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MALDI-TOF-MS: A Fast Diagnostic Tool for Plant Diseases

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Abstract

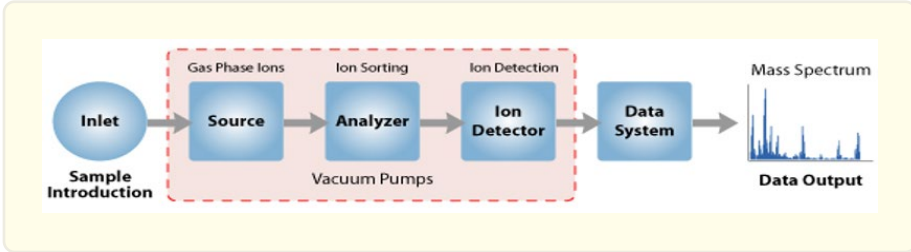
Microorganisms are best recognized by 16S and 18S rRNA gene sequencing. Microbial identification and diagnosis may be possible with matrix-aided laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). MALDI-TOF MS identifies microorganisms utilizing intact cells or cell extracts. It's fast, sensitive, and cheap. Microbiologists have used MALDI-TOF MS for microbial identification and strain typing, epidemiological studies, biological warfare agent detection, water- and food-borne pathogen detection, antibiotic resistance detection, and blood and urinary tract pathogen detection (Neelja Singhal et al., 2015). Citrus Huanglongbing (HLB) is caused by the phloem-limited plant pathogenic bacteria Candidatus Liberibacter species. HLB is difficult to identify early since freshly infected trees don't show symptoms. In order to establish a rapid detection method and assess the metabolite differences between healthy and HLB-affected Newhall navel oranges, we used matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) to study asymptomatic and symptomatic leaf extracts compared to healthy leaves. Using MALDI-TOF-MS and multivariable analysis, healthy, asymptomatic, and symptomatic leaves were distinguished. The training set's discrimination accuracy and cross-validation analysis success rate were 100% according to discriminant analysis (DA). 2020 (Yongquan Liu et al.). For agricultural and therapeutic applications, fungi are rich in biologically active natural compounds. Bioactive fungal natural compounds must be discovered and accurately identified for optimal use. fungal biocontrol In vitro, *Purpureocillium lilacinum* inhibited Botrytis cinerea, an airborne plant pathogenic fungus that causes gray mold disease in many vegetables and fruits. The co-culture of two fungi on agar plate demonstrated that *P. lilacinum* suppressed *B. cinerea's* growth, indicating that it may create bioactive secondary metabolites against B. cinerea. The discovery of a novel antifungal lipopeptaibol (leucinostatin Z) from P. lilacinum against *B. cinerea* was made using matrix-assisted laser desorption ionization-time of flight mass spectrometry imaging mass spectrometry (MALDI-TOF-IMS). Leucinostatin Z's planar structure was confirmed by LC-HRESI-MS-MS analysis. MALDI-TOF-IMS is a revolutionary method for discovering new bioactive chemicals in fungi by directly observing bioactive natural products on growth media between two fungi colonies. 2020 (LiuR et al.)

Introduction

Food production and the ever-increasing demands of a growing population have become a major concern around the world, making food security a pressing issue of our time. An additional 70 per cent of food production is required by 2050, according to a recent estimate, to meet the needs of the growing population. It is important to increase agricultural output per unit of accessible land by doing two primary things: boosting productivity through the use of modern techniques and reducing crop loss. By lowering crop failure rates, we can boost food output overall.

There are many causes of declining agricultural output, but pests and pathogens are major contributors to global crop losses. Between 20% and 40% of agricultural output, loss can be attributed to pathogen infections. High-tech crop disease detection and prevention are crucial for minimizing disease-induced damage in crops during growth, harvest, and post-harvest processing, maximizing agricultural production, and guaranteeing agricultural sustainability.

There are many ways for the early detection and diagnosis of plant diseases, including visual observation, cultural and morphological approaches, serological methods, DNA/RNA assays, spectroscopic-based techniques, and others, all of which are essential for effective disease control. Direct approaches, such as molecular and serological methods, are more commonly used for high-throughput analysis, which is necessary when a large number of samples must be evaluated.



Spectroscopy has great potential in the era of omics, particularly with the emerging idea of proteomics. Pathogens can be identified using mass spectrometry by looking at their protein makeup. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has shown promise as a method for identifying and diagnosing microorganisms in recent years (Singhal et. al., 2015).

