily potassium ions at m/z 39. A negative ion mass spectrum of a single cell of the same species is shown in Fig. 4. We have also shown in this figure some of the ions that have been studied by tandem mass spectrometry with arrows indicating the fragment ions that were detected in collisionally-induced dissociation experiments. Many of the prominent ions in these spectra appear to contain phosphate or potassium. With UV laser ablation, we have not been successful in generating appreciable numbers of ions with m/z greater than 300 Da. We assume that the ions we detect are mostly fragments of the large molecular constituents of the cell membranes disrupted by the high intensity laser pulses.[4]

Positive ion mass spectra are shown in Fig. 5 for three species of bacteria together with spectra of three types of nonbiological particles that might be encountered in the environment. Because of the large variation in the spectra of individual cells, even of the same species, we have presented spectra averaged over several hundred single particle measurements. The similarity of the bacterial mass spectra and the differences from the nonbacterial particles are apparent. We have teamed with John S. Wagner's group at Sandia National Laboratory to explore statistical discrimination of individual particle mass spectra.

We have observed a strong similarity between the averaged laser ablation mass spectra of bacterial cells and the lipopolysaccharides extracted from cells of the same species. Results for *E. coli* positive ions are shown here. Similar results were observed for *Pseudomonas aeruginosa.*

In summary, a method for the real-time detection of airborne bacteria by laser ablation mass spectrometry in an ion trap has been developed. Microparticles are sampled directly from the air by a particle inlet system into the vacuum chamber of a mass spectrometer. An incoming particle is detected as it passes through two CW laser beams and a pulsed laser is triggered to intercept the particle for laser ablation/ionization and subsequent mass analysis in the ion trap mass spectrometer. Either positive or negative ions can be studied and ions of a particular value of m/z can be further characterized by tandem mass spectrometry in the ion trap. Statistical methods to discriminate between bacteria and other airborne particles appear feasible. We are currently exploring alternative means of sampling and ionization to extend the mass range to higher values of m/z.

## ACKNOWLEDGEMENT

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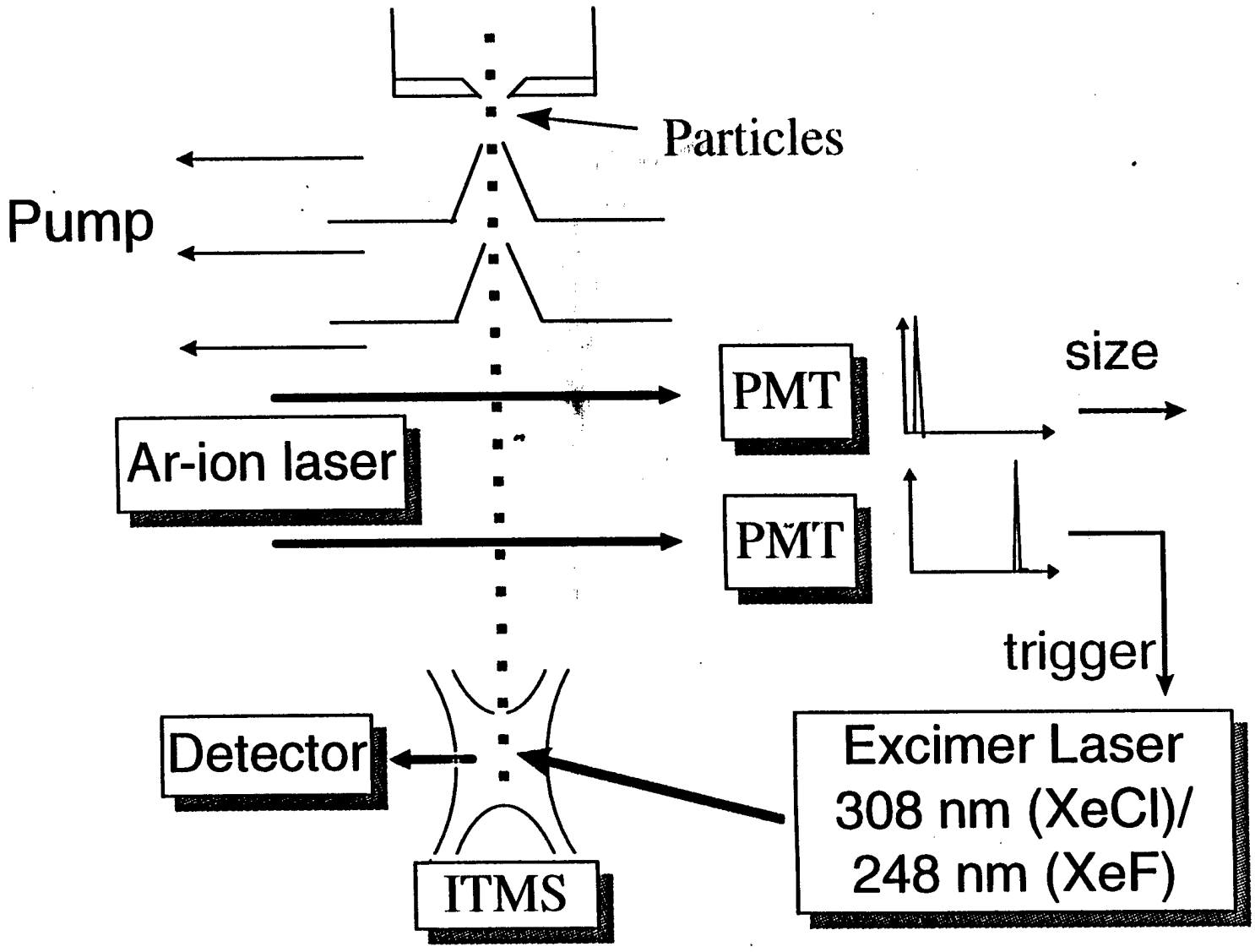

