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Popular Article

MALDI – TOF: A Modern Technique for Identification of Microorganism

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What is MALDI-TOF MS?

Mass spectrometry has been commonly used by research laboratories and it is regarded as a promising technique as to the rapid identification of microorganisms. Mass spectrometry applied to the identification of micro- organism. MS is another method that may be of great help to microbiological diagnosis. Furthermore, it has been widely used as a research tool, mainly in proteomic and lipid analyses. Several techniques based on ionization and subsequent biomolecular detection have been developed and the matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) has been one of the mostly applied.

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has emerged as a rapid, highly accurate, and cost-effective method for routine identification of a wide range of microorganisms. Mass spectrometry is an analytical technique in which chemical compounds are ionized into charged molecules and ratio of their mass to charge(m/z) is measured. Fresh colonies (< 24h) are preferentially selected for performing bacterial identification by MALDI-TOF MS.

How Does MALDI-TOF MS Work?

In clinical microbiology, direct colony testing, is not as user friendly for clinical microbiology applications and thus is generally reserved for processing hazardous or difficult-to-lyse organisms. Direct colony processing is easiest, fastest, and least expensive. A colony is “picked” from a culture plate to a “spot” on a MALDI-TOF–MS target plate. The addition of a formic acid solution to the MALDI plate may be used to improve the quality of the generated mass spectrum, which may be particularly helpful for certain types of organisms, such as yeasts. After drying, the target plate is placed in the mass spectrometer’s ionization chamber. In MALDI (matrix-assisted laser desorption ionization), a matrix (e.g., alpha-cyano-4-hydroxycinnamic acid dissolved in 50% acetonitrile and 2.5% trifluoroacetic acid) assists in the desorption and ionization of microbial analytes through the energy of a laser. The matrix isolates analyzed molecules and protects them from fragmentation by the laser. As a result of being “shot” by the laser, microbial and matrix molecules are desorbed, with the majority of energy being absorbed by the matrix, converting it to an ionized state.

Through random collision in the gas phase, charge is transferred from matrix to microbial molecules. The ionized microbial molecules are accelerated, based on mass-to-charge ratio, into a TOF (time-of-flight) mass analyser, a tube under vacuum. Ion's travel toward an ion detector, with smaller analytes reaching the detector first, followed by progressively larger analytes. A mass spectrum is generated, representing the number of ions of a given mass impacting the ion detector over time

How Does the Analysis Work?

For clinical microbiology applications, highly abundant microbial proteins such as ribosomal proteins are the main contributors to the generated mass spectrum, although specific proteins are not identified and their mass and abundance are merely profiled. In general, mass spectra are unique to individual organism-types, with peaks specific to genera, species, and strains. The mass spectrum of the test isolate is compared to a database of reference spectra or deconvoluted spectra to determine relatedness to spectra in the database; the most closely related organisms are identified with a value provided as to the level of confidence in identification. Depending on how high the value is, the organism may be identified at the family, genus, or species level.

Applications in Microbial Diagnosis

MALDI-TOF MS in Bacteriology: Clinical microbiology laboratories require rapid, reliable, and cost-effective methods for identification of potential pathogens in clinical samples so that appropriate antimicrobial therapy maybe initiated early. A number of researchers have shown that MALDI-TOF MS can be used for early identification of bacteria in blood cultures, urinary tract infections (UTIs), cerebrospinal fluids, respiratory tract infections, stool samples etc.

Food-and Water-Borne Bacteria: Rapid identification of pathogenic microorganisms is important to ensure safety and quality of water and food products. MALDI TOF MS has been shown to be useful for early detection of bacterial hazards which might contaminate drinking water.

Environmental Bacteriology: Tests based on biochemical traits usually fail to identify microbes isolated from environmental samples, as the diversity of microbes in these habitats is enormous. Various studies have shown that whole cell MALDI-TOF MS can be used as an efficient tool to identify and characterize isolates which originate from specific ecosystems.

Researchers have reported the use of MALDI-TOF MS in identification of microbes isolated from sewage sludge.

Biological Warfare: Fast and reliable identification of microbes which pose threats as agents of bioterrorism is required, not only to combat biological-warfare attacks, but also to prevent natural out breaks caused by these organisms. Recently various researchers reported MALDI-TOF MS as a simple, rapid and reliable approach to identify highly pathogenic organisms like Brucella spp., Coxiella burnetti, Bacillus anthracis, Francisella tularensis, and Y. pestis.

Detection of Antibiotic Resistance in Bacteria: MALDI-TOF MS has been shown to generate PMF capable of discriminating line ages of methicillin-resistant S. aureus strains Similarly, MALDI-TOF MS has been shown to be of great use in identifying vancomycin resistant enterococci.

Bacterial Strain Typing and Taxonomy: MALDI-TOF MS for rapid characterization of pathogen. They also determined strain-specific differences and it was a powerful tool for the identification of clinical isolates. MALDI-TOF MS was used for rapid identification often different species, when the results of species identification obtained by MALDI TOF MS were compared with the phenotypic/genotypic identification systems, a 100% consonance was achieved.


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