

MALDI-TOF-MS

a new identification tool for
microbiologists?

What is MALDI?

Matrix

Sample support

Instrumentation figures by kind courtesy of Shimadzu Biotech. Mass spectra produced using the Kratos Kompact MALDI mass spectrometer.

Figure 3 Reproducibility

%Int.

1000 2000 3000
Mass/Charge

Figure 4
Different species of the Enterob

C.f.
K.f.
E.f.

Mass/Charge

Figure 6 Differences between EMRSA
and EMRSA 18 (PGPE 1) and potentia

Mass/Charge

Matrix assisted laser desorption ionisation time-of-flight mass spectrometry (**MALDI-TOF-MS**) is a powerful analytical technique whose use in microbiology has been recently recognised



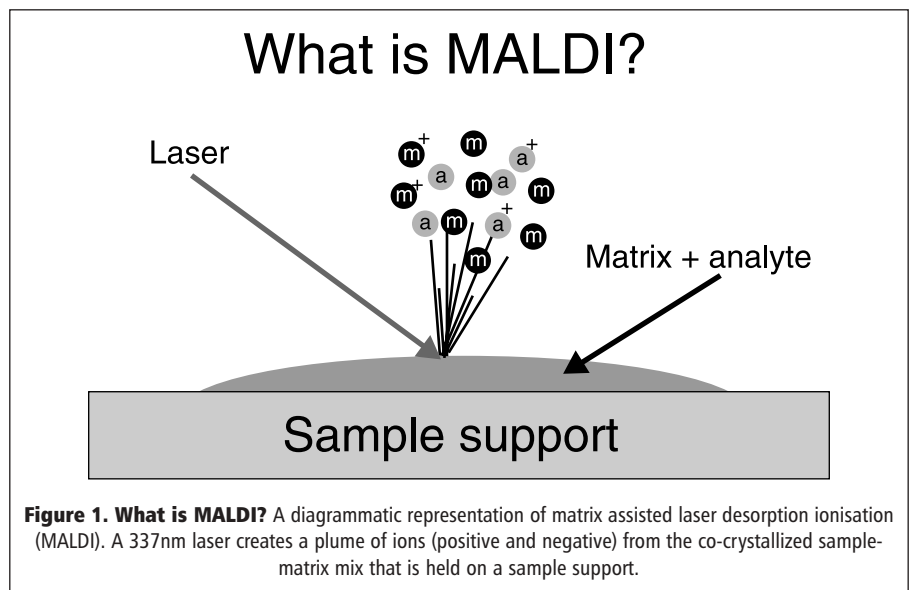
The potential uses of **MALDI-TOF-MS** include rapid microbial identification, rapid sub-typing, direct detection of biomarkers

from samples, identification of possible vaccine components, detection of adhesion / virulence markers and detection of antibiotic resistance markers to name but a few. One of the most profound advantages of this instrumentation is the speed of analysis, with a mass spectrum being generated in a matter of minutes.

Mass spectrometry, however, is *not* a new tool and has been used by chemists and biochemists for years to identify unknown compounds, quantify known materials and elucidate the structure and chemical properties of molecules according to their atomic and molecular mass. The basic components of a mass spectrometer consist of an ionisation source, an analyser and a detector kept under a high vacuum of the order of 10^{-5} mBarr. The resultant ions are separated in the analyser according to their mass/charge ratio (m/z) ratio and collected by the detector. In the detector, the ions generate an electrical signal that is proportional to the

number of ions and a data system records these signals and converts them into the mass spectrum. The expansion of ionisation methods, development of bench top instruments and major improvements in data analysis has greatly reduced the analysis time, increased the sensitivity and created a cost effective analytical tool that is extremely attractive to a non-experienced operator. The relative size of a mass spectrometer ranges from that of a microwave or coffee machine to large research instruments some of which require large amounts of laboratory space.

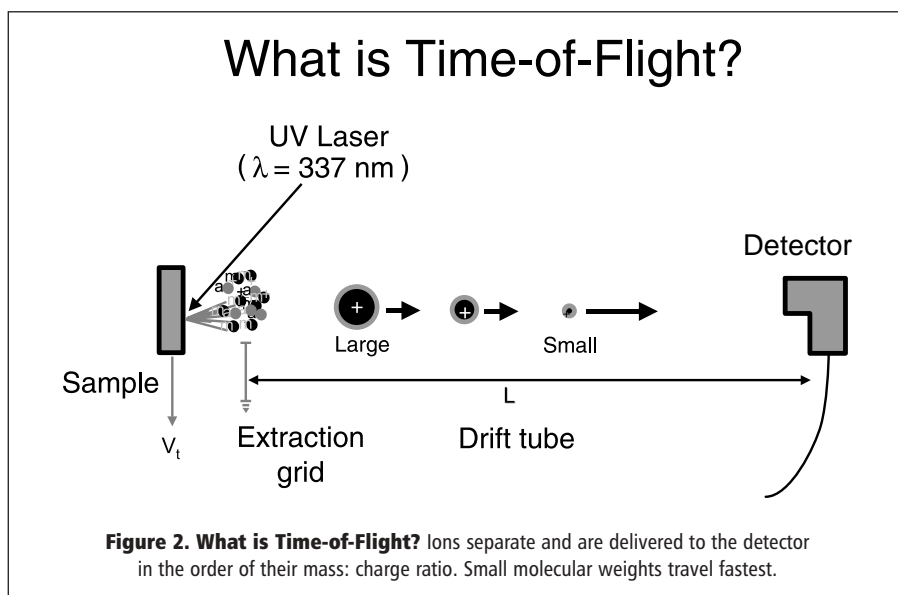
In **MALDI-TOF-MS**, sample preparation is relatively simple. Purified or crude sample is co-crystallised with an excess of organic matrix onto a target plate that is placed into the mass spectrometer. In most microbiological applications, a UV laser (337nm) is used to generate the ions. The matrix helps with ionisation and is thought to absorb most of the photon energy that is converted into a plume of vapourised material. Both positive and negative ions are formed and either can be used for analysis (Figure 1). Positive ion detection is most commonly used in microbiological ☒



applications, as negative ion detection is relatively inefficient for the majority of components detected, namely proteins and carbohydrates. Alternatively DNA and acidic compounds form negative ions more efficiently. Sinapinic acid and α -cyano-4-hydroxycinnamic acid are commonly used for the ionisation of proteins and 3-hydroxypicolinic acid more frequently used for DNA. After ionisation the ions are extracted into a time-of-flight analyser where they separate and are sequentially delivered to a detector in order of their mass:charge ratio (m/z), those with small molecular weights travelling the fastest (Figure 2).

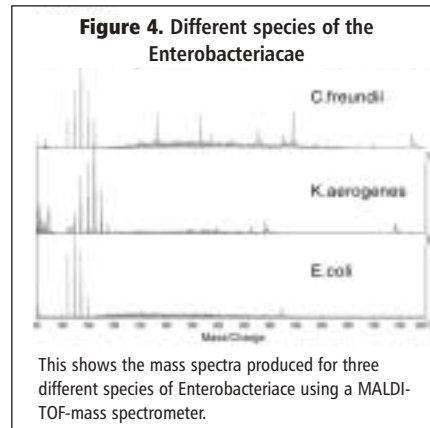
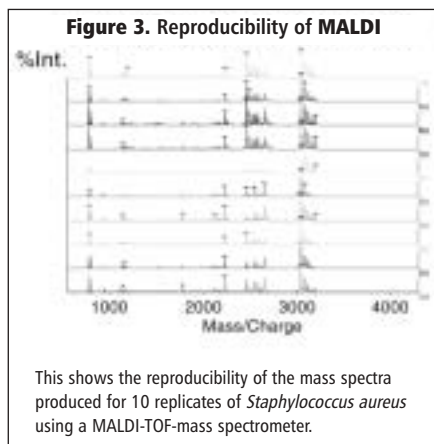
complex mixtures that could provide evidence for chemotaxonomic classification. The versatility and rapidity of analysis led to the further development of a number of **MALDI-TOF** methods involving bacteria and incorporation of sophisticated mathematical algorithms into the computer software helped analyse the complex data output. Currently **MALDI-TOF-MS** has been applied successfully to a wide variety of microbial applications including the analysis of bacterial RNA and DNA, the detection of recombinant proteins, the characterisation of unknown proteins, bacterial proteomics, the detection of virulence markers, and the very rapid

species or sub-type of a species it may be possible to identify organisms directly from the sample. Studies have shown that there are subtle differences between epidemic MRSA phage type 15 (EMRSA 15) and EMRSA 16, seen at high frequency in the United Kingdom (2,3). Typical spectra derived from EMRSA 15 (pulse field gel electrophoresis type 5 - PFGE 5) and EMRSA 16 (PFGE 1) are shown in figure 6. Work is currently being undertaken to establish if the technique can be used to detect these strains directly from clinical specimens or using short-term cultures. If this proves possible it may be that same day detection and simultaneous sub-typing directly from specimens is not too far away. Although this is also the aim of DNA amplification techniques, the speed of analysis provided by **MALDI-TOF-MS** is a very attractive alternative. Several groups of researchers worldwide are currently evaluating this technique for a variety of applications within microbiology, primarily in epidemiological and identification areas. This is a very active area of research in the UK and USA amongst the National Defence Departments for the detection of biomolecules used in biological warfare.



The general use of **MALDI-TOF-MS** for the characterisation of large biomolecules has shown a marked increase since the late 1980s and within microbiology this initially led to successful applications involving the analysis of isolated and purified bacterial proteins which could be rapidly identified in a database. It quickly became evident that **MALDI-TOF-MS** could be applied directly to crude cellular fractions or cellular suspensions to produce reproducible data from these

characterisation of bacteria at the genus, species, and strain level. Recently this technique has been successfully applied to intact bacterial cells, (Intact cell mass spectrometry-ICMS) providing a reproducible spectrum within minutes (See figure1). Briefly, bacterial cells are applied to a sample slide (often directly from primary isolation media) and the matrix added. The slide is placed into the instrument and the sample-matrix mix is bombarded with a laser to create gas phase ions that are then pulsed into the flight tube. A series of these m/z values provide a spectrum within minutes that is a characteristic fingerprint of the surface of the bacterial cell. The spectra obtained have been shown to be reproducible (See figure 3) and sufficiently different to allow bacterial speciation (See figures 4 and 5). In some cases, there is sufficient discrimination in the spectra to allow subtle differences to be detected within species allowing simultaneous sub-typing of the organism from a single analysis (See figure 6). Peaks within the spectrum are characteristic for a particular strain and if sufficient analysis is undertaken to identify 'bio-markers' for a particular



References

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Edwards-Jones, V, Claydon, M A; Evason, D J; Walker, J; Fox, A; and Gordon, D B. (2000) **Rapid discrimination between methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* using Intact Cell Mass Spectrometry.** *Journal of Medical Microbiology*; 49; 345-351.

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Walker, J; Borrow, R; Goering, R V; Egerton; Fox, A J; and Openheim B A. (1999) **Subtyping of MRSA isolates from the North West of England: a comparison of standardised pulse field gel electrophoresis with bacteriophage typing including an interlaboratory study.** *Journal of Medical Microbiology*; **48**, 297-301.

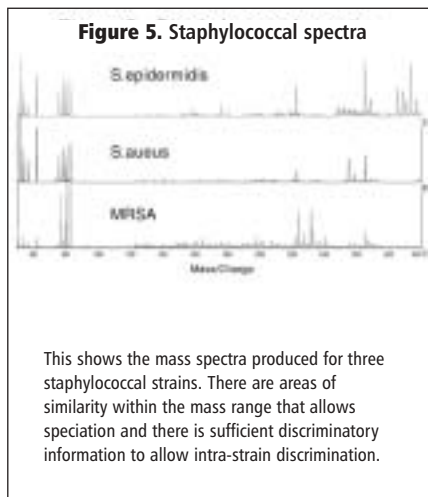
For further information on the use of mass spectrometry for the characterisation of microorganisms the following reviews are highly recommended.

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■ Fenselau, C and Demirev, P A (2001). **Characterisation of intact microorganisms by MALDI Mass Spectrometry.** *Mass Spectrometry Reviews*; **20**, 157-171.

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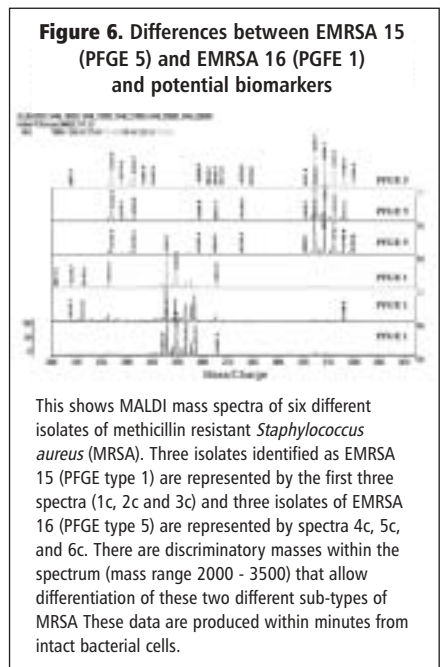
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Thank you to **Shimadzu Biotech** for the supply of the instrumentation figures. Mass spectra were produced using the **Kratos Kompact MALDI** mass spectrometer.

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Typhoid carriers: a salmonella gene mutation?

Salmonella enterica causes approximately 16 million cases of typhoid fever worldwide, killing around 500,000 per year. One in thirty of the survivors, however, become carriers, such as infamous **Typhoid Mary** who caused several typhoid outbreaks in New York City at the beginning of the last century.



In carriers the bacteria remain hidden inside cells and the gall bladder, causing new infections as they are shed from an apparently healthy host. The factors that enable the bacteria to establish chronic infection were unclear.

However, in a paper published in the Proceedings of the National Academy of Science, researchers at the Institute of Food Research in Norwich and the Karolinska Institute in Sweden found that the change of a single base pair in one *Salmonella* gene can determine if the bacteria cause short-term

illness or a long-term carrier state. The authors stumbled upon the striking change in infectivity while investigating a mutant strain that produces persistent infection in mice.

Tracing the mutation to the genome, the scientists found it caused a single base change in the gene coding for the enzyme polynucleotide phosphorylase (PNPase). This enzyme normally decreases the production of virulence factors by

breaking down the messenger RNA essential for the translation of the genetic code into the *Salmonella* virulence factors. The mutant enzyme is less active, allowing greater production of virulence factors and, therefore, persistent infection.



Dr Jay Hinton of the Institute of Food Research said, 'This is a new mechanism for controlling the expression of *Salmonella* virulence factors, and it's the first time that this type of gene regulation has been linked with the carrier state of typhoid.'

Resources on the World wide web about Typhoid include:
<http://history1900s.about.com/library/weekly/aa062900a.htm>
www.netdoctor.co.uk/travel/diseases/typhoid.htm
www.netdoctor.co.uk/travel/diseases/typhoid.htm