Particles
Pump
PMT
size
Ar-ion laser
PMT
trigger
Detector
Excimer Laser
308 nm (XeCl)/
248 nm (XeF)
ITMS

# Flight Time vs Particle Size

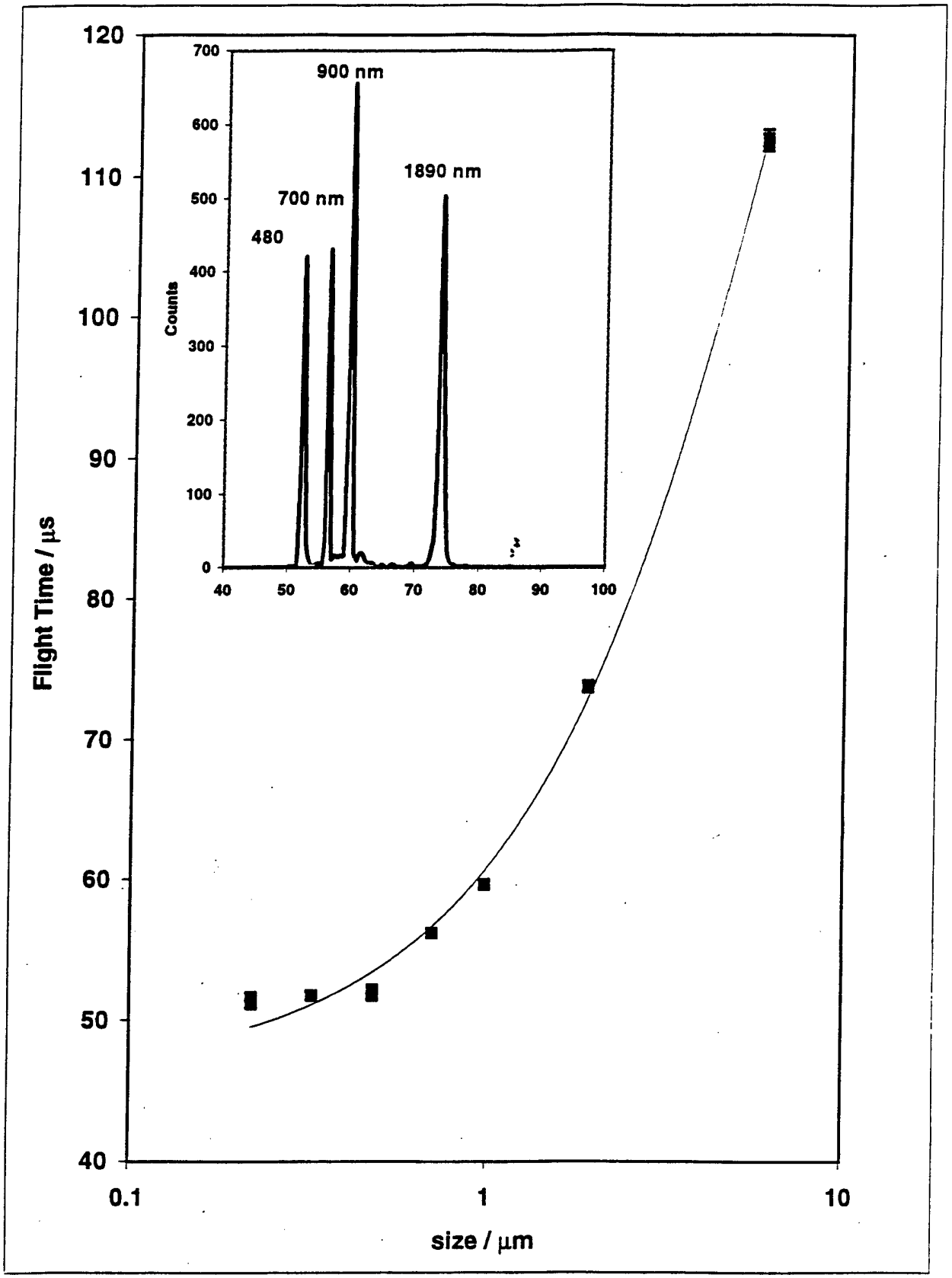



