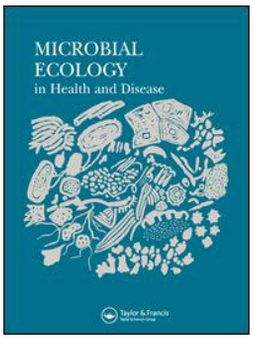


Microbial Ecology in Health and Disease

ISSN: (Print) 1651-2235 (Online) Journal homepage: informahealthcare.com/journals/zmeh20

The Application of Matrix-Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry to Profile the Surface of Intact

Haroun N. Shah, Carrina J. Keys, Saheer E. Gharbia, Kathryn Ralphson, Frank Trundle, Ian Brookhouse & Ian Martin A. Claydon

To cite this article: Haroun N. Shah, Carrina J. Keys, Saheer E. Gharbia, Kathryn Ralphson, Frank Trundle, Ian Brookhouse & Ian Martin A. Claydon (2000) The Application of Matrix-Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry to Profile the Surface of Intact, Microbial Ecology in Health and Disease, 12:4, 241-246, DOI: 10.1080/08910600050216237

To link to this article: https://doi.org/10.1080/08910600050216237

Published online: 11 Jul 2009.

The Application of Matrix-Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry to Profile the Surface of Intact Bacterial Cells

Haroun N. Shah[1], Carrina J. Keys[1], Saheer E. Gharbia[2], Kathryn Ralphson[3], Frank Trundle[3], Ian Brookhouse[3] and Martin A. Claydon[4]

From the [1]Identification Services Unit, National Collection of Type Cultures, PHLS Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, [2]Department of Life Sciences, University of East London, Romford Road, London E15 4LZ, [3]Kratos Analytical, Wharfside, Trafford Wharf Road, Manchester M17 1GP, and [4]Department of Biological Sciences, Manchester Metropolitan University, Chester Street, Manchester M1 5GD, UK

Correspondence to: Haroun N. Shah, Identification Services Unit, National Collection of Type Cultures, PHLS Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT. Tel: +44 208 200 4400 Ext. 3749, Fax: +44 181 205 7483

Microbial Ecology in Health and Disease 2000; 12: 241–246

Matrix-Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry (MALDI-TOF-MS) as a tool for differentiating bacterial species was examined using reference strains representing gram-positive and gram-negative taxa. Initially, the effect of differences in medium composition on spectral profile was examined. The results indicated that growth on Columbia blood agar resulted in a larger spectrum of ionized residues and was therefore used for the cultivation of all strains in the rest of the study. The stability of the obtained mass spectral profiles against differences in batch and media processing suggested that no significant alterations to the profiles occurred in response to changes in media sources. The established conditions from these initial experiments were used to standardize subsequent experiments. The MALDI-TOF-MS profiles of 15 reference strains were compared and species characteristic markers were identified. The potential of using MALDI-TOF-MS as a tool for probing clonal diversity was examined using well characterized but clonally variable isolates of *Bacteroides fragilis*. Comparative analysis of the profiles of 20 strains revealed 5 clusters within the species but compared to other taxa such as *Bacteroides merdae* and *Salmonella arizonae* they form a closely related lineage. These results obtained strongly support the potential to use MALDI-TOF-MS as a tool for exploring bacterial surfaces for characteristic biomarkers and species-specific signatures.

Revised 2 March 2000

INTRODUCTION

An increasing number of methods have been proposed for the rapid identification of bacterial species from clinical, environmental and pharmaceutical products. Species and strain characteristic markers are essential for the success of such techniques. Drawbacks are due to tedious or lengthy sample preparation, need for specialized training and expensive reagents. Further complication is associated with the analysis of profiles and data generated in the absence of computational software to archive data, compare results and identify unknowns. Matrix Assisted Laser Desorption/ Ionisation Time of Flight Mass Spectrometry (MALDI-TOF-MS) is a novel technique that is emerging as a potentially powerful tool in microbiology (1–7). The technique enables ionisation of cell surface molecules of intact bacteria with the use of a matrix solution, which is bombarded by a laser to create gas-phase ions. Depending upon the mode in which the instrument is set, negative or positive ions move towards a detector at different speeds and are separated according to their mass/charge ratio. The resulting mass spectrum is a profile of the ionisable components of the cell envelope. MALDI-TOF-MS was shown to produce profiles of gram-positive and gram-negative bacteria while different matrix analytes resulted in variation in surface ionisation profiles (5). It was also reported that reproducible mass spectral profiles were obtained over a period of several months for a given isolate. To investigate the potential use of MALDI-TOF-MS for the identification of bacteria through comparison of unknowns to an archived database, it is essential to assess the diversity of spectra produced by MALDI-TOF MS, the reproducibility of the technique and the effect of the

