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Rapid chemotaxonomy of pathogenic bacteria using in situ thermal hydrolysis and methylation as a sample preparation step coupled with a field–portable membrane-inlet quadrupole ion trap mass spectrometer

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Abstract

A field-portable, pyrolysis membrane-inlet quadrupole ion trap mass spectrometer has been used to characterize four pathogenic bacteria (*Bacillus anthracis, Brucella melitensis, Yersinia pestis,* and *Francisella tularensis*). Moreover, a variety of strains were included, prepared under various growth conditions and a range of growth stages. In these analyses, an *in situ* thermal hydrolysis-methylation procedure was used during pyrolysis with the reagent tetramethylammonium hydroxide. Mass spectra generated from the analysis of the four pathogens contained information related to the biochemical composition of the sample (i.e. biomarkers) including mass spectral peaks derived from methyl esters of fatty acids, DNA/RNA, and peptide/protein fragments. Using multivariate statistics, bacterial mass spectral fingerprints were analyzed to determine the variance in the data and the contribution of biomarker origin (i.e. lipid, protein, nucleic acid, etc.) for bacterial differentiation. An optimum 98.3% correct classification rate was obtained using cross validation with linear discriminant analysis (on four replicates each of 54 bacterial samples) using only biomarkers of lipid origin and the bacterial spore biomarker dipicolinic acid. (Int J Mass Spectrom 190/191 (1999) 331–342) © 1999 Elsevier Science B.V.

Keywords: Mass spectrometry; Quadrupole ion trap; Bacteria; Biomarkers; Chemotaxonomy; Microorganisms

1. Introduction

The advent of the quadrupole storage ion trap [1] has sparked a multitude of research in ion and ion–molecule chemistries [2–4] and fundamental and applied mass spectrometry [5–7]. The applications of ion trap mass spectrometers have taken advantage, among others, of the unique ion storage and ion manipulation capabilities (MS^n) and the compact size of the analyzer. Incorporation of soft-ionization techniques [8,9] along with high-mass scanning capabilities [10] have allowed the application of ion trap mass spectrometers for the analysis of biomolecules extending mass ranges up to 72 000 Da.

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Dedicated to J.F.J. Todd and R.E. March in recognition of their original contributions to quadrupole ion trap mass spectrometry.

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Recently, the need for rapid characterization of microorganisms has become important, for which a variety of methods have been used. Methods that employ mass spectrometry have been utilized for many years. Anhalt and Fenselau combined pyrolysis and mass spectrometry for the analysis of bacterial phospholipids and ubiquinones in 1975 [11]. Several other researchers have combined pyrolysis and mass spectrometry for the characterization of microorganisms [12–17]. These methods analyze bacterial components or fragments that are characteristic of each bacterium and appear in their mass spectra for the detection and possible identification of the microorganism.

Direct clinical and laboratory applications permit the use of research-based mass spectrometers for which instrumentation size and power consumption requirements are not limiting factors, such as triple quadrupole mass spectrometry, Fourier transform mass spectrometry (FTMS), and time-of-flight mass spectrometry (TOF-MS) systems. However, there is an increased need to perform real-time, on-site measurements for bacterial detection and identification [18]. The compact size of the quadrupole ion trap mass analyzer and higher operating pressures make it a prime candidate for a field-portable mass spectrometry instrument. Advancements in vacuum pump technology, computers, and electronics have allowed considerable reductions on the size, weight, and power consumption of mass spectrometry-based field-portable instruments. However, for the analysis of microorganisms, there is still a need for the development of rapid, automated sample preparation methodology that can be readily interfaced between the sample collection unit (e.g. aerosol collector) and the mass analyzer (the ion trap mass spectrometer, in this case).

A major focus of our research effort has been centered around the development of a membrane-inlet quadrupole ion trap mass spectrometer using pyrolysis as the sample pretreatment method. This instrument samples directly from air without sample preparation, is field portable, requires low power, and can perform tandem mass spectrometry (MS/MS) analyses to improve specificity and selectivity in the presence of a complex environmental background. In this instrument, pyrolysis occurs at atmospheric pressure in air, and samples are distilled or degraded at around 450 °C. The pyrolysis products (or pyrolysates) are then transferred into the mass spectrometer through a semipermeable membrane.

Considerable research has been performed toward the development of analytical methods for microorganism analysis and a biomarker database. In our research group specifically, the in situ thermal hydrolysis and methylation (THM) products of fatty acids [19,20], dipicolinic acid [21], DNA/RNA [22], carbohydrates, ubiquinones, phospholipids, amino acids, peptides [23–25], and proteins [26] have been examined. *In situ* THM has been used with this instrumentation in order to increase the selectivity and specificity of the biomarker detection scheme for compounds with acidic hydrogens ($pKa < 12$). Methylation enhances permeability through the nonpolar silicone membrane, increasing the volatility and decreasing the polarity of the involved compounds. In this study we apply mass spectral biomarker information for the differentiation of four pathogenic bacteria (*Bacillus anthracis*, *Francisella tularensis*, *Brucella melitensis*, and *Yersinia pestis*) using the in situ THM methodology for sample preparation in conjunction with a field-portable pyrolysis quadrupole ion trap mass spectrometer.

