MicrobeLynx™ FOR IDENTIFICATION OF BACTERIAL SPECIES

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MicrobeLynx™ in combination with bio-informatics provides a powerful tool for determining the species and typing of microorganisms. This mass-fingerprinting technique for bacteria offers greater sensitivity, selectivity and speed of analysis compared to classical identification methods.

The technology applies biopolymer mass spectrometry techniques to analyses of intact bacteria and actinomycetes for rapid fingerprinting. The entire process from sample preparation to result takes only a few minutes for each purified test microorganism.

PRINCIPLES

It allows a unique population of macromolecules expressed on the surface of bacteria to be rapidly sampled and characterized by molecular weight. The resulting mass spectrum provides a unique physico-chemical fingerprint for the species tested and can be reliably matched against databases of quality-controlled reference mass spectrum.

The MALDI micro MX (*Figure 1*) is a bench top matrix assisted laser desorption ionization time of flight mass spectrometer (MALDI-TOF MS) which generates high quality MALDI MS data. MALDI is the soft technique for ionizing large molecules for analysis in a mass spectrometer (Bright *et al.*, 2002).

The linear mode (*Figure 2*) is appropriate for bacterial fingerprinting. Measurement of high molecular weight samples are enhanced by a retractable post-acceleration dynode. Ions are incident on the detector at the top of the flight tube. When high mass detection is enabled, the post-acceleration dynode automatically swings into the ion path.

OPERATIONAL CONCEPTS

The flow chart in *Figure 3* summarizes the operation of the MALDI micro MX in obtaining data.

A sample of purified microbe is mixed with small UV absorbing organic acid, *e.g.* α-cyno-4-hydroxycinnamic acid (matrix). The sample/matrix mixture is allowed to air dry. The matrix forms crystals which contain the sample molecules (*Figure 4*).

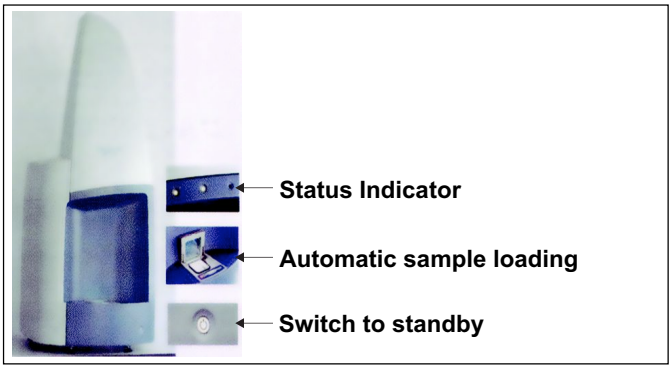

Figure 1. External view of the MALDI micro MX instrument. The key areas are highlighted.

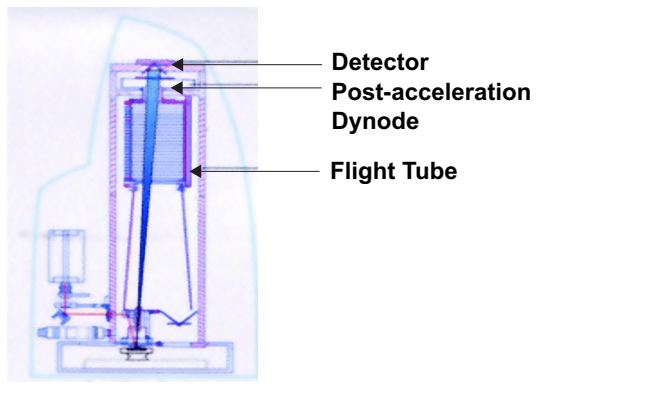

Figure 2. Linear mode of operation by the MALDI micro MX.

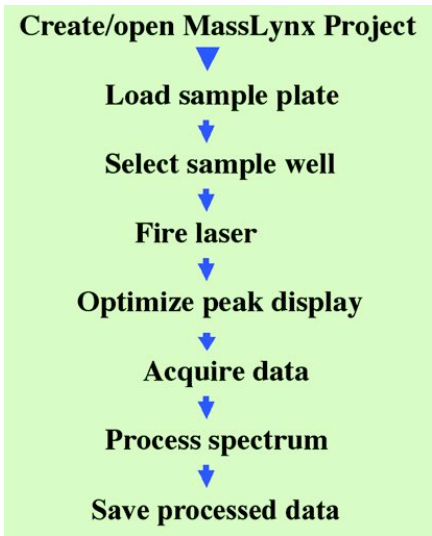

Figure 3. Operation concept of the MALDI micro MX.

