PosterREPRINT

Waters

QUALITY ASSURANCE TESTING FOR THE RAPID IDENTIFICATION OF BACTERIA USING MATRIX-ASSISTED LASER/DESORPTION IONISATION TIME-OF-FLIGHT MASS SPECTROMETRY (MALDI TOF MS)

H. Sutton[1], D. Dare[1], J. Bright[1], V. Edwards-Jones[1], C.J. Keys[2], H.N. Shah[2], T. Mckenna[3] and M. Lunt[3]
[1]Manchester Metropolitan University, Manchester, UK. [2]PHLS Central Public Health Laboratory, London, UK.
[3]Waters Corporation, Manchester, UK.

Presented at IUMS, Paris, France, 27th July - 1st August, 2002

Introduction

Intact cell matrix assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF-MS) produces a characteristic mass spectral fingerprint using surface ions desorbed from the intact bacterial cell [1,2,3 & 4]. The characteristic fingerprints are produced within minutes and yield sufficient data to identify bacteria to species and in some cases to strain. There is also evidence that this method has the potential for some species to simultaneously sub-type [5 & 6]. Previous parallel studies have shown that the mass spectral fingerprints produced are reproducible between operators and instruments provided that appropriate protocols are followed [6 & 7].

This study chose eight organisms for quality assurance testing; *Bacteroides fragilis, Pseudomonas aeruginosa, Escherichia coli, Lactobacillus rhamnosus, Bacillus firmus, Corynebacterium jeikeium, Micrococcus lylae* and *Vibrio parahaemolyticus*.

All eight organisms were repeatedly grown and analyzed on a weekly basis using different batches of media, following the same protocols used to produce the database entries. This was carried out to ensure that the protocols, instrumentation and software produce consistent results, independent of the batch of media used.

Method

Bacterial Strains and growth conditions

- Eight NCTC strains of bacteria were used for this study see **Table 1** for detailed list.
- All eight NCTC strains previously laid down on Project beads (Lab M, Bury, Lancashire, UK) were revived by streaking a single bead onto Columbia Blood (5% v/v) agar (CBA, Supplied by the Public Health Laboratory Service accredited laboratories in Chester).
- Incubation: 24 hours at 37°C on CBA in an aerobic atmosphere, except *Bacteroides fragilis*; 24 hours at 37°C on CBA in an anaerobic atmosphere (Oxoid, AnaeroGen™, 3.5L AN 35).
- Two further sub-cultures were made prior to MALDI-TOF-MS analysis.
- Different batches of CBA were used for each test run, the same batch for all eight organisms each week.

Bacterial preparation for MALDI-TOF-MS analysis

- Using a 1mL culture loop, several bacterial colonies were applied to 12 target plate wells. (Twelve wells per strain)
- Samples air-dried for at least 1 hour.
- Samples overlaid with 1mL aliquot of matrix, either:

  α-cyano-4-hydroxycinnamic acid (Sigma-Aldrich Chemical Company), for Gram-negative bacteria.

  5-chloro-2-mercaptobenzothiazole (Sigma-Aldrich Chemical Company), for Gram-positive bacteria.
- Then allowed to air dry.
- Matrix solvent acetonitrile: methanol: water (1:1:1) with 0.1% (v/v) formic acid and 0.01M 18-crown-6.

Waters

Method

- Analysis performed using a M@LDI-Linear time of flight mass spectrometer (Waters Corporation, Manchester, UK).
- A nitrogen laser giving a 337nm output of 3ns pulse width.
- Laser fluence was set to just above the threshold for ion production in the positive ion detection mode.
- Acceleration voltage of +15kV
- On loading each target plate, automatic, accurate indexing of the sample/reference wells was performed.
- Mass calibration performed using the average molecular weights from a standard peptide mixture (bradykinin, angiotensin I, gul-fibrinopeptide B, rennin substrate tetra decapeptide, ATCH (18-39 clip) all at 1pmol/µL, bovine insulin 2pmol/µL and ubiquitin 10pmol/µL)
- Data acquisition mass range was from m/z 800 to 3000 Da.
- Bacterial mass fingerprints, and spectra from reference wells, for lock mass calibration, were automatically acquired using the MAXspec real-time data selection algorithm to optimize the bacterial fingerprint in the mass range 600-3000 Da

Data analysis using Micromass MicrobeLynx™ software

- Replicates of twelve spectra, per bacterial strain, were compared for reproducibility using the root mean square (RMS) value; this value is obtained by comparing each replicate in turn with the average of the other 11 replicates.
- An RMS rejection value of three was used to identify outliers significant at the 0.1% level.
- Any outliers were excluded from the database search.
- The remaining replicates were then combined to give a representative average spectrum.
- The average spectrum was then searched against the Manchester Metropolitan University Database, which contains some 1000 bacterial fingerprints, covering a wide range of genera.
- The search uses a pattern recognition algorithm within which all the mass and intensity data in the spectrum is used to give the best database match with a probability score.
- A display of the test spectrum and the differences from the best database matches are presented in a browser format, **Figure 1**.