2. Experimental

2.1. Reagents

All chemicals were purchased from Sigma Chemical Co. (St. Louis MO) and were used without further purification. Bacterial samples were obtained as gamma-killed freeze-dried cells from the Armed Forces Institute of Pathology (Washington, DC). These samples were prepared as 10 mg/mL water suspensions. For mass spectral analysis, 15 μL of the bacterial suspension was coinjected with 5 μL of 1.0 M tetramethylammonium hydroxide (TMAH) in water, immediately prior to pyrolysis. TMAH and similar derivatization reagents have been used to increase the volatility of analytes, allowing analysis by gas chro-

matography and mass spectrometry [27,28]. An automated TMAH injection procedure is currently being developed for samples which are collected directly from the atmosphere into the pyrolysis unit.

2.2. Instrumentation

All analyses were performed using an air buffered quadrupole ion trap mass spectrometer fitted with an infrared pyrolyzer (Bruker-Franzen, Bremen, Germany) [29]. The quartz pyrolysis chamber is cylindrical, 4 cm in length with an inner diameter of 3 mm and an outer diameter of 4 mm. Centered in this chamber is a quartz frit (3 mm thick), where the sample is deposited. During pyrolysis, a temperature of 450 °C was maintained for 55 s. Pyrolysates were transferred through a 3 deactivated fused silica transfer line using air as the carrier gas at a flow rate of 1.5 L/min. Logistical purposes originally required this separation of the pyrolysis unit from the ion trap instrumentation. Pyrolysis was performed in air at atmospheric pressure. The transfer line was held at a temperature of 180 °C and the pyrolysis base unit was held at a temperature of 200 °C. A temperature of 200 °C was also used for the silicone membrane which interfaces the transfer line to the ion trap mass spectrometer. The quadrupole ion trap pressure was maintained at 5×10^{-5} Torr. A 70 eV positive electron ionization was used for all analyses. Ionization times were controlled and varied from 50 to 15 000 μs to prevent space charge effects from occurring in the trap [30]. An ion cooling time of 50 μs was used prior to scanning.

2.3. Pattern recognition

Factor analysis plots are generated using the RESOLVE software, developed at the Colorado School of Mines [31]. Mass spectra were collected as a set of raw intensities over the same mass range. Spectra were normalized to the total intensity to correct for variation in the total ion current. The data was also mean centered, so that principal component analysis (PCA) information is related to the variance in the data, rather than the variance from zero. To accomplish this, the average mass spectrum of the entire data set is subtracted from each individual mass spectrum (peak by peak) prior to PCA.

Linear discriminant analysis (LDA) was used to maximize intergroup (and minimize intragroup) variance. This was accomplished by using linear combinations of the principal components to further differentiate the data, as several principal components often contribute to the variance of the mass spectral fingerprints. Linear discriminant score plots, also known as canonical variate plots, were used to interrogate the linear discriminant functions and relate mass spectral differentiation to specific mass spectral peaks and intensities.

3. Results

Table 1 lists the bacteria studied. Several strains were analyzed for each species. Growth media and stages are known to affect certain enzyme activity within microorganisms. Therefore, various media and growth stages were examined for each bacterium to determine if the mass spectra would be affected. Fig. 1(a)–(d) show typical mass spectra for each of these bacteria when coinjected with TMAH. The observed mass spectral peaks are related to compounds such as fatty acid methyl esters (FAMEs), DNA methyl esters, and methylated peptide/protein fragments. Previous work has demonstrated the utility of FAMEs for classification of bacteria using pyrolysis mass spectrometry [19,20]. Table 2 lists FAME biomarker mass spectral ions which have been used for bacterial identification. In this mass spectrometer system, $[M + 1]^+$ protonated molecular ions are usually observed for fatty acid methyl esters as a result of self-chemical ionization (self-CI) occurring in the ion trap mass spectrometer [32]. Good reproducibility has been observed for both the degree of self-CI and methylation using TMAH. Self-CI is avoidable, provided small enough amounts of analyte are present in the trap. This is, of course, dependent on the specific functional groups in the analyte(s) present. In order to maintain a consistent amount of self-CI both the quantity of neutrals and reaction time are kept con-