Fig. 2

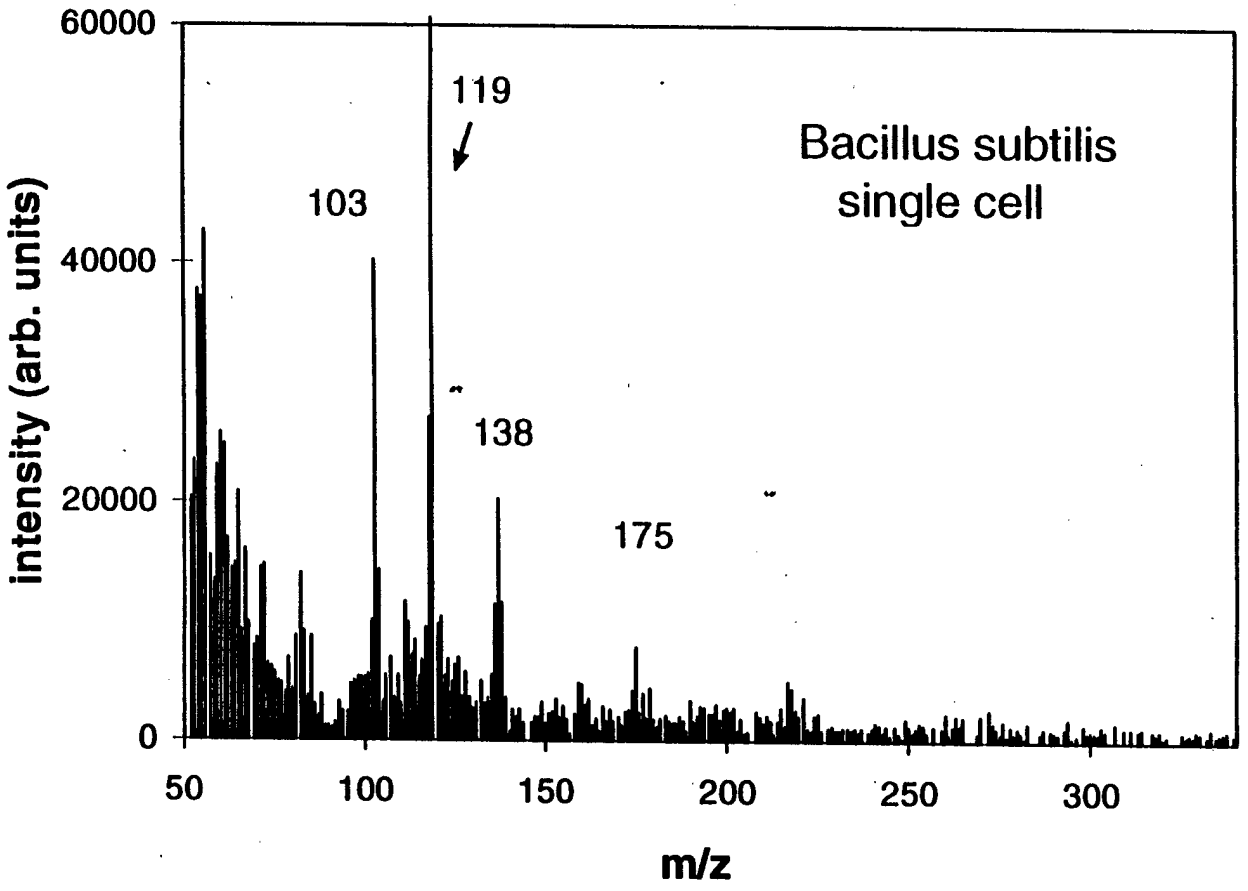

60000
40000
20000
0
intensity (arb. units)
119
103
138
175
Bacillus subtilis
single cell
50
100
150
200
250
300
m/z