 ISSN 0891-060X

culture medium on the mass spectral profiles. The aim of this study, therefore, was to investigate these parameters with the ultimate aim of using this as means of rapid identification of bacteria and characterisation of virulence determinants and specific epitopes.

MATERIALS AND METHODS

Bacterial cultures and media

The following reference strains from the National Collection of Type Cultures (NCTC) were used in these studies; *Serratia marcescens* NCTC 10036, *Shigella boydii* NCTC 10024, *Pasteurella multocida* NCTC 8771, *Bacillus coagulans* NCTC 3991, *Staphylococcus aureus* NCTC 10655, *Streptococcus pneumoniae* NCTC 11900, *Streptococcus sp.* Group B NCTC 9415 and *Vibrio damsela* NCTC 11646. *Salmonella arinonae* NCTC 7336, *Bacteroides merdae* NCTC 13052, *Bacteroides fragilis* NCTC 8560, NCTC 11295, NCTC 9343 and 16 clinical isolates obtained from the Anaerobe Reference Unit, Anaerobe Reference Unit, Public Health Laboratory Cardiff, UK.

The effect of differences in culture medium on the MALDI-TOF mass spectral profiles was investigated using 6 of the above reference strains; NCTC 10036, NCTC 10024, NCTC 8771, NCTC 3991, NCTC 10655 and NCTC 9415. They were grown on CBA plates obtained from four different Public Health Laboratory (PHL) sources in England. These were Dorchester PHL, Chester PHL, Taunton PHL and the Central Public Health Laboratory in Colindale, London, UK. All strains were grown for 24–48 hours prior to analysis; all media constituents were from Oxoid Ltd Basingstoke, Hants, UK), while blood was supplied by TCS Microbiology (Bucks, UK).

To study the effect of media composition on the MALDI-TOF-MS profile, *Shigella boydii*, NCTC 10024, grown on Columbia Blood Agar (CBA) and Nutrient Agar (NA) and analysed using the above described procedure.

Sample Preparation and Mass Spectral Analysis

Cultures were grown overnight at 37°C on NA or CBA plates. 1 ωl disposable plastic loops were used to remove a small amount of growth from the surface of several single colonies on a plate. This was placed on a stainless steel 20 sample slide (Kratos Analytical, Manchester,TO-406, UK) and 0.7 ωl of matrix solution containing acetonitrile, water and methanol (1:1:1) and 0.01 M 18-Crown-6 ether and 0.1% formic acid (v/v), saturated with 5-Chloro-2-mercaptobenzothiazole (CMBT) or α-cyano 4-hydroxy-cinnamic acid (CHCA) was then overlaid onto the inoculated target. The saturated solution was 3.0 mg CMBT in 1 ml matrix solution for gram-positive organisms and 14.0 mg CHCA in 1 ml matrix solution for gram-negative organisms. The suspensions were allowed to dry naturally. All reagents were obtained from Sigma Aldrich (Poole, Dorset, UK). A Kratos Kompact Alpha™ Mass Spectrometer with 337 nm laser and 3 ns pulse width, sampling 100 profiles across the width of the sample and pulsed extraction, was used for the analysis of all samples. A combined calibration file was generated to cover the mass range 500–6000 daltons. The data was collected in a linear positive ion mode. A modified Jaccard coefficient and UPGMA was used to analyse and interpret data which was then presented schematically in the form of a dendrogram as percentages of differences (% Dissimilarity).