Table 1
Bacteria examined, growth conditions and stages

# code	Organism strain	Media	Growth stage	Media codes
1 A	Bacillus anthracis-Vollum	LD	½log	LD = Leighton-Doi
2 S	Bacillus anthracis-Vollum	LD	spore	CAD = Casein acid digest
3 A	Bacillus anthracis-Vollum	CAD	½log	BB = Brucella broth
4 S	Bacillus anthracis-Vollum	CAD	Spore	BA = Brucella agar
5 A	Bacillus anthracis-Zimbabwe	LD	½log	BLOOD = Blood agar
6 S	Bacillus anthracis-Zimbabwe	LD	spore	TSB = Tryticase Soy broth
7 A	Bacillus anthracis-Zimbabwe	CAD	½log	TSA = Trypticase Soy agar
8 S	Bacillus anthracis-Zimbabwe	CAD	spore	IVCHOC = IsoVitalex Chocolate
9 A	Bacillus anthracis-Ames	LD	½log	MHB = Muller-Hinton broth
10 S	Bacillus anthracis-Ames	LD	Spore	
11 A	Bacillus anthracis-Ames	CAD	½log	
12 S	Bacillus anthracis-Ames	CAD	Spore	
13 A	Bacillus anthracis-Sternes	LD	½log	
14 S	Bacillus anthracis-Sternes	LD	Spore	
15 A	Bacillus anthracis-Sternes	CAD	½log	
16 S	Bacillus anthracis-Sternes	CAD	Spore	
17 B	Brucella melitensis-melitensis/WILD	BA	Top	
18 B	Brucella melitensis-melitensis/WILD	BB	½log	
19 B	Brucella melitensis-melitensis/WILD	BB	Top	
20 B	Brucella melitensis-melitensis/REV-1	BA	Top	
21 B	Brucella melitensis-melitensis/REV-1	BB	½log	
22 B	Brucella melitensis-melitiensis/REV-1	BB	Top	
23 B	Brucella melitensis-Suis	BA	Top	
24 B	Brucella melitensis-Suis	BB	½log	
25 B	Brucella melitensis-Suis	BB	Top	
26 B	Brucella melitensis-abortus/WILD	BA	Top	
27 B	Brucella melitensis-abortus/WILD	BB	½log	
28 B	Brucella melitensis-abortus/WILD	BB	Top	
29 B	Brucella melitensis-abortus/S19vac	BA	Top	
30 B	Brucella Melitensis-abortus/S19vac	BB	½log	
31 B	Brucella melitensis-abortus/S19vac	BB	Top	
32 B	Yersinia pestis-195/P India	Blood	Top	
33 Y	Yersinia pestis-195/P India	TSB	½log	
34 Y	Yersinia pestis-195/P India	TSB	Top	
35 Y	Yersinia pestis-La Paz	Blood	Top	
36 Y	Yersinia pestis-La Paz	TSB	½log	
37 Y	Yersinia pestis-La Paz	TSB	Top	
38 Y	Yersinia pestis-Nair Kenya	Blood	Top	
39 Y	Yersinia pestis-Nair Kenya	TSB	Top	
40 Y	Yersinia pestis-A1122 California	Blood	Top	
41 Y	Yersinia pestis-A1122 California	TSB	½log	
43 Y	Yersinia pestis-EV76	Blood	Top	
44 Y	Yersinia pestis-EV76	TSB	½log	
45 Y	Yersinia pestis-EV76	TSB	Top	
46 F	Francisella tularensis-Type A/Utah	IVCHOC	log	
47 F	Francisella tularensis-Type A/Utah	BCYE	log	
48 F	Francisella tularensis-Type A/Utah	MHB	Stationary	
49 F	Francisella tularensis-Type A/Utah	MHA	Stationary	
50 F	Francisella tularensis-Palaeartica	IVCHOC	Log	
51 F	Francisella tularensis-Palaeartica	MHB	Log	
52 F	Francisella tularensis-Palaeartica	MHA	Log	
53 F	Francisella tularensis-LVS	IVCHOC	Stationary	
54 F	Francisella tularensis-LVS	BCYE	Log	
55 F	Francisella tularensis-LVS	MHB	Stationary	
56 F	Francisella tularensis-LVS	MHA	Log	

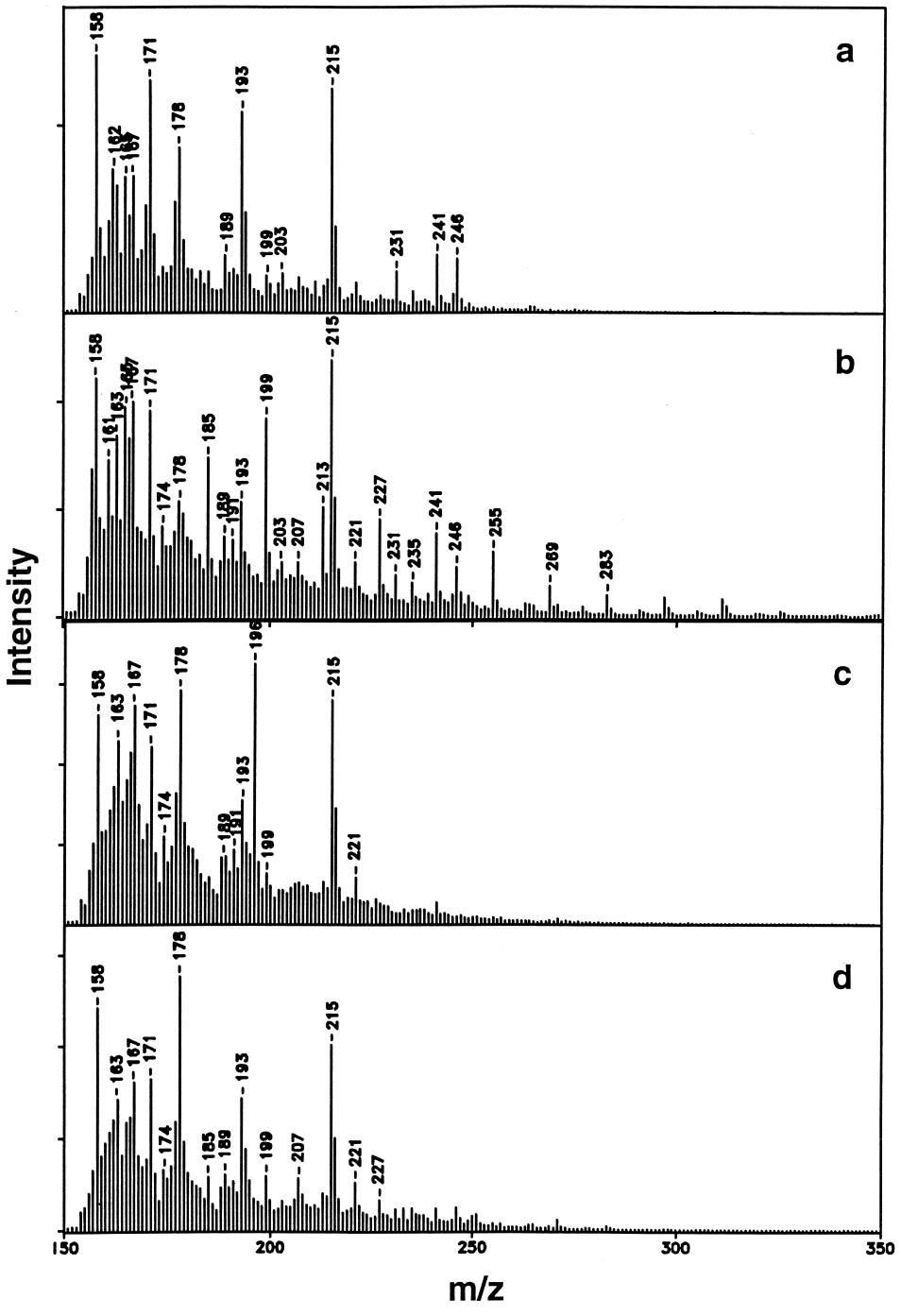


Fig. 1. Pyrolysis mass spectra of bacteria coinjected with TMAH: (a) *Brucella melitensis*; (b) *Francisella tularensis*; (c) *Bacillus anthracis*; (d) *Yersinia pestis*.

Table 2
FAME ions useful for bacterial identification[a]

FAME	*m*/*z*	FAME	*m*/*z*	FAME	*m*/*z*
C12:0 ME*	214	C16:1 ME*	268	C19:0 ME*	312
		M–31	237	M–31	281
C14:0 ME*	242	M–32*	236		
		M–74	194	cyC19:0 ME*	310
C15:0 ME*	256			M–31	279
M–57	225	C17:0 ME*	284	M–32*	278
		M–31	253	M–74	236
aC15:0 ME*	256				
M–57	199	iC17:0 ME*	284	C20:0 ME*	326
		M–43	241	M–31	295
iC15:0 ME*	256	cyC17:0 ME*	282	C21:0 ME*	340
M–43	213	M–31	251	M–31	309
		M–32*	250		
C16:0 ME*	270	M–74	208	C22:0 ME*	354
M–31	239			M–31	323
M-29	241	C18:0 ME*	298		
−14	227	M–31	267	C24:0 ME*	382
−14	213				
−14	199	C18:1 ME*	296	C24:1 ME*	380
−14	185	M–31	265	M–32*	348
		M–32*	264	M–74	306
		M–74	222		

[a]Entries marked with an asterisk can also be measured as the $(M + 1)^+$ ion in the ion trap mass spectrometer.

stant. In addition the total number of ions produced in the trap is kept constant by varying the ionization time according to the quantity of sample present. Obviously, this issue can be circumvented by using a chemical ionization system [33,34].

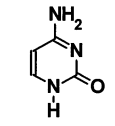
The intact methylated DNA bases that have also been identified and are listed in Table 3. DNA bases can undergo varying degrees of methylation, as each base contains multiple methylation sites. Thus several peaks can often be observed which are identified as derivatized DNA bases.

Peptides and proteins are rarely observed as intact species in this membrane–inlet system, due to their relative polarity [26]. Also, these species are often thoroughly fragmented and/or oxidized during the pyrolysis process creating a wide distribution of products. No molecular ions are observed for proteins in this system. Most peaks of proteinaceous origins, therefore, have been observed below *m*/*z* 150, the lowest mass scanned in these analyses. However, some peaks of proteinaceous origin have been observed during the analysis of protein standards, including *m*/*z*'s 165, 167, 179, 189, 191, 200, 215, 231, and 246. The chemical structure of most of these ions is under investigation, but are known to be related to amino acids which contain relatively nonpolar moieties such as aromatic or ring functionalities [e.g. tyrosine (*m*/*z* 246, 231), phenylalanine (*m*/*z* 215), tryptophan (*m*/*z* 189)]. Oxidative pyrolysis conditions and thermal hydrolysis and methylation combined with membrane–inlet transmission enhancement of previously unobserved nonpolar compounds make their identification complex [26].

Using this biomarker information, bacteria can be classified via their fatty acid, DNA, and protein content. Because of the large number of variables involved, factor analysis techniques were employed for bacterial classification. PCA is a variable reduction method which allows interrogation of the variance in the data in a viewable dimensionality. As mentioned previously, linear discriminant analysis can be subsequently employed to further separate and categorize the mass spectra.

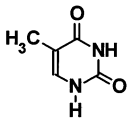
Figure 2 is a LDA score plot (components 1 and 3)

Table 3
DNA bases and their methyl derivatives



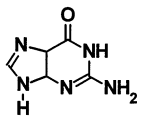
Cytosine

MW 111
Derivative masses 125, 139, 153



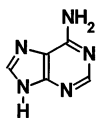
Thymine

MW 126
Derivative masses 140, 154



Guanine

MW 151
Derivative masses 165, 179, 193, 207



Adenine

MW 135
Derivative masses 149, 163, 177

using 13 principal components for the analysis. Mass spectra are labeled with one letter codes: A = *Bacillus anthracis* ½-case log phase, S = *Bacillus anthracis* sporulated, B = *Brucella melitensis*, F = *Francisella tularensis* and Y = *Yersinia pestis*. Four replicates of the 54 samples listed in Table 1 are plotted using all of the peaks in the scanned mass spectral range of 150–400 Da. In Fig. 2, the samples show well defined clustering. Loading plots are used to interrogate the LDA score plots. Fig. 3(a) shows the loading plot for discriminant component 1. Bacterial mass spectra on the positive side of the component 1 axis in the LDA score plot (Fig. 2) are relatively enriched in the peaks on the positive side of the loading plot [Fig. 3(a)]. The composition of the loading is mainly based on FAME ions and $(M + 1)^+$ FAME molecular ion peaks as listed in Table 2. *Francisella tularensis* is the bacterium most enriched

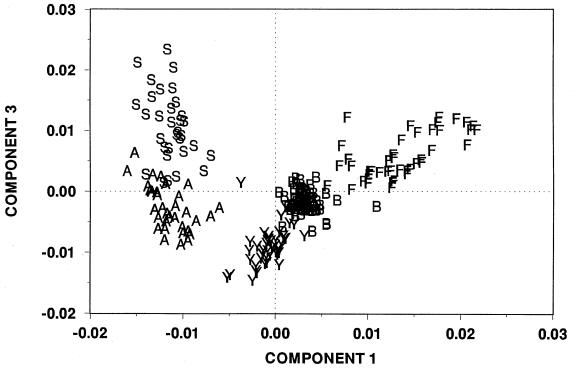


Fig. 2. Linear discriminant analysis plot of bacterial mass spectra *m*/*z* 150–400, components 1 and 3. A = *Bacillus anthracis* ½log phase, S = *Bacillus anthracis* sporulated, B = *Brucella melitensis*, F = *Francisella tularensis* Y = *Yersinia pestis*.

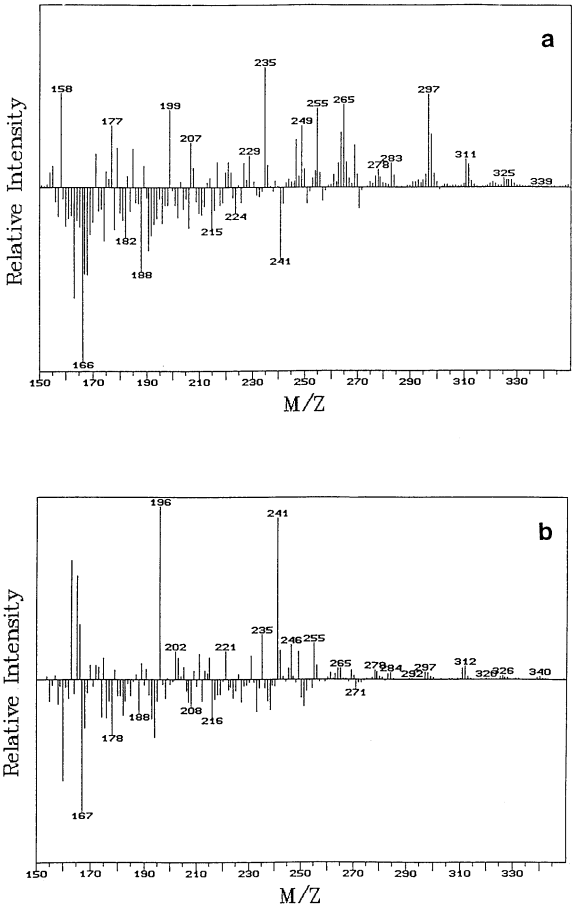


Fig. 3. Discriminant loading plot for (a) component 1 and (b) component 3 in Fig. 2.

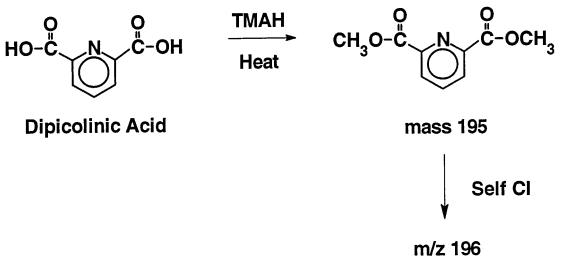


Fig. 4. Methylation step to form dipicolinic acid dimethyl ester (M + 1), a biomarker for bacterial spores.

in these fatty acids, including some higher molecular weight FAMEs such as C18:1 ME, cyC19:0 ME, C22:0 ME and C24:0 ME [35]. These peaks are also observed in the mass spectrum of *F. tularensis* [Fig. 1(b)]. The *Bacillus anthracis* species contain lower amounts of these FAMEs. These results correlated well with previous analyses of extracted/methylated lipids from bacterial pathogens with a Curie-point pyrolysis triple quadrupole mass spectrometer instrument [18].

Component 3 in the LDA score plot shown in Fig. 2 is primarily responsible for the separation of sporulated from nonsporulated *B. anthracis*. Fig. 3(b) shows the loading plot for this component. The ion at *m*/*z* 196 represents the protonated molecule of dipicolinic acid dimethyl ester. Dipicolinic acid is produced by certain sporulated bacteria and has been well established to be a useful biomarker for the presence of bacterial spores [15,21]. Fig. 4 shows the methylation and ionization steps by which the ion at *m*/*z* 196 is formed. Without the methylation step, the molecular ion of dipicolinic acid is not observed. Rather, a thermal degradation product, pyridine is observed at *m*/*z* 79. Fig. 5(a) and (b) are pyrolysis mass spectra of *B. anthracis* harvested at ½ log and sporulated

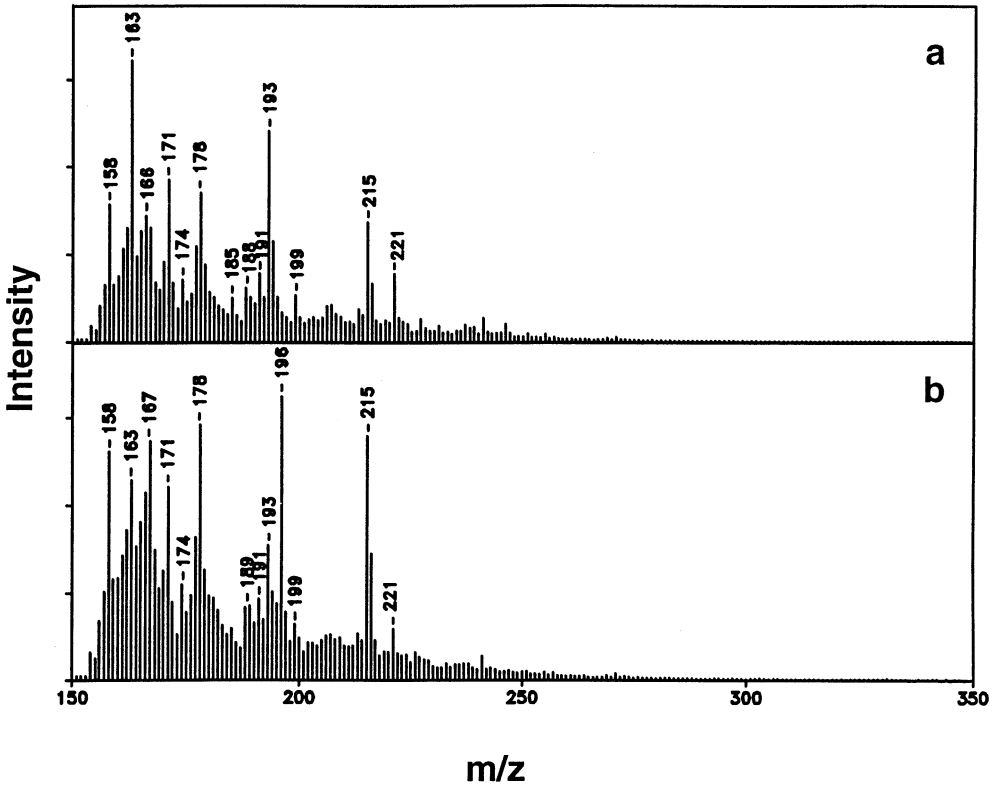


Fig. 5. Pyrolysis mass spectra of *Bacillus anthracis* harvested at (a) ½log and (b) sporulated phase.

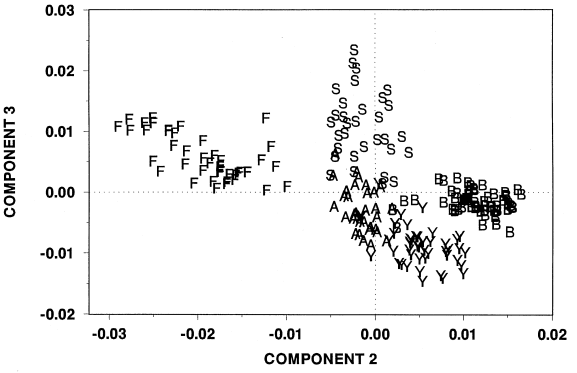


Fig. 6. Linear discriminant analysis plot of bacterial mass spectra m/z 150–400, components 2 and 3.