Fig 1

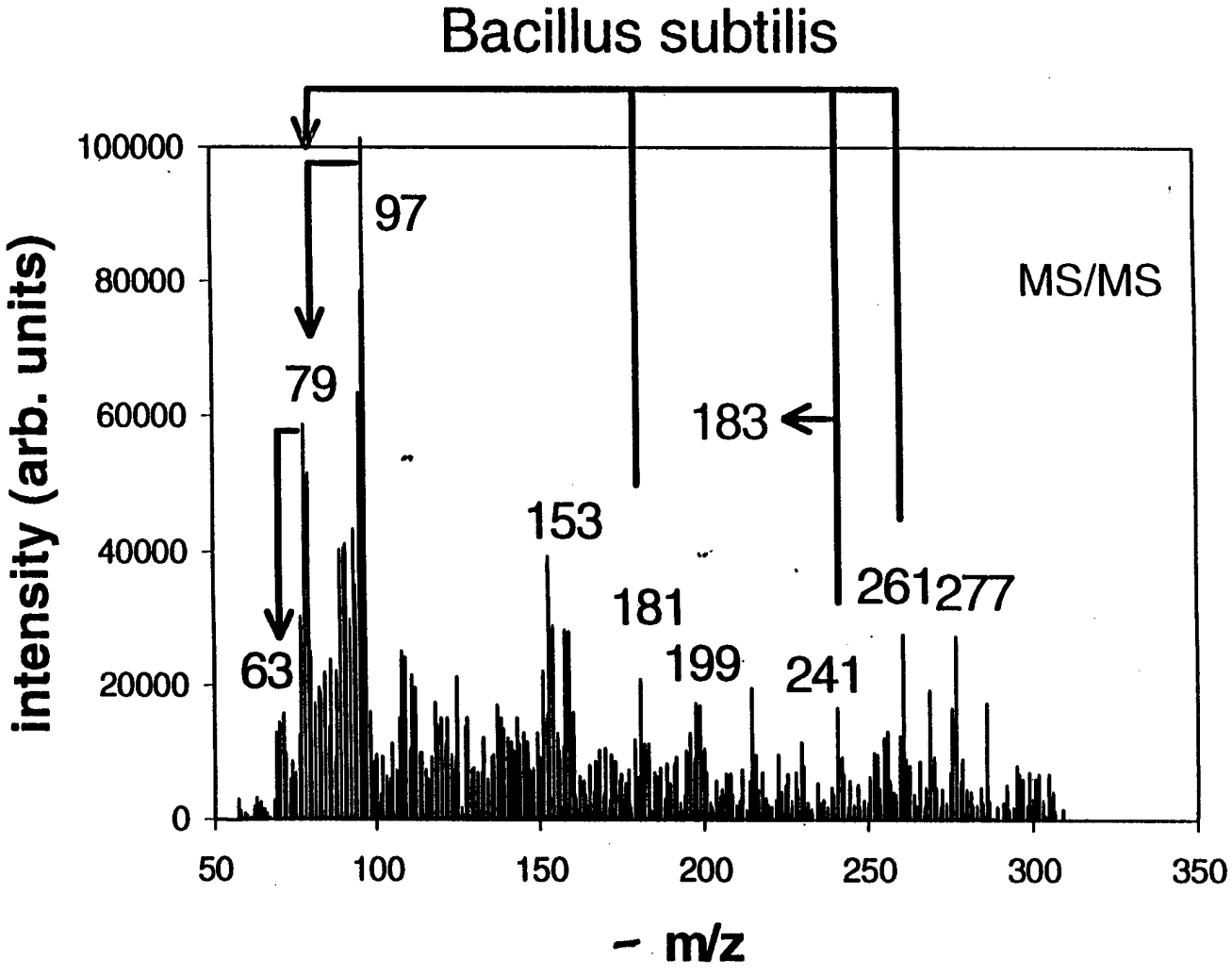
