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Bacterial identification using a SCIEX 5800 TOF/TOF MALDI research instrument and an external database

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ABSTRACT

In our current study we were identifying 26 bacterial isolates using a SCIEX 5800 TOF/TOF MALDI instrument and an external database. The results were compared with the results of a Vitek® MS system and in case of discrepancies at the species level 16s rRNA sequencing was performed for further verification.

1. Introduction

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) uses a soft ionization method where sample integrity is not significantly compromised. Thanks to its suitability for the detection of proteins in the 2–20 kDa mass range and the relatively low cost of the instruments needed, linear MALDI-TOF MS has become a growing technology for bacterial identification in clinical microbiology (Picaud et al., 2018; Singhal et al., 2015).

Microbiological identification is achieved by matching the respective mass spectra mainly representing highly abundant ribosomal proteins against a database. Databases are a main component of commercial MALDI platforms used in clinical diagnostics (De Carolis et al., 2014; Singhal et al., 2015). However, such platforms are costly and therefore not easily accessible for researchers (Singhal et al., 2015).

The SCIEX 5800 TOF/TOF (AB Sciex, Framingham, MA, 01701, USA) MALDI instrument is a research instrument that possesses several types of TOF analysers. These include a reflecting analyser for increased resolving power, mass accuracy and sensitivity in smaller molecules of up to 5–10 kDa (https://patents.google.com/patent/US7589319B2/en), a TOF/TOF analyser for the investigation of molecular structures (Kafka et al., 2011), and a linear analyser.

The linear analyser is preferred for analysing high mass ions such as intact proteins and may be used for bacterial identification (Picaud et al., 2018). However, our SCIEX 5800 TOF/TOF MALDI instrument does not have a respective database for bacterial identification. Therefore, we were evaluating the use of an external database. For this purpose, 26 bacterial isolates comprising several different bacterial species were investigated. The results were compared against the results obtained using the VITEK®MS (bioMérieux, 69,280 Marcy-l'Etoile, France) platform, a system routinely used in clinical diagnostics laboratories (De Carolis et al., 2014; Deak et al., 2015; Rychert et al., 2013).

2. Methods, results and discussion

Bacterial isolates were kept at −80 °C in Brain Heart Infusion broth (Becton, Dickinson and Company, Sparks, MD 21152 USA) with 25% glycerol. Isolates were cultured from frozen stocks and a single clone was subcultured for bacterial typing using LB agar plates (Luria-Bertani agar, Becton, Dickinson and Company, Sparks, MD 21152, USA).

The calibrant was prepared as follows: Two 1 µl loops full of the *E. coli* ATCC 25922 strain were suspended in 30 µl of 70% formic acid (Optima for LC/MS grade, Thermo Fisher Scientific, Fair Lawn, NJ 07410, USA) and vortexed for 1 min. Thirty microliters of acetonitrile (Acetonitrile, Lichrosolv®, hypergrade for LC-MS, Merck Milipore, 290 Concord Road, Billerica, MA 01821, USA) were added, and after vortexing for another minute the mixture was centrifuged at 10,000*g* for 2 min. Four microliters of the supernatant were transferred to a tube, and 2 µl of apomyoglobin 100 pmol/µl (MALDI-MS standard, Sigma-Aldrich, St Louis, MO, USA), reconstituted in 100 µl of 0.1% of trifluoroacetic acid (TFA analytical standard, Sigma-Aldrich, St Louis, MO, USA) were added. The calibrant mixture was diluted 1:10 in sinapinic acid matrix consisting of 10 mg of sinapinic acid (matrix

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substance for MALDI-MS, Sigma-Aldrich, St Louis, MO, USA), 500 µl acetonitrile, 480 µl of ultrapure water and 20 µl of trifluoroacetic acid (analytical standard, Sigma-Aldrich, St Louis MO 63178).

The identification of the isolates was obtained by either the following extraction method: Two 1 µl loops full of the respective bacterial isolate were suspended in 1 ml of ultrapure water and centrifuged at 10,000*g* for 2 min. The pellet was resuspended in 30 µl of 70% formic acid and vortexed for 1 min. Thirty microliters of acetonitrile were added, and after vortexing for 1 min, samples were left on the shaker for 5 min and centrifuged at 10,000*g* for 2 min. The supernatant was diluted 1:10 in sinapinic acid matrix. Apomyoglobin 100 pmol/µl 0.1% TFA was also diluted 1:10 in sinapinic acid matrix.