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After drying, the target is placed in the MALDI-TOF mass spectrometer (*Figure 5*). The microorganisms in the matrix are illuminated with a pulse from a nitrogen laser (337 nm). The matrix absorbs energy from the laser, and the macromolecules from the surface of the microorganisms are desorbed and ionized (Bright *et al.*, 2002). It then expands into the gas phase and at the same time carries with it undamaged analyst molecules (*Figure 6*).

The resulting ionized macromolecules are mass analysed and the results reported as a mass spectrum, a plot of mass (X axis) versus abundance (Y axis) (*Figure 7*). The mass-fingerprint of the test microorganism is then submitted to the MicrobeLynx™ search algorithm, which challenges an appropriately selected database from a range of quality controlled bacterial reference mass spectra (Dare *et al.*, 2003).

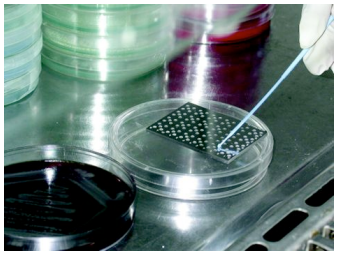

Figure 4. Smearing of bacteria colony on target plate.

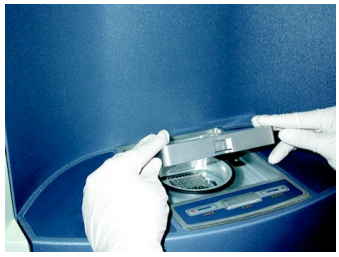

Figure 5. Loading the sample into MALDI-TOF MS.

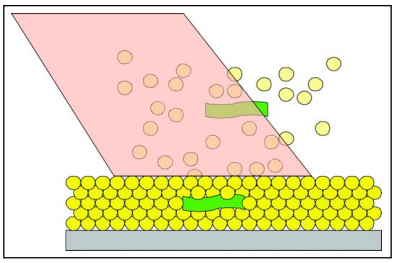

Figure 6. Ionization of matrix carries the analyse molecules.

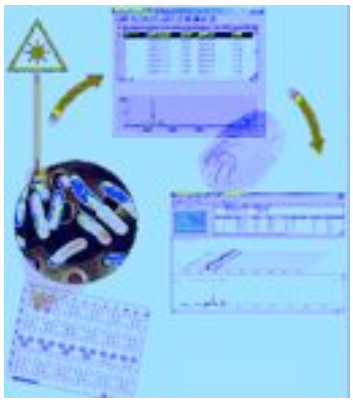

Figure 7. Bacterial ionized macromolecules analysed as a mass spectrum.

DATABASES

Quality controlled collections of mass-fingerprints of well, clinical, environmental and food borne bacteria are accessible at http://www.hpa.org.uk/srmd/index_srmd.htm.

Rapid identification of a microorganism from its characteristic mass-fingerprint is easy with the MicrobeLynx™ pattern recognition Search Algorithm. MicrobeLynx™ applies a probabilistic mathematical strategy which compresses all the mass/intensity data in the test spectrum to a lower dimensional space, to give, in seconds, the identity of the bacterium with the best database match (Dare *et al.*, 2003).

COST

MPOB will charge a minimal cost per species or strain of bacteria or actinomycetes identified.

BENEFITS

- Sensitive and rapid identification readily achieved from a small colony for mass-fingerprinting of culturable bacteria in crude palm oil, soil under oil palm and in palm insect pests;
- Selective, can discriminate between genetic transformants, antibiotic-sensitive and resistant strains, vegetative, mother and spore cells of bacilli, and can identify conventionally problematic micro-organisms, *e.g. Porphyromonas* spp; and
- Cost-effective with minimal operator training.

REFERENCES

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DARE, D J; SUTTON, H E; KEY, C J; SHAH, H N; WELLS, G and MCDOWALL, M A (2003). Optimization of a database for rapid identification of intact bacterial cells of *Escherichia coli* by MALDI-TOF MS. 51st American society of Mass Spectrometry Conference, Montreal, Canada.

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