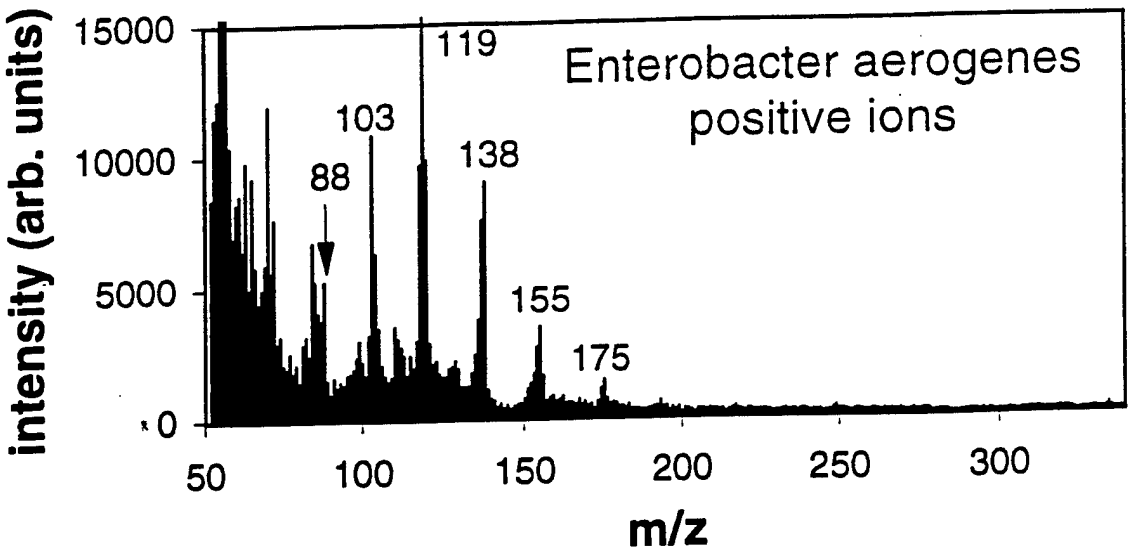
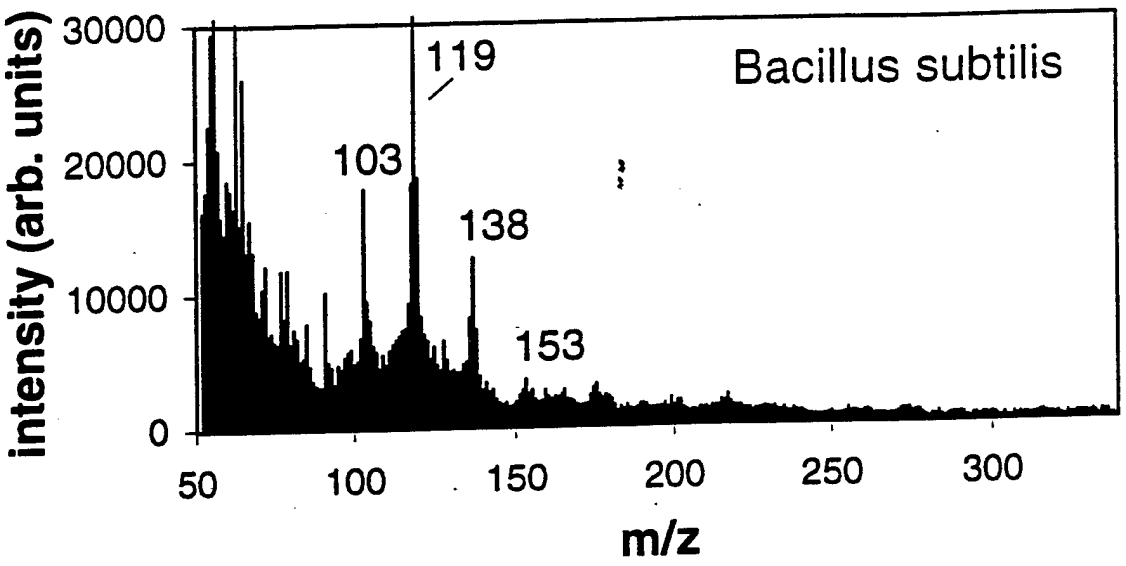