A droplet of each sample was spotted onto a sample spot on an Opti-TOF 384 MALDI plate insert (AB Sciex, Framingham, MA, 01701, USA) in quadruplicates as a dilution curve of a sample/matrix ratio of 1:10, 1:20, 1:30 and 1:40.

Alternatively the following direct streak method was applied: For each isolate, a sterilised wooden splint was used to pick up a small amount of bacteria and spread in a thin layer onto a sample spot on an Opti-TOF 384 MALDI plate insert. Samples were overlayed with 0.5 µl of formic acid where necessary for optimisation of spectra, leaving the samples to dry before adding a droplet of sinapinic acid matrix.

The calibrant and apomyoglobin were spotted onto four sample spots each for control and preliminary calibration. The calibrant was also spotted onto at least four calibration spots in order to run the calibration batch.

The MALDI-TOF instrument used was a 5800 TOF/TOF (AB Sciex, Framingham, MA, 01701, USA) instrument with the following settings: The calibration batch was run as an internal calibration with a mass tolerance of ± 800 ppm. The minimum signal to noise of four was applied and the maximum outlier error was 500 ppm. The following reference masses were used: 4365.3 (SUB = L36_1), 7274.5 (SUB = L29), 8476.78 *m/z* (apomyoglobin + +) and 16952.56 m/z (apomyoglobin +). The mass range of the acquisition method was 3000 to 20000 m/z, and the focus mass was 12000 m/z. The laser intensity was set at 3800 with a total of 2500 shots per spectrum. The spectra were processed using baseline subtraction (peak width 30). A minimum signal to noise ratio of three was applied for peak detection with the local noise window width being 200 m/z. The resolution was 750. The low mass gate was enabled.

Raw data files were either exported as text files using PeakView® 2.2 (https://sciex.com/products/software/peakview-software) or converted to mzxml files using a t2d file converter made by Tom Gao (http://www.pepchem.org/) and sent to Mabritec Switzerland (www.mabritec.com). The spectra were processed using MALDIquant (Gibb and Strimmer, 2012) with the following parameters: The peak intensities were transformed using square root, and the data was smoothed using the Savitzky-Golay filter with a polynomial order of 10 and a half window size of 20. The baseline correction method used was the statistics-sensitive non-linear iterative peak-clipping (SNIP) algorithm with 100 iterations, and the peak detection was done using the median absolute deviation (MAD) method with half window size of 20 and a signal to noise ratio of three. The processed spectra were subsequently compared against the SARAMIS™ (Spectral ARchive And Microbial Identification System database, Anagnostec GmbH, 14476 Potsdam, Germany) and PAPMID™ (Putative Assigned Protein Masses for Identification Database, Mabritec AG, 4125 Riehen, Switzerland) databases as follows: Acquired spectra were compared against the superspectra of the SARAMIS™ database. If confidence values were less than 90%, spectra were automatically compared against the entire SARAMIS™ database containing more than 110,000 spectra of more than 3100 bacterial species, as well as the PAPMID™ database. The PAPMID™ database is based on calculated ribosomal masses from more than 82,000 genomes to date, covering more than 8,100 bacterial species and verified with bacterial strains (Toh et al., 2015).

The bacterial isolates were also identified using the VITEK® MS v3.2.0 system (bioMérieux, 69280 Marcy-l'Etoile, France). Samples were spotted as per the VITEK® MS Workflow User Manual – Clinical Use, with the exception of the use of a sterile wooden toothpick instead of a loop for spotting.

The results of the two MALDI methods were compared, and where there were inconsistencies at the species level, the respective isolates were subjected to 16S rRNA sequencing. For this purpose, the DNA was extracted using the Qiagen DNeasy UltraClean Microbial Kit (QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany) and sent to Macrogen, Korea for sequencing and contig assembly. The following universal amplification and sequencing primers were used: 27F (AGA GTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) for amplification and 785F (GGATTAGATACCCTGGTA) and 907R (CCGTCAATTCMTTTRAGTTT) for sequencing. (www.macrogen.com). The obtained contigs were subsequently blasted against the EZBioCloud (https://www.ezbiocloud.net/) database for identification.

For two isolates, *rpoB* and *hsp60* genes were also sequenced, using amplification and sequencing primers as previously described (García-González et al., 2018). Files were assembled using the MacVector program (https://macvector.com). All nucleotide sequences including the 16s rRNA sequences were subsequently blasted against the NCBI BLAST database, (https://blast.ncbi.nlm.nih.gov) using the megablast program to find bacterial species with high sequence similarities.