RESULTS AND DISCUSSION

The bacterial cell envelope plays a central role in adherence to host tissues, attachment to other bacteria and interaction with the environment. Epitopes within the cell envelope alter in response to environmental stimuli. Methods for detecting such changes are difficult and usually involve the mechanical disruption of cells and subsequent isolation of surface components by tedious procedures. There is a need for non-invasive procedures to characterise such surface biomarkers for identification and assessment of bacterial responses to stimulants and environmental pressure. MALDI-TOF Mass Spectrometry enables direct examination of intact cells and may therefore have wide application in microbiology. Here we explore its potential value as a tool for microbial identification and examine some of the parameters that are likely to affect the interpretation of the data.

We selected a range of reference strains from NCTC that varied in their morphological features, gram stain and cell envelope composition, to determine the extent to which characteristic mass spectral signatures could be obtained for different species. The results obtained confirmed previous observations that differences in the ionisation of surface molecules occur between gram-positive and gram-negative bacteria in response to the analyte used (2). A wider range of mass spectral ions with clear mass peaks was obtained for gram-positive bacteria using CMBT, while gram-negative patterns were better resolved using CHCA.

Colonial morphology of bacterial isolates is a function of cell envelope structure and may be markedly affected by medium composition. The addition of blood, growth supplements or selective reagents to a culture medium alters the expressed phenotype of cells and will therefore result in differences in mass spectral profiles. Consequently, part of this study was undertaken to examine the effect of differences in the selected growth media on the range of mass ions released from bacterial cells. Major differences were detected in the obtained profiles of colonies grown on CBA compared to those cultured on NA. The former possessed a wider range of mass ion over the detected mass range (ca 500–2100 Da) while nutrient agar grown cultures were poorly ionized and resulted in a low mass/charge range (ca. 500–1400 Da), (see Fig. 1). Hence all further experiments were undertaken using CBA as the growth medium.

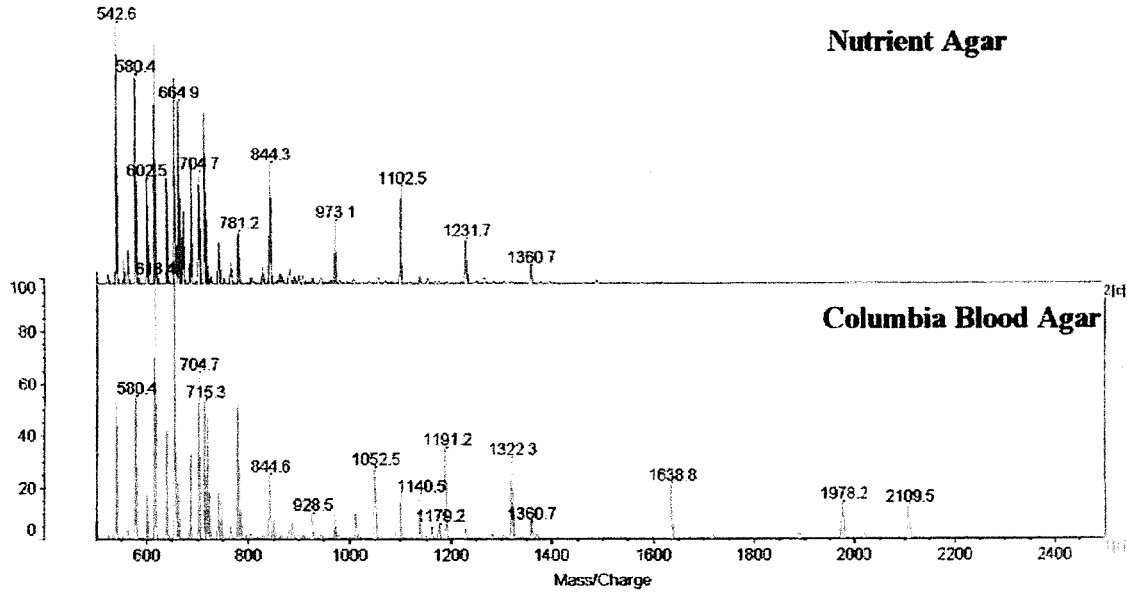


Fig. 1. Comparison of the mass spectral profiles obtained from the analysis of *Shigella boydii* NCTC 10024, when grown on two different types of media, Columbia blood agar and nutrient agar.

Six reference strains; *Serratia marcescens* NCTC 10036, *Shigella boydii* NCTC 10024, *Pasteurella multocida* NCTC 8771, *Bacillus coagulans* NCTC 3991, *Staphylococcus aureus* NCTC 10655 and *Streptococcus* sp. Group B NCTC 11646 were examined for the consistency of the profiles in response to growth on CBA from four media production centres as stated in Methods and Materials (above). The mass spectral profiles of each species remained unchanged indicating that differences in culture media preparation or batches do not significantly alter the obtained profiles. An example of the results obtained is shown in Fig. 2 which compares the mass spectral profiles for *Pasteurella multocida* NCTC 8771 grown on CBA plates prepared by the four centres. Cluster analysis (data not shown) of these spectra revealed negligible differences between spectra.

Comparative analysis of the test strains grown on CBA was undertaken to ascertain whether distinct mass spectral profiles were associated with each organism. Fig. 3(a-f) show examples of gram-positive and gram-negative organisms obtained on this medium over the mass range ca. 500–3,000 daltons. Peaks below 500 daltons were mainly matrix-derived and were excluded. Here the profile of each

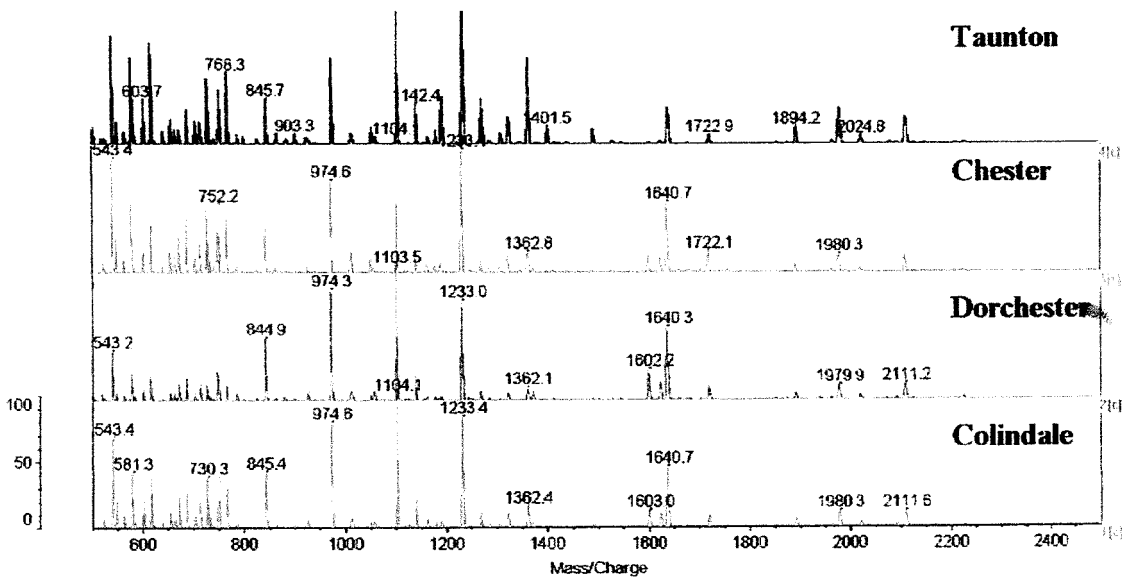


Fig. 2. Comparison of the mass spectral profiles obtained for *Pasturella multocida*, NCTC 8771, when grown on Columbia blood agar media obtained from four different media production centres.