Bacillus subtilis
intensity (arb. units)
100000
80000
60000
40000
20000
0
50
100
150
200
250
300
350
m/z
MS/MS
97
79
63
153
181
199
183
241
261
277

Fig 4


Enterobacter aerogenes
positive ions
intensity (arb. units)
15000
10000
5000
0
119
103
88
138
155
175
50
100
150
200
250
300
m/z


Bacillus subtilis
intensity (arb. units)
30000
20000
10000
0
119
103
138
153
50
100
150
200
250
300
m/z

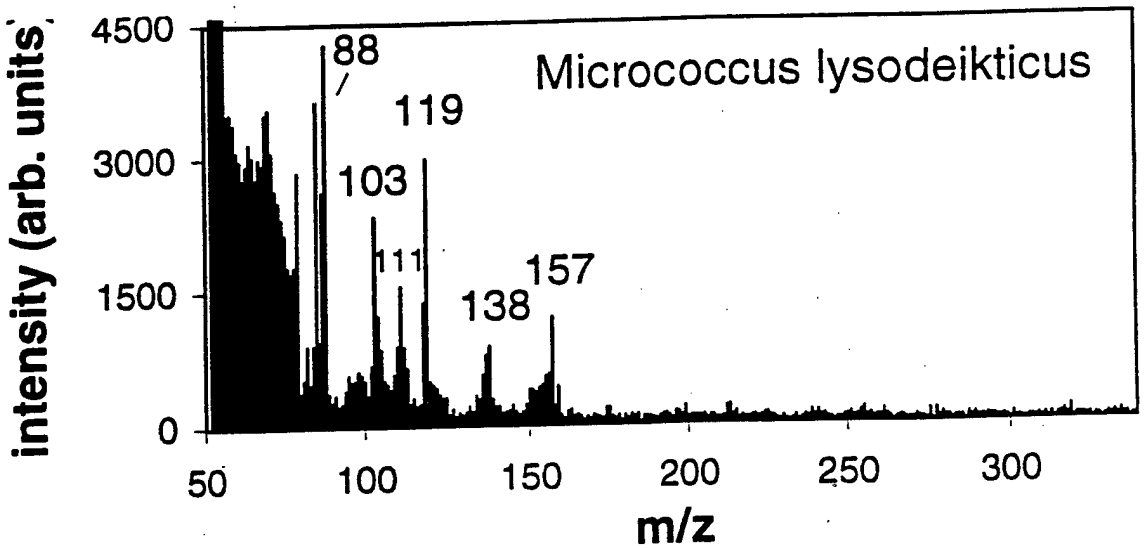

Micrococcus lysodeikticus
intensity (arb. units)
4500
3000
1500
0
88
119
103
111
138
157
50
100
150
200
250
300
m/z