At the genus level, all 26 isolates showed consistent results between the two MALDI methods, and 13 bacterial genera were detected in total. At the species level, 21 out of the 26 isolates showed consistent results between the two MALDI methods (Table 1), all of which had a confidence value of 99.9% when using the VITEK® MS system.

Five isolates were identified as a different species within the same genus when using the VITEK® MS or the TOF/TOF 5800 - MABRITEC identification method. This was the case for the two *Aeromonas* isolates which had ambivalent results using the VITEK® MS system, one *Staphylococcus* isolate, and two *Enterobacter* isolates, which, although identifed as belonging to the *E. cloacae* complex (Hoffmann and Roggenkamp, 2003) with both MALDI methods, had divergent results at the species level (Tables 1 and 2). The *Staphylococcus* isolate had a confidence value of 99.9% using the VITEK® MS system, however, the *Aeromonas* and *Enterobacter* isolates had a confidence value of no more than 50%.

16S rRNA sequencing confirmed the results of the TOF/TOF 5800 - MABRITEC identification method for the two *Aeromonas* isolates. The result of the VITEK® MS system was confirmed for the *Staphylococcus* isolate (Table 2).

For the two *Enterobacter* isolates, the results of neither of the two MALDI methods could be confirmed using 16s rRNA sequencing. *Hsp60* and *rpoB* gene sequencing was subsequently performed. All sequences were combined and blasted against the NCBI BLAST database for sequence similarities in bacterial species. The species with the highest similarity were in agreement with the results of both MALDI methods (Table 2) with a Query coverage of 99% and 100% identity for both isolates. No other species had a similarity this high. The species identified with each MALDI method all belonged to the *Enterobacter cloacae* complex (Table 2). Species identification within the complex has been described to be difficult and it may be due to the rapidly changing nature of the nomenclature of the complex (Hoffmann and Roggenkamp, 2003; Paauw et al., 2008) that our database search did not give unequivocal results.

3. Conclusion

Here we have shown that we were able to use the SCIEX TOF/TOF 5800 instrument for bacterial identification with comparable accuracy to a commercial system. This was done with the help of an external database and without the need to purchase a platform for bacterial identification or set up a database in house.

Table 1
Bacterial isolates tested.

Isolate	VITEK® MS ID	TOF/TOF 5800 – MABRITEC ID	Origin	Reference
EC2 TEM	*Escherichia coli*	*Escherichia coli*	Australia	
QTR-47	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	Qatar	(Zowawi et al., 2014)
102145	***Aeromonas hydrophilia / caviae***	***Aeromonas hydrophilia***	Australia	
94652	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	Australia	
102149	***Aeromonas sobria / veronii***	***Aeromonas veronii***	Australia	
105545	*Citrobacter freundii*	*Citrobacter freundii*[a]	Singapore	
94670	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	Australia	
126007	*Staphylococcus warneri*	*Staphylococcus warneri*	Australia	
93781	*Proteus mirabilis*	*Proteus mirabilis*	Australia	
IMP 110	***Enterobacter cloacae***	***Enterobacter hormaechei*** subsp. ***steigerwaltii***	Australia	(Sidjabat et al., 2015)
IMP 11	***Enterobacter cloacae***	***Enterobacter asburiae***[a]	Australia	(Sidjabat et al., 2015)
OMN-35	*Klebsiella oxytoca*	*Klebsiella oxytoca*	Oman	
SA-111	*Acinetobacter baumannii*	*Acinetobacter baumannii*	Saudi Arabia	(Zowawi et al., 2015)
T210	*Acinetobacter nosocomialis*	*Acinetobacter nosocomialis*	Thailand	
T217	*Acinetobacter pitii*	*Acinetobacter pittii*	Thailand	
C0369	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	Australia	(Sidjabat et al., 2016)
C0619	***Staphylococcus pasteuri***	***Staphylococcus warneri***[b]	Australia	(Sidjabat et al., 2016)
C0382	*Staphylococcus aureus*	*Staphylococcus aureus*	Australia	(Sidjabat et al., 2016)
C0366	*Staphylococcus warneri*	*Staphylococcus warneri*	Australia	(Sidjabat et al., 2016)
C3156	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*	Australia	(Sidjabat et al., 2016)
C2798	*Staphylococcus aureus*	*Staphylococcus aureus*	Australia	(Sidjabat et al., 2016)
C2077	*Staphylococcus aureus*	*Staphylococcus aureus*	Australia	(Sidