Introduction to MALDI-TOF Mass Spectrometry

Principles of MALDI-TOF
Overview

• Matrix Assisted Laser Desorption/Ionisation – Time of Flight (MALDI-TOF) mass spectrometry used to detect and characterize mixtures of organic molecules.
• In micro used as a rapid, accurate and cost-effective method for ID of microbes
• 2 commercially available:
  • Vitek MS (bioMerieux)
  • MALDI Biotyper CA system (Bruker Daltonics)
Mass Spectrometry

• Mass spectrometry (MS) measures mass-to-charge ratio (m/Q) of ions.

• Results presented as a mass spectrum - a plot of the ion signal as a function of the mass-to-charge ratio.

• Spectra used to determine
  • elemental or isotopic signature
  • the masses of particles
  • the chemical ID or structure
Three components of MS

• An ion source
  • Sample (solid, liquid, or gaseous) is ionized - for solids via electrons or MALDI

• A mass analyzer
  • Ions then separated based on mass-to-charge ratio
  • Done by acceleration and subjecting to an electric or magnetic field
  • Ions with a lower mass will reach the detector first.

• A detector
  • records either charge or current produced when ion passes by or hits a surface
  • produces a mass spectrum (mass-to-charge ratio)
  • Usually an electron multiplier
MALDI – TOF MS

1. An ion source
   • Laser and ionization chamber to ionize sample and transfer into a gas phase
   • Uses laser energy-absorbing matrix to create ions from large molecules

2. A mass analyser
   • separates ionized analytes according to their mass (all same charge)
   • TOF uses electric field to accelerate ions & measure time to reach detector.

3. A detection device to monitor separated ions

Add Formic Acid and Dry; Add Matrix and Dry
Procedure

• Combine isolated colony (analyte), formic acid & matrix on MALDI plate

• Solvents vaporize, leaving only the recrystallized matrix with analyte embedded
Matrix

• Matrix isolates molecules from each other, protecting them from fragmentation and enabling desorption by laser energy

• Consists of:
  • Small crystalised acid molecules – usually sinapinic acid, alpha-cyano (alpha matrix) or DHB acid.
  • Purified water
  • Organic solvent (alcohol or acetonitile)
  • Trifluoroacetic acid 2.5%
Ion Source - Laser

- Uses UV lasers (nitrogen laser light, wavelength 337nm)
- Laser pulses fired at the matrix crystals in the dried-droplet spot.
- Matrix absorbs the laser energy converting it to an ionised state
- Charge is transferred to analyte (random collision in the gas phase)
- Ionised analyte and matrix molecules are desorbed from the plate.

Shimadzu, Principles of MALDI-TOF MS, 2020
Mass Analyser – Time of Flight

• Ionized microbial molecules accelerated through a positively charged electrostatic field into time of flight (TOF) tube
• Inside vacuum tube ions travel toward an ion detector
• Small analytes travel the fastest (generating mass spectrum)
• Ions emerge from the mass analyser and hit the ion detector → generate a mass spectrum representing the number of ions of a given mass impacting the detector over time

Carroll, MCM 12 ed, 2019
Results

• Mass spectrum provides profile unique to individual types of microbes, with peaks specific to genera and species
• Once acquired compared to a database of reference spectra
• A value - percentage or score is produced
Results
Results

• ID is started immediately after mass spectrum available.

• During the run the appearance of sample and QC positions in the MALDI plate display reflects the success of the measurement and ID a each position
  • If spectrum measurement successful - left half of the sample is green.
  • If measurement fails - left half of the sample is orange
  • Colouring of right half of sample position indicates the score value of ID