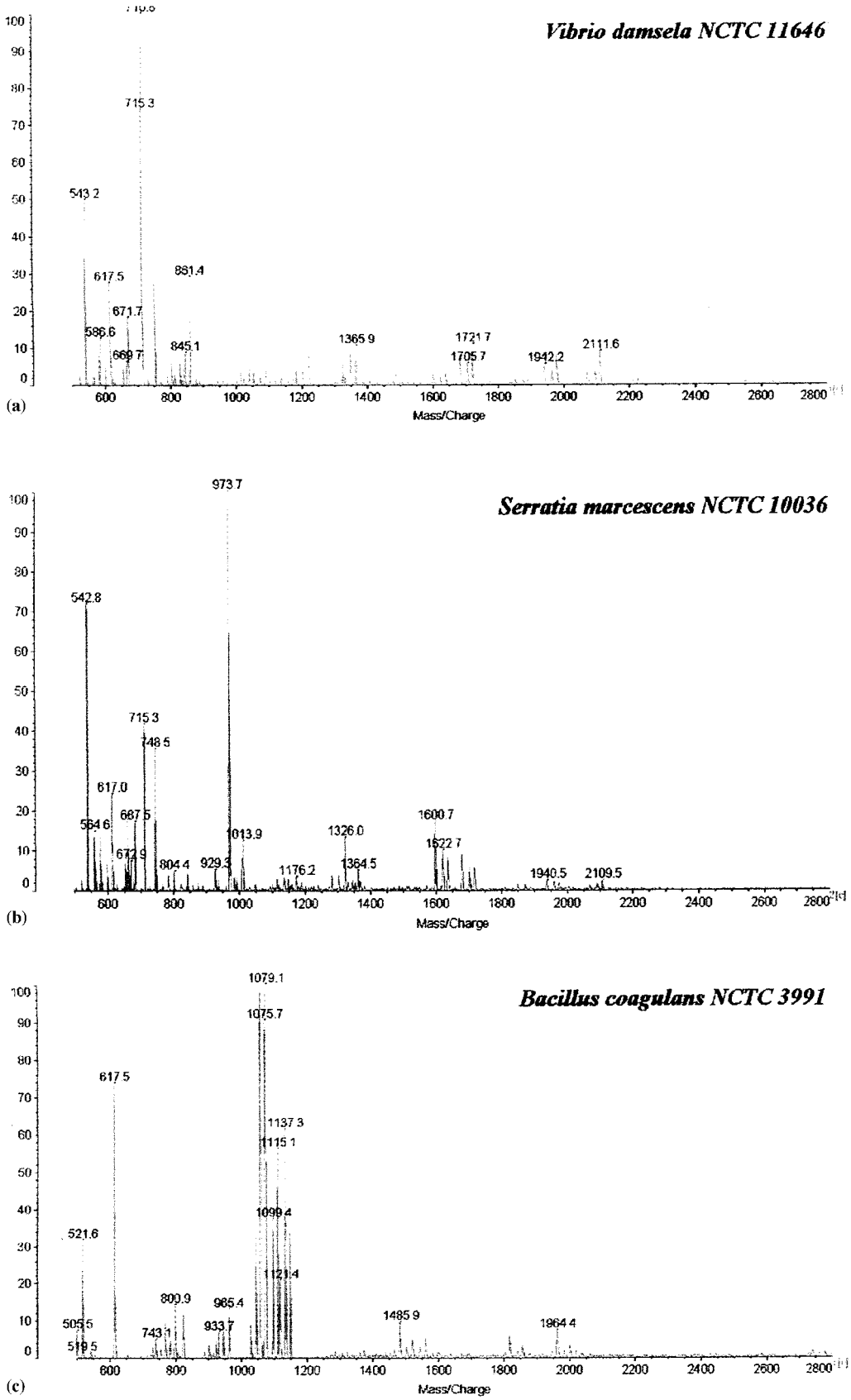
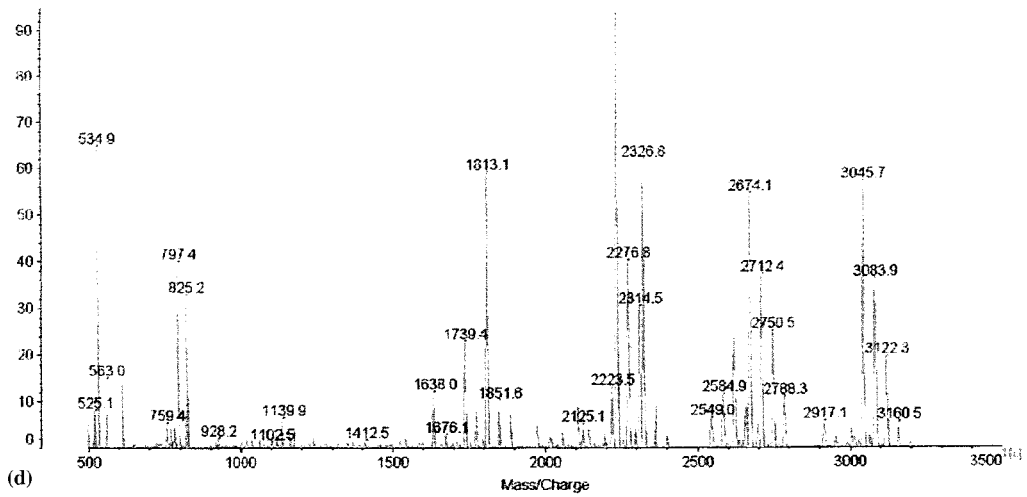
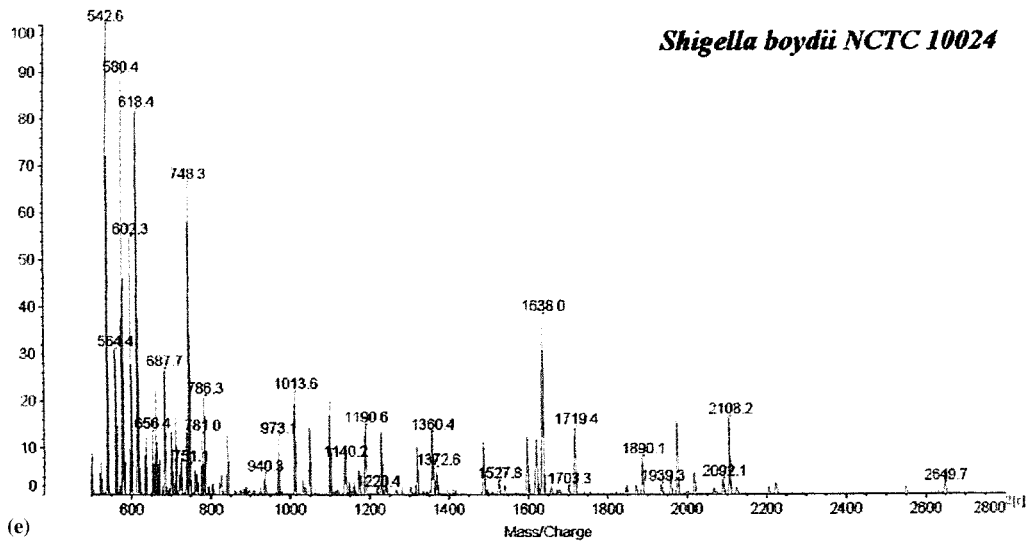