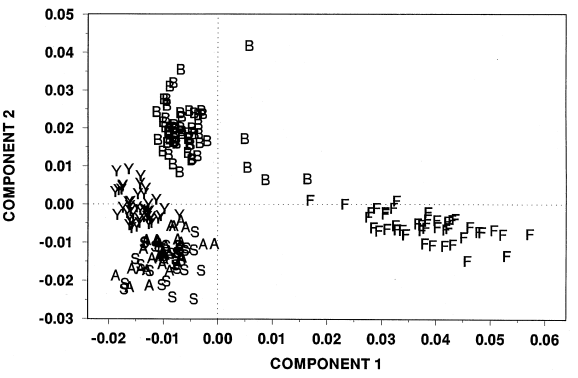


Fig. 7. Linear discriminant analysis plot of bacterial mass spectra using fatty acid related peaks, components 1 and 2.

phases, coinjected with TMAH. Clearly the sporulated form of the bacterium exhibits the dipicolinic acid biomarker.

The combination of other principal components in a LDA plot can yield to the better differentiation of two specific bacteria. In Fig. 6 a LDA score plot of components 2 and 3 shows that *F. tularensis* can be further separated from *B. melitensis* and *Y. pestis* along component 2. Hence, different combinations of components can be used to enhance the differentiation between two bacteria within a predefined set of bacteria.

LDA analyses were also performed on the same data set using only the mass spectral peaks related to fatty acids, DNA, and protein fragments. This approach can help reduce the contribution from chemical noise by eliminating peaks which have no taxonomic significance [13,19]. Fig. 7 shows a LDA score plot of components 1 and 2 using only the peaks related to the fatty acid methyl esters and the M + 1 molecular ions as described in Table 2. Again, there is good grouping of the bacterial species with the exception of *B. anthracis* species harvested at the ½ log and sporulated phases (A and S in Fig. 7, respectively). This is not surprising since the dipicolinic acid peak (m/z 196) was removed from the data set. However, the majority of the variance among the different bacterial species mass spectra occurs as a result of the FAME profiles of the bacteria. Bacterial differentiation at the species level is still possible, although the growth stage of the *Bacillus anthracis* is more difficult to discern using only FAME related peaks. Fig. 8(a) and (b) show the loading plots for components 1 and 2 (in Fig. 7) using FAME related ions only. These results are consistent with fatty acid methyl ester extract analyses performed previously [18,19] with the added advantage that whole cell analysis with in situ THM requires a sample preparation and analysis time of less than 10 min/sample.

Fig. 9 is an LDA score plot using DNA/RNA related peaks only, as listed in Table 3. Although the group clustering is not as distinct as when the fatty acid data is included, significant taxonomic information is still observed. It is interesting to note that there is variance between the sporulated and nonsporulated *Bacillus anthracis* samples. Since the DNA of a specific species is identical whether the cell occurs in the vegetative or sporulated state, the variance was attributed to a varying degree of thermal hydrolysis and methylation. This is not surprising, given the morphological differences between the vegetative and sporulated stages. The in situ THM mass spectra of sporulated bacteria showed less complete methylation of the DNA bases. The rugged nature of the bacteria spore seems to require more thermal energy for the in situ THM process. However, this is not detrimental to the analysis and identification of bacterial spores. As long as consistent mass spectral fingerprints are generated, the growth stage of *Bacillus anthracis* can be predicted with confidence. Fig. 10(a) and (b) are the

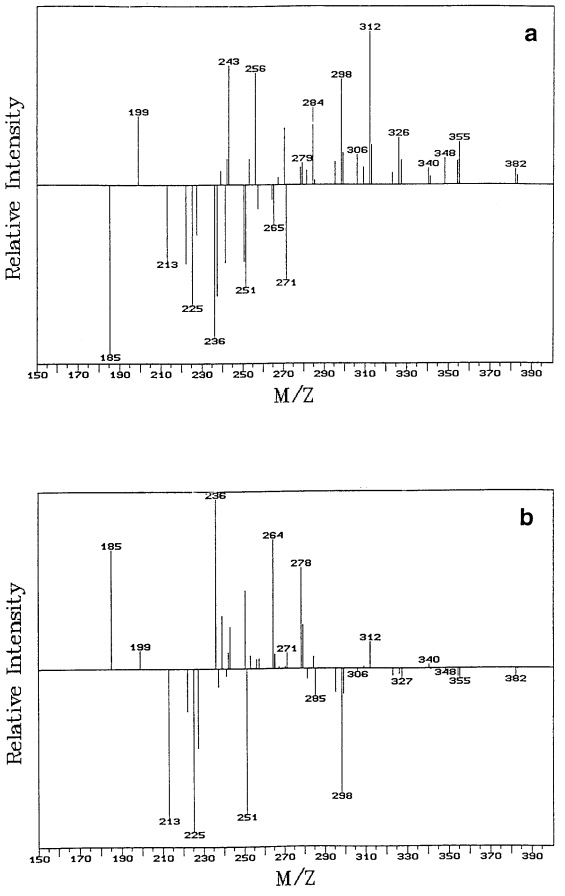


Fig. 8. Discriminant loading plot for (a) component 1 and (b) component 2 in Fig. 7.

loading plots of factors 1 and 2, using only the DNA related peaks.

Cross-validation linear discriminant analysis was used in an attempt to predict the ability of factor analysis for the classification of unknown bacteria. This method removes one sample at a time, treats it as an unknown and then predicts its category using the patterns generated from LDA plots of the remaining data set, for which the categories are known. Using this method, 208 of the 216 bacterial mass spectra were correctly classified using cross-validation discriminant analysis and the full mass range scanned (150–400 Da). This corresponds to a 96.3% correct classification rate. Of the 8 misclassifications 4 were *B. anthracis*, which were misclassified based on

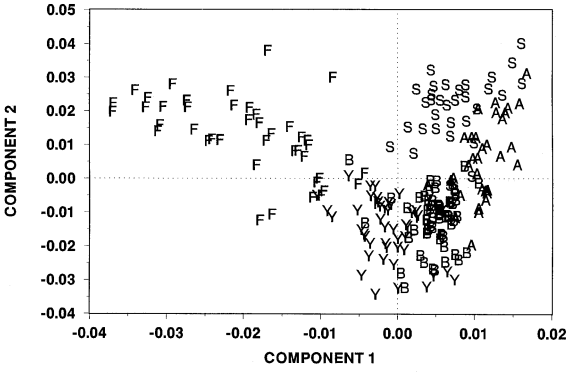


Fig. 9. Linear discriminant analysis plot of bacterial mass spectra using DNA related peaks only, components 1 and 2.

growth stage (½log and sporulated phases or A versus S in the LDA plots). Using only the fatty acid peaks a classification rate of 97.2% was obtained. DNA and protein information was more limited. A 86.1% correct classification rate was obtained using only DNA peaks and 87.5% of the samples were correctly classified using protein related peaks. Using all of the defined biomarker peaks (FAMEs, DNA, protein, DPA) a 95.8% correct classification rate was achieved.

The best results were obtained when the FAME related peaks were combined with the dipicolinic acid dimethyl ester (M + 1) biomarker peak at *m*/*z* 196. In this case, a correct classification rate of 98.3% was obtained, with 212 of the 216 correctly classified. Only one sample was incorrectly identified at the genus level (Yersinia instead of Brucella). The other three were misclassifications of *Bacillus anthracis* growth stage, but were still correctly classified as *Bacillus anthracis*. DNA information pertaining to the differentiation of *Bacillus anthracis* on growth stage did not improve the results when added to the FAME and DPA data. For this analysis a 94.4% correct classification rate was obtained.

4. Conclusions

Biomarker information has been used for the classification of four pathogenic bacteria using a membrane–inlet pyrolysis quadrupole ion trap mass spec-

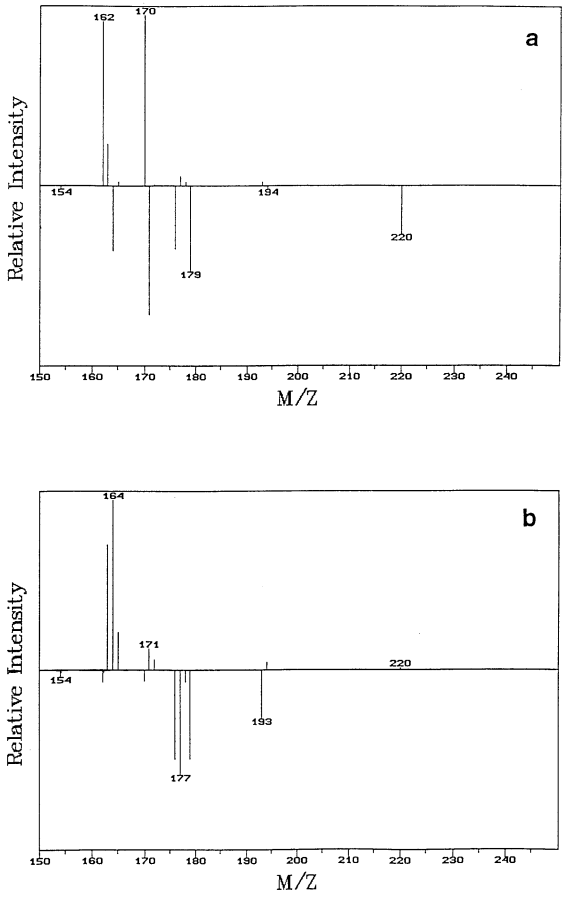


Fig. 10. Discriminant loading plot for (a) component 1 and (b) component 2 in Fig. 9.

trometer and in situ thermal hydrolysis and methylation. Four replicates of 55 samples of four pathogenic bacteria were analyzed. Various growth media and stages were also included in the samples. However, most mass spectra were still classified correctly during data analysis. This is particularly significant because for rapid field detection of microorganisms to be successful, the identification scheme must be capable of correctly recognizing a wide range of bacteria, regardless of the subspecies/strains and growth conditions which might be encountered. Biomarker mass spectral ions used in this study were related to the fatty acids, DNA, and protein present in the bacteria. An optimal classification rate of 98.3% was obtained using cross-validation linear discriminant analysis with only FAME and methylated dipicolinic acid biomarker ions. A 96.3% correct classification rate was obtained using all of the ions in the full mass spectral range scanned (150–400 Da).

The combination of in situ THM as a sample inlet technique with quadrupole ion trap as a sample analysis method provides the possibility of rapid ($<$ 10 min total analysis time per sample), selective and reproducible field analysis for microorganisms. The portability (small size), low power requirements and higher operational pressures of the ion trap mass spectrometer make this combination ideal for an automated field portable mass spectrometer.

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