Spectroscopy of Masses

Principle and instrumentation: The first step in the mass spectroscopic analysis of compounds is the production of gas phase ions of the compound, primarily by electron ionization. These ions are then analyzed by a mass spectrometer to determine the amount of a given compound in a sample and the identity of any unknown compounds. This ion fragments into smaller molecules. The molecular ion is fragmented into its fundamental product ions, which are then fragmented again, and so on. The mass spectrometer uses the ions' mass-to-charge ratio to sort them out, and it detects them in proportion to their quantity. This results in the creation of the molecule's mass spectrum. Ion abundance versus mass-to-charge ratio is shown to show the outcome. Ions reveal details about the composition and structure of the molecule from which they were formed.

The three main parts of the instrument are

An Ion Source is a device used to generate gaseous ions from the material under investigation.

Analyser, which separates ions into their mass and charge components for study.

Third, a detector system to record the abundance of each resolved ionic species by detecting the ions.

Mass spectrometry can be broken down into subtypes like MALDI-TOF-MS which use a unique ion source and mass analyzer.

Matrix-assisted Laser Desorption/Ionization (Maldi)

To produce ions from big molecules with little fragmentation, the MALDI ionization process uses a laser energy-absorbing matrix. Biomolecules including DNA, protein, polypeptides, and sugar can be analyzed with this method because of its gentle ionization (Ahmad et al., 2012).

Mass spectrometry techniques based on measuring the time it takes for ions to travel from a source to a detector are collectively referred to as TOF (Time of Flight). The Time-Of-Flight Mass Spectrometer (TOF -MS) is the most common instrument for use with MALD.

Using this method, technology creates a unique mass spectral fingerprint that can be compared to a massive database of mass spectra. Bioinformatics pattern profiling allows for precise microbiological identification down to the genus and species levels, because to the fact that the spectral fingerprints are unique signatures for each microbe. Mass fingerprints, or a mass spectrum, are a graph of abundance versus mass-to-charge (m/z) ratio.

The Developing Role of Maldi in Cancer Diagnosis

In 1985, researchers Franz Hillenkamp, Michael Karas, and others came up with the term matrix-assisted laser desorption ionization (MALDI). When treated with a pulsed 266 nm laser, a mixture of the amino acids alanine and tryptophan proved to be the most effective in generating ionic species. Laser energy was absorbed by the tryptophan, which then helped ionize the non-absorbing alanine. In 1987, Koichi Tanaka and colleagues employed the "ultra-fine metal plus liquid matrix approach," which involved the combination of 30 nm cobalt particles in glycerol with a 337 nm nitrogen laser for ionization, and they achieved a major advance in big molecule laser desorption ionization. In a study using this laser and matrix, Tanaka was able to ionize biomolecules as large as the 34,472 Da protein carboxypeptidase-A. In 2002, Tanaka was awarded a quarter of the Nobel Prize in Chemistry for his discovery that proteins can be ionized by adjusting the wavelength and matrix of a laser. First introduced to the public in the early 1990s, when 337 nm nitrogen lasers became widely available at affordable prices, this instrument

A Basic Explanation of How MALDI-TOF Mass Spectrometry Functions

There are two stages to the MALDI TOF process: 1. the ionisation phase and 2. the detection stage.

2. The Flying Time

Transition to the Ionization State

Lasers are initially used to blast the samples while they are securely fastened in a crystalline matrix on a target plate. The molecules in the sample are ionised as they evaporate into the vacuum. The charged particles are then accelerated by applying a high voltage.

Time-of-flight mass spectrometry is the next stage.

First, in the linear mode, the linear detector will be hit by particles only a few ns after ionisation. More massive molecules will arrive later than their lighter counterparts. Direct determination of molecular masses is made possible by measuring their flight times. Starting at the ionisation instant, the increasing peaks in the spectrum represent increasing particle masses along the time axis.

2. The particles are redirected to a second detector when operating in reflector mode. The reflector does double duty: it both concentrates the masses and increases their range. Because of these two factors, the resulting resolution is superior to that of the linear mode.

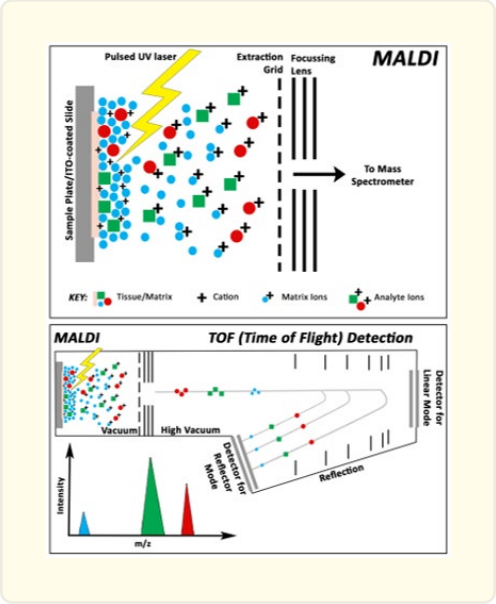
The MALDI TOF MS apparatus includes a sample target plate, matrix, laser, variable voltage grid, vacuum system, flight tube, detectors, and a data system. Matrix and the laser are two of the most crucial elements. Accurate detection requires a suitable matrix and laser combination.

Matrix: A material composed of crystalline molecules that coexist with the test specimen. In order to make a matrix solution, the molecules of the matrix are first dissolved in a combination of ultra-purified water and an organic solvent like acetonitrile (ACN) or ethanol. Generating [M+H] ions typically requires the addition of a counter-ion source, such as Trifluoroacetic acid (TFA). Matrix solutions are commonly used in scientific research; sinapinic acid in a mixture of ACN: water: TFA (at 20 mg/mL) is a great example (50:50:0.1).

The matrix serves a dual purpose

It serves as a solvent for the analyte, decreasing intermolecular interactions and preventing the analyte molecules from aggregating so that (1) the laser beam's photon energy may be converted into excitation energy and (2) the energy of the analyte molecules can be measured.

A significant absorption property of light at the wavelength of the laser flux is one desirable feature of a typical MALDI matrix.



Microcrystallization Capability of The Sample

In order to participate in a photochemical reaction and have the sample molecules ionised with high yields, the matrix-sample material must have two properties: a low sublimation temperature, which allows for the formation of an instantaneous high-pressure plume of matrix-sample material during the duration of the laser pulse; and a high melting point.

Nitrogen lasers (337 nm) and frequency-tripled and -quadrupled Nd: YAG lasers are common UV lasers used in laser MALDI procedures. Table1. A Compendium of MALDI-Related Lasers (355 nm and 266 nm respectively).

For the purpose of preparing samples

Direct cell profiling is a method for identifying certain bacteria using MS, but for others, complete cell lysates or crude cell extracts must be made.

One microbe colony is isolated, "spotted" onto a sample plate, and then the matrix solution is added on top of that. This is called "direct cell profiling" (Ilina et al., 2009). Ethanol-incubated agar extract containing formic acid was used to cultivate the fungus. After adding acetonitrile and centrifuging the resulting mixture, the supernatant was analyzed by MALDI-TOF MS (Cassagne et al., 2014).

Maldi-Tof and Its Potential Uses

Biochemistry: Proteins isolated using SDS-PAGE, size exclusion chromatography, affinity chromatography, strong/weak ion exchange, isotope coded protein labelling (ICPL), and 2-dimensional gel electrophoresis are identified using MALDI in proteomics.

Some synthetic macromolecules, such as catenanes and rotaxanes, dendrimers, and hyper branched polymers, can be quickly analyzed and verified using this method, thanks to organic chemistry.

In *polymer chemistry*, the molar mass distribution can be calculated using MALDI.

In the field of *medicine*, MALDI/TOF spectra are frequently used in conjunction with other analytical and spectroscopic methods.

Plant pathologists have tried to implement the use of maldi in the detection of plant pathogens for large numbers of samples because of its widespread adoption in clinical microbiology for the quick identification of microorganisms. It has many applications in plant pathology, including pathogen detection, pathogen-associated protein analysis, and host-pathogen interaction protein analysis.

How is Maldi Tof ms Distinctive from Similar Techniques?

Simple sample preparation, fast measurement periods, easy creation of reference spectra, and low costs per sample are all significant benefits of MALDI-TOF MS, which uses entire cells or crude, acidic extracts and mass spectra to identify particular species.

MALDI -TOF MS has a wide range of applications and can be combined with other extraction or processing processes to isolate and detect unique biomarkers, metabolites, or biochemical functions.

MALDI-TOF The mass spectral (MS) spectrum of a certain microbe is a taxon-specific characteristic of that organism that is unaffected by factors such as location, growth conditions (which should not vary greatly), or sample preparation technique. This method not only allows for the recognition of consistent phenotypic patterns reflecting taxonomic identities, but it also allows for the identification of new isolates as members of established species if their type strains have been previously examined. Taxonomic and inter- and intra-specific diversity can be studied thanks to the ease and speed with which a large number of isolates can be identified (Feli and Dellaglio, 2007).

It is important to note that MALDI-TOF MS has applications beyond species identification, specifically in strain typing. As demonstrated by Kumar et al. (2004), MALDI-TOF MS has the potential to be an effective method for distinguishing between beta-hemolytic streptococci strains and for characterizing untypable strains of the streptococci group. Despite the fact that several researchers have standardized protocols for the identification of some fungi species also using MALDI TOF MS, progress in identifying fungi by MALDI-TOF MS in the medical mycology laboratory has lagged behind that of identifying bacteria. This is because fungi are more biologically complex and thus more difficult to study in general.

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