Bruker, MBT Compass User Manual, 2018
## Table of Detected Species

<table>
<thead>
<tr>
<th>ID</th>
<th>Position</th>
<th>Detected Species</th>
<th>Score</th>
<th>Comment</th>
<th>Description</th>
<th>Confidence</th>
<th>Export State</th>
<th>Species to Export</th>
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<tbody>
<tr>
<td>A1</td>
<td></td>
<td><em>Escherichia coli</em></td>
<td>2.08</td>
<td>closely related to <em>Shigella</em> and not <em>E.</em></td>
<td>Description of <em>A1</em></td>
<td>high</td>
<td>&lt;Best Match&gt;</td>
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<tr>
<td>A2</td>
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<td><em>Escherichia coli</em></td>
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<tr>
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<td><em>Escherichia coli</em></td>
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<td></td>
<td>Description of <em>A6</em></td>
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<tr>
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<td><em>Escherichia coli</em></td>
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<td></td>
<td>Description of <em>A7</em></td>
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<tr>
<td>A8</td>
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<td><em>Escherichia coli</em></td>
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<td></td>
<td>Description of <em>A8</em></td>
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<td>&lt;Best Match&gt;</td>
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<td></td>
<td>Description of <em>B1</em></td>
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<td>&lt;Best Match&gt;</td>
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<td>B2</td>
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<td><em>Escherichia coli</em></td>
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<td>Description of <em>B2</em></td>
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<tr>
<td>B3</td>
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<td></td>
<td>Description of <em>B3</em></td>
<td>high</td>
<td>&lt;Best Match&gt;</td>
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<tr>
<td>B4</td>
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<td><em>Escherichia coli</em></td>
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<td>Description of <em>B4</em></td>
<td>high</td>
<td>&lt;Best Match&gt;</td>
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</tr>
</tbody>
</table>

Bruker, MBT Compass User Manual, 2018
MALDI Scores

• Higher the log (score), higher the similarity between mass spectrum of isolate & the database entry in the reference library

<table>
<thead>
<tr>
<th>Consistency</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>High (A)</td>
<td>Best match is a high-confidence ID&lt;br&gt;Second-best match is:&lt;br&gt;• high-confidence ID identical sp ID to best match&lt;br&gt;• low-confidence ID identical to genus in best match&lt;br&gt;• non-identification</td>
</tr>
<tr>
<td>Low (B)</td>
<td>Requirements for high consistency not met.&lt;br&gt;Best match is a high- or low-confidence ID&lt;br&gt;Second-best match is:&lt;br&gt;• high- or low-confidence ID identical to genus in best match&lt;br&gt;• non-identification</td>
</tr>
<tr>
<td>None (C)</td>
<td>Requirements for high or low consistency not met</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Range</th>
<th>Interpretation</th>
<th>Symbols</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00 - 3.00</td>
<td>High Confidence Identification</td>
<td>(+++)</td>
<td>green</td>
</tr>
<tr>
<td>1.70 - 1.99</td>
<td>Low Confidence Identification</td>
<td>(+)</td>
<td>yellow</td>
</tr>
<tr>
<td>0.00 - 1.69</td>
<td>No Organism Identification Possible</td>
<td>(-)</td>
<td>red</td>
</tr>
</tbody>
</table>

Bruker, MBT Compass User Manual, 2018
<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Sample ID</th>
<th>Organism (best match)</th>
<th>Score Value</th>
<th>Organism (second best match)</th>
<th>Score Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (+++)(A)</td>
<td>ID of A1</td>
<td><em>Escherichia coli</em></td>
<td>2.68</td>
<td><em>Escherichia coli</em></td>
<td>2.30</td>
</tr>
<tr>
<td>A2 (+++)(A)</td>
<td>ID of A1</td>
<td><em>Escherichia coli</em></td>
<td>2.75</td>
<td><em>Escherichia coli</em></td>
<td>2.35</td>
</tr>
<tr>
<td>A3 (+++)(A)</td>
<td>ID of A3</td>
<td><em>Cupriavidus necator</em></td>
<td>2.61</td>
<td><em>Cupriavidus necator</em></td>
<td>2.15</td>
</tr>
<tr>
<td>A4 (+++)(A)</td>
<td>ID of A4</td>
<td><em>Staphylococcus aureus</em></td>
<td>2.29</td>
<td><em>Staphylococcus aureus</em></td>
<td>2.27</td>
</tr>
<tr>
<td>A5 (+++)(A)</td>
<td>ID of A5</td>
<td><em>Escherichia coli</em></td>
<td>2.69</td>
<td><em>Escherichia coli</em></td>
<td>2.30</td>
</tr>
<tr>
<td>A6 (-)(C)</td>
<td>ID of A6</td>
<td>No Organism Identification Possible</td>
<td>1.41</td>
<td>No Organism Identification Possible</td>
<td>1.38</td>
</tr>
<tr>
<td>A7 (+++)(A)</td>
<td>ID of A7</td>
<td><em>Proteus mirabilis</em></td>
<td>2.67</td>
<td><em>Proteus mirabilis</em></td>
<td>2.66</td>
</tr>
<tr>
<td>A8 (-)(C)</td>
<td>ID of A8</td>
<td>No Organism Identification Possible</td>
<td>1.10</td>
<td>No Organism Identification Possible</td>
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</tr>
<tr>
<td>ID</td>
<td>Position</td>
<td>Detected Species</td>
<td>Score</td>
<td>Comment</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>----------</td>
<td>---------------------------------------</td>
<td>-------</td>
<td>----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>ID of A5</td>
<td>A5</td>
<td>Escherichia coli</td>
<td>2.69</td>
<td>closely related to Shigella and no...</td>
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<td>ID of A6</td>
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<td>No Organism Identification Possible</td>
<td>1.41</td>
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</tr>
<tr>
<td>ID of A7</td>
<td>A7</td>
<td>Proteus mirabilis</td>
<td>2.67</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Detected Species**