Vibrio damsela NCTC 11646
715.3
543.2
617.5
881.4
671.7
586.6
669.7
845.1
1365.9
1731.7
1705.7
1942.2
2111.6
(a)
Mass/Charge
Serratia marcescens NCTC 10036
973.7
542.8
715.3
748.5
617.0
687.5
564.6
672.9
804.4
929.3
1013.9
1176.2
1326.0
1364.5
1600.7
1622.7
1940.5
2109.5
(b)
Mass/Charge
Bacillus coagulans NCTC 3991
1079.1
1075.7
617.5
1137.3
1115.1
1099.4
521.6
1121.4
809.9
985.4
505.5
519.5
743.1
933.7
1485.9
1964.4
(c)
Mass/Charge

Fig. 3.





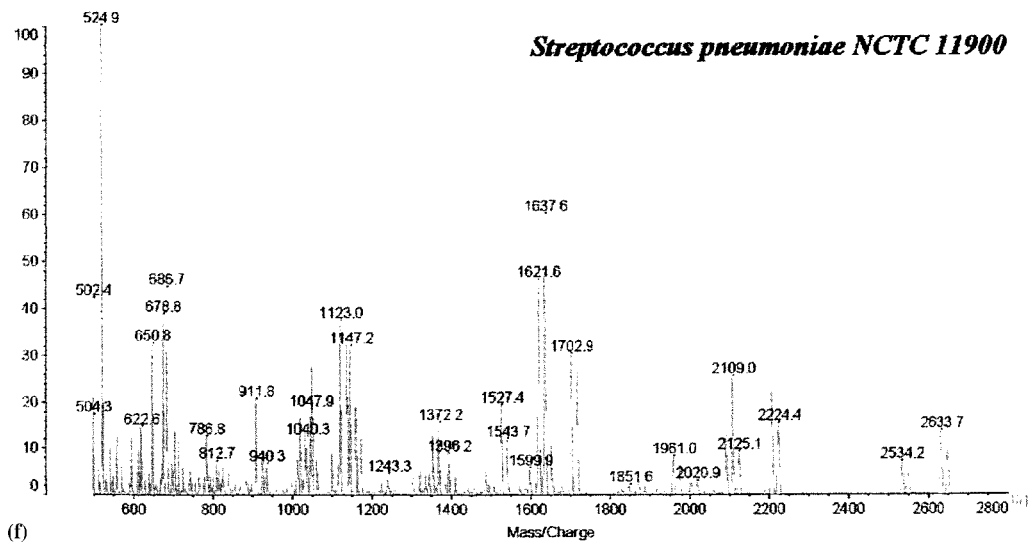


Fig. 3. Examples of mass spectral profiles of Gram-positive and Gram-negative organisms maintained on Columbia Blood Agar. The displayed mass range (500–3,000 daltons) reveals distinct genus-characteristic profiles that may be used as diagnostic biomarkers.

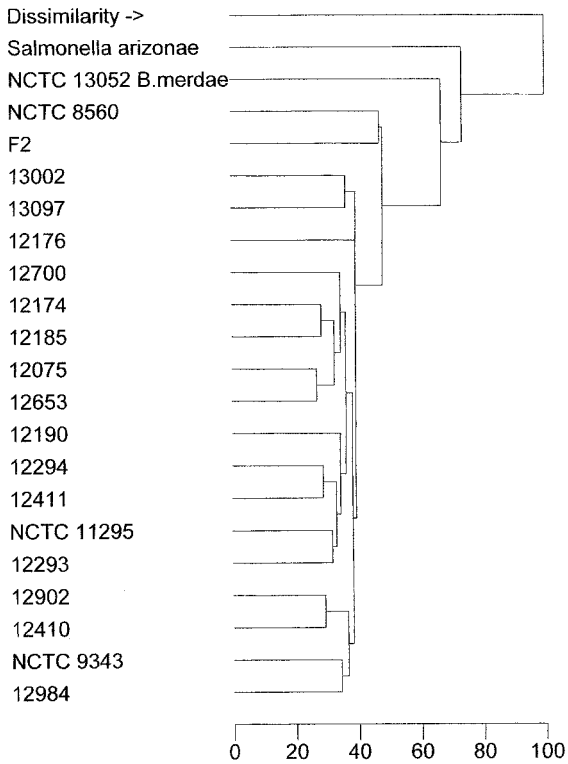


Fig. 4. Cluster analysis of three NCTC reference and 16 clinical isolates of *Bacteroides fragilis* revealed intraspecies dissimilarity which is reflected in their MALDI-TOF mass spectral profiles. Although all *B. fragilis* strains were shown to be closely related when compared to *Salmonella arizonae* and *Bacteroides merdae*, at least five lineages are apparent among the isolates compared.