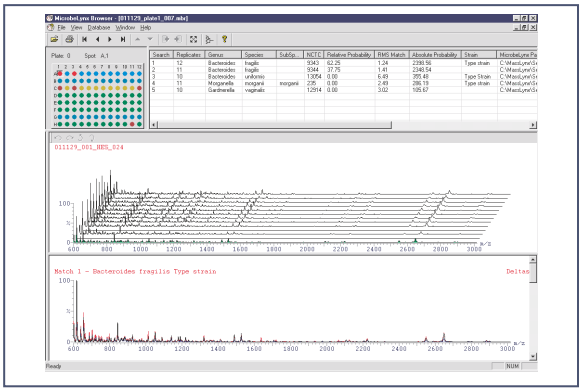

Figure 1. Browser results for quality assurance test of Bacillus fragilis 9343 searched against MMU database; red wells indicate spectra excluded from database search

Waters

Results

The following three organisms all matched to strain *B. fragilis, C. jeikeium* and *M. lylae*. With the exception of *B. fragilis* in week 6, which matched to species 1st and strain 2nd (**Table1**).

Organism name	NCTC number	Week Number						Number of matches in top 5, against database of 1000 entries
		1	2	3	4	5	6	
Bacteroides fragilis	9343	✓	✓	✓	✓	✓	✓#	6
Corynebacterium jeikeium	11913	✓	✓	✓	✓	✓	✓	6
Micrococcus lylae	11037	✓	✓	✓	✓	✓	✓	6
Bacillus firmus	10335	✓	✓	x*	✓	3rd	3rd#	5
Vibrio parahaemolyticus	10903	5th	✓#	✓	4th	✓	3rd	6
Pseudomonas aeruginosa	10332	4th	✓	✓	4th	5th#	3rd	6
Escherichia coli	9001	5th	4th#	x	2nd ®	x	2nd#	4
Lactobacillus rhamnosus	10302	x[1]	x[1]	x	x*	x	x	0

✔ First match correct to strain unless other wise stated.
* Contamination observed
\# Species
x[1] incorrect culture conditions

Table 1. Shows total number of matches against database of 1000 entries

For *Bacillus firmus* the search matched to strain within the top 3, in all but the third week (**Table1**). Two samples were contaminated, however the spectra are mathematically similar at 0.1% significance level (i.e. RMS < 3, **Figure 2**)

Vibrio parahaemolyticus and *Pseudomonas aeruginosa* matched to strain/species within the top 5. Although for *V. parahaemolyticus* the RMS values are >3 for weeks 1 & 3, and For *P. aeruginosa* the RMS values were generally >3 for weeks 3-6.

Escherichia coli **Figure 3** shows that the replicates for weeks 1 to 6 were very similar although significantly different from the database entry; this difference is with respect to peak intensity.

Lactobacillus rhamnosus, the dendogram clusters weeks 1, 2 & 3 together two samples were grown in CO_2 and the remaining sample had an RMS >3,(**Figure 4**). Weeks 5 and 6 are mathematically similar to the database with RMS values <3 even though the search failed to match these samples, failure to match these samples is due to the QA spectra being more intense in comparison to the database spectra. Week 4 is significantly different due to sample contamination (**Figures 4 & 5**).

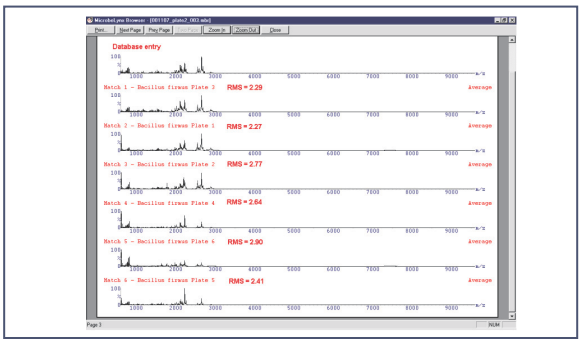

Figure 2. Bacillus firmus; Comparison of database entry against six QA replicate spectra

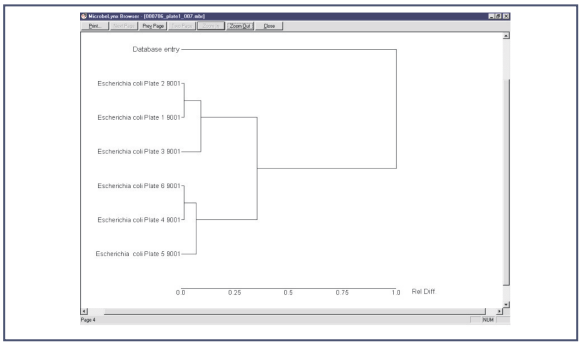

Figure 3. Dendogram comparing Escherichia coli database entry against six QA replicate spectra

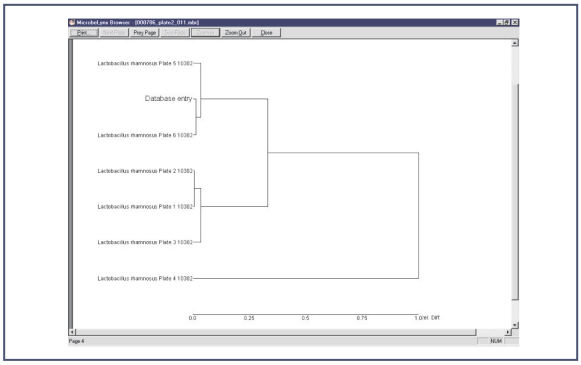

Figure 4. Dendogram comparing Lactobacillus rhamnosus database entry against six QA replicate spectra

Waters

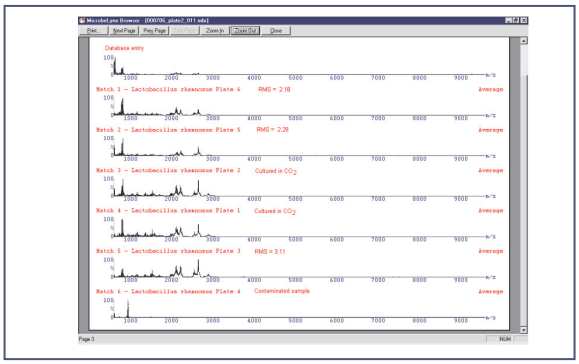

Figure 5. Lactobacillus rhamnosus; Comparison of database entry against six QA replicate spectra

Conclusion

- Protocols for bacterial growth must be followed, as changes in culture conditions affects the spectral pattern/biomarkers.
- Spectral reproducibility is good, provided that protocols are followed and the instrument calibration is within the set parameters.
- The bacterial fingerprint/biomarker pattern is not affected by different batches of accredited media.
- The software is able to successfully compare spectra, present the best matches with a probability score, and correctly identify an organism to strain/species from a core database of some 1000 organisms.
- Bacterial Identification to strain/species in the top 5 matches can be achieved to 91%, for pure samples grown under equivalent culture conditions to the database entries.
- Future work should include refinement of the search e.g. normalising intensities to improve matches.

References

1. Claydon, M. A., Davey S N, Edwards-Jones V., & Gordon D. B., 1996, The rapid identification of intact microorganisms using mass spectrometry, *Nature Biotech.*, **14**,1584-1586.
2. Holland, R. D., Wilkes, J. G., Rafii, F., Sutherland, J. B., Persons, C. C., Voorhees, K. J., Lay, J. O., 1996, Rapid identification of intact whole bacteria based on spectral patterns using matrix-assisted laser desorption with time-of-flight mass spectrometry, *Rapid Commun. Mass Spectrom.*, **10**, 1227-1232.
3. Krishnamurthy, T., Ross, P. L., Rajamani. U., 1996, Detection of pathogenic and non-pathogenic bacteria by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, *Rapid Commun. Mass Spectrom.* ,**10**, 883-888.
4. Welham, K. J., Domin, M. A., Scannell, D. E., Cohen, E., Ashton, D. S., 1998, The characterisation of microorganisms by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry, *Rapid Commun. Mass Spectrom.*, **12**, 176-180.
5. Edwards-Jones, V., Claydon, M. A, Evason, D . J., Walker, J., Fox, A., & Gordon, D .B., 2000, Rapid discrimation between methicillin sensitive and methicillin resistant *Staphylococcus aureus* using Intact Cell Mass Spectrometry, *J Med Micro.*, **49**, 345-351.
6. Walker, J., Fox, A. J., Edwards-Jones, V. & Gordon, D. B., 2002, Intact cell mass spectrometry (ICMS) used to type methicillin resistant *Staphylococcus aureus*; media effects and inter-laboratory reproducibility *J. Microbiological Methods*, **48**, 117-126
7. Dare, D. J., Bright, J. J., Morgan, M. M., Edwards-Jones, V, Keys, C. J., Shah, H. N., McKenna M. & Lunt, M., 2001, Rapid Identification of Bacteria by Intact Cell Matrix Assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry:Inter-laboratory Study, 101st General Meeting of the American Society for Microbiology, Florida, USA.

PosterREPRINT

Author to whom all correspondence should be addressed:
Thérèse McKenna
Waters Corporation
(Micromass UK Limited)
Floats Road, Wythenshawe
Manchester, M23 9LZ
Tel: + 44 (0) 161 946 2400
Fax: + 44 (0) 161 946 2480
e-mail: therese.mckenna@micromass.co.uk

WATERS CORPORATION
34 Maple St.
Milford, MA 01757 U.S.A.
T: 508 478 2000
F: 508 872 1990
www.waters.com

Made in the United Kingdom

Waters
RIGHT ON TIME.

MICROMASS®
MS TECHNOLOGIES

Certificate No: 951387