- Proteus mirabilis DSM 18254 DSM
- Proteus mirabilis 9482_2 CHB
- Proteus mirabilis DSM 30115 DSM
- Proteus mirabilis DSM 46227 DSM
- Proteus mirabilis DSM 788 DSM
- Proteus mirabilis DSM 50903 DSM
- Proteus mirabilis RV412_A1_2010_06b LBK
- Proteus mirabilis 13210_1 CHB
- Proteus mirabilis (PX) 22086112 MLD
- Proteus vulgaris DSM 13625 DSM

- ID of A8 | A8       | No Organism Identification Possible    | 1.10  | is a member of Pseudomonas pu...              |
Advantages

• Rapid (≤3 minutes per isolate)
• Inexpensive - low reagent cost
• Small amounts of organism are required
• Direct sample ID possible
• Reduced labour
• Accurate ID

<table>
<thead>
<tr>
<th>Microorganism group</th>
<th>Number of processed samples</th>
<th>Number of correct identifications</th>
<th>Number of incorrect identifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-fermenting Gram-negative bacteria</td>
<td>229</td>
<td>215 (93.89%)</td>
<td>14 (6.11%)</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>265</td>
<td>263 (99.25%)</td>
<td>2 (0.75%)</td>
</tr>
<tr>
<td>Other Gram-negative bacteria</td>
<td>204</td>
<td>195 (95.59%)</td>
<td>9 (4.41%)</td>
</tr>
<tr>
<td>Gram-positive bacteria</td>
<td>230</td>
<td>224 (97.39%)</td>
<td>6 (2.61%)</td>
</tr>
<tr>
<td>Yeasts</td>
<td>225</td>
<td>219 (97.33%)</td>
<td>6 (2.67%)</td>
</tr>
<tr>
<td>Total number</td>
<td>1153</td>
<td>1116 (96.79%)</td>
<td>37 (3.21%)</td>
</tr>
</tbody>
</table>

* Of the 1153 samples, 0.61% (7 samples) provided low-confidence identifications; 1.91% (22 samples) could not be identified; and 0.69% (8 samples) gave a false result.
Limitations

• Databases are proprietary unlike publicly available sequence databases
• Difficulties in ID with some organisms
• Difficulty analysing mixed cultures
• Identifying organisms from liquid cultures
• Low identification scores - repeat testing for 10% of isolates
• Growth on some media may be associated with low scores
• Small/mucoid colonies may fail ID
• ID of biosafety level 3/4 organisms
• Requires room temperature (20-25 °C)
• Human error
Difficulties in Identification

• Misidentification rare
• Can occur with closely related organisms
  • E. coli and Shigella
  • Discrimination between species from same complex eg. E cloacae complex
• Salmonella can only be ID to genus level - No typing
• Difficulties with some species - alpha haemolytic strep
• Mycobacteria & filamentous fungi
Considerations for other organisms

• Mycobacteria:
  • Requires processing to kill tested bacteria, break down cell envelopes, disrupt clumped cells
  • Can ID most clinically relevant species
  • MTB complex ID to complex level only
  • Some related mycobacterium species not well differentiated (M. chimaera and M. intracellulare)

• Enhanced databases ID Nocardia – often specific extraction processes needed

• Fungi:
  • Can identify yeast well
  • Filamentous fungi limited - variable phenotypes & protein spectra vary with growth conditions
  • Available for aspergillus, fusarium & mucorales
Common Sources of Error

• Colony inoculation in erroneous target plate locations
• Testing impure colonies
• Smearing between spots
• Failure to clean target plates
• Entry of wrong results
Conclusion

• MALDI-TOF MS utilizes:
  • Laser & matrix as an ion source
  • TOF (electric field) as a mass analyser
  • Ion detector

• Provides a rapid, accurate and cost-effective method for ID of many bacteria & yeast

• Several limitations which operators need to be aware of when reporting & troubleshooting