Fig 5a

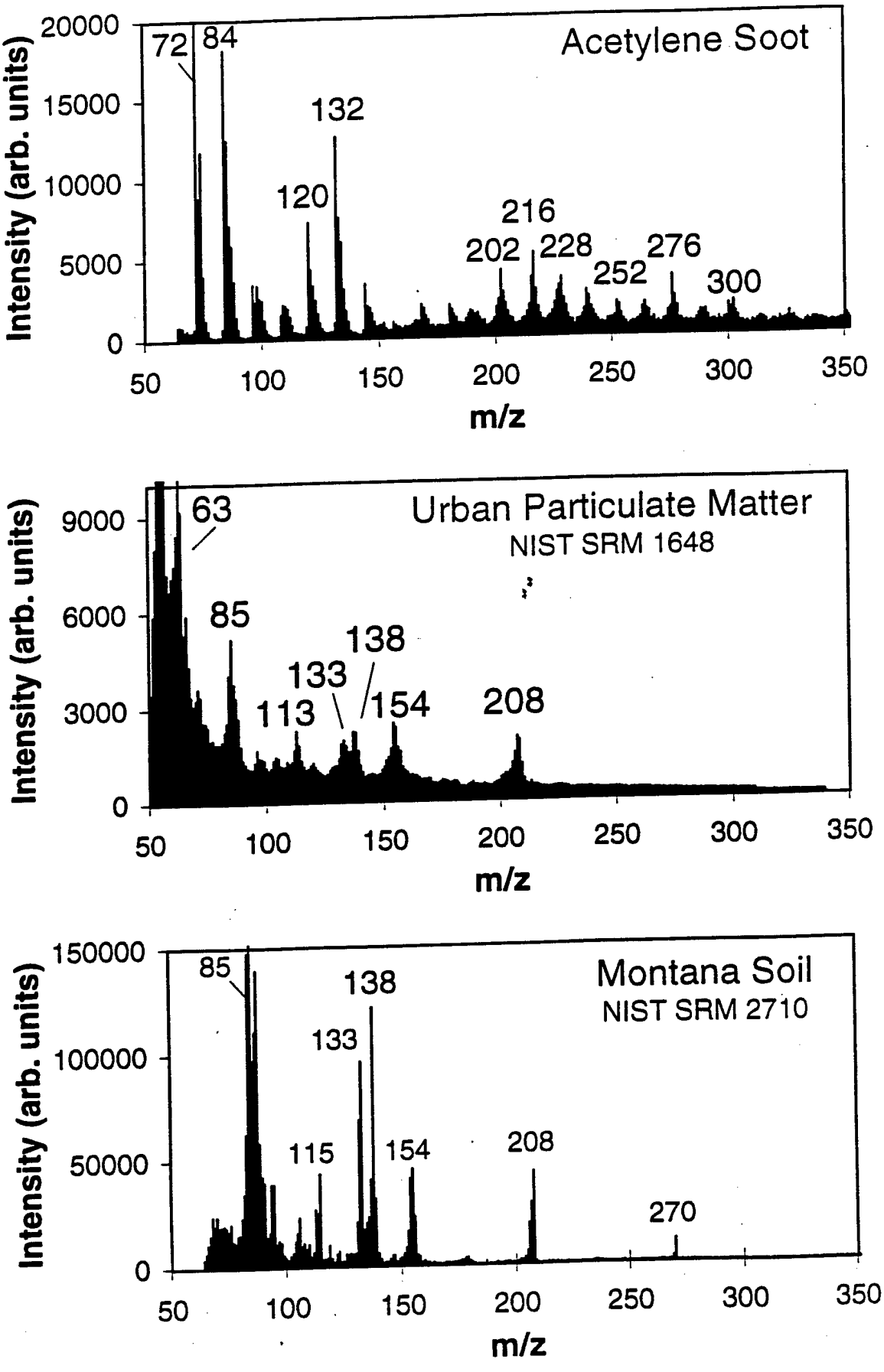

Acetylene Soot
Intensity (arb. units)
20000
15000
10000
5000
0
72
84
132
120
216
202
228
252
276
300
50
100
150
200
250
300
350
m/z
Urban Particulate Matter
NIST SRM 1648
Intensity (arb. units)
9000
6000
3000
0
63
85
138
133
113
154
208
50
100
150
200
250
300
350
m/z
Montana Soil
NIST SRM 2710
Intensity (arb. units)
150000
100000
50000
0
85
138
133
115
154
208
270
50
100
150
200
250
300
350
m/z

Fig 5b

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