species was visually compared prior to confirmation using the cluster analysis algorithm. Repeated experiments carried out over several months to examine the stability of the mass spectral profile for a particular species revealed stable and reproducible patterns providing procedures were rigorously standardized and adhered to. Comparing the generated profiles, some mass ions were common to more than one species, however, a sufficient number of unique mass ions were observed to enable unambiguous differentiation of each species tested. These early results therefore, clearly indicate that a distinctive mass spectral profile can be obtained for a given species, thus the potential exists to compare an unknown to a given spectrum for rapid identification. However, within bacterial species, genetic variation occurs which may not be reflected in the expressed phenotype. techniques as multilocus enzyme electrophoresis, SDS-PAGE patterns or nucleic acid restriction patterns are used to discern such heterogeneity. To test whether MALDI-TOF-MS can be used to probe intraspecies clonal variation to a known heterogeneous species, *Bacteroides fragilis* was examined. In previous studies, this species has been subjected to a considerable range of analyses including serotyping, multilocus enzyme electrophoresis, DNA-DNA hybridisation and ribosomal RNA restriction analysis which resulted in the report of several related clusters within the species (see review, (8)). MALDI-TOF-Mass profiles resolved at least 5 distinct lineages each represented by one of the reference strains which were closely related compared to *B. merdae* (a member of the same genus) and *Salmonella arizonae* which is phylogenetically distant. Fig. 4 shows a dendrogram of the 16 clinical isolates and three NCTC reference strains of *Bacteroides fragilis*. The data from this study are in accord with previous findings in revealing substantial intraspecies heterogeneity.

The results of this study suggest that a database of archived mass listing of profiles obtained under standardized culture conditions and combined profiles of several strains of each species may allow species- specific identification. This should be achievable since it was demonstrated unequivocally that MALDI-TOF-MS reveals characteristic mass spectral fingerprints of bacterial species. A further application would be to monitor changes in cell envelope in response to environmental pressure and stimuli and comparing topological features of intact bacterial cells in response to mutational modification and acquisition of plasmids and mobile elements.

ACKNOWLEDGEMENTS

We are grateful to Dr. Valerie Hall for supplying the clinical isolates of *Bacteroides fragilis* and Mr. Oliver Schmid for technical support.

REFERENCES

1. Claydon MA, Shah HN, Borman P, Evason D, Gordon DB. Differentiation of biochemically inert, poorly characterised members of the genus *Porphyromonas gingivalis* by mass spectrometry of intact cells. Microbial Ecol. Health Dis. 1998; 10: 124.
2. Claydon MA, Davey SN, Edward-Jones V, Gordon DB. The rapid identification of microorganisms using mass spectrometry. Nature Biotechnol. 1996; 14: 1584–6.
3. Demirev PA, Ho Y-P, Ryzhov V, Fenselau C. Microorganism Identification by Mass Spectrometry and Protein Database Searches. Analyt. Chem. 1999; 71: 2732–8.
4. Hillenkamp F, Karas M. Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry of Lipopolymers. Analyt. Chem. 1991; 63: 1193–202.
5. Holland RD, Wilkes JG, Rafii F, Sutherland JB, Persons CC, Vorhees KJ, Lay Jr. JO. Rapid identification of Intact Whole Bacteria Based on Spectral Patterns using Matrix-assisted Laser Desorption/Ionization with Time-of-Flight Mass Spectrometry. Rapid Commun. Mass Spectrom. 1996; 10: 1227–32.
6. Holland RD, Duffy CR, Raffii F, Sutherland JB, Heinze TM, Holder CL, Vorhees KJ, Lay Jr. JO. Identification of Bacterial Proteins Observed in MALDI TOF Mass Spectra from Whole Cells. Analyt. Chem. 1999; 71: 3226–30.
7. Liang X, Zheng K, Quian MG, Lubman DM. Determination of Bacterial Protein Profiles by Matrix-assisted Laser Desorption/Ionization Mass Spectrometry with High-Performance Liquid Chromatography. Rapid Commun. Mass Spectrom. 1996; 10: 1219–26.
8. Shah HN, Gharbia SE, Duerden BI. *Bacteroides*, *Prevotella* and *Porphyromonas*. In: Collier L, Balows A, Sussman M, eds. Topley and Wilson's Microbiology and Microbial Infections. London: Arnold, 1998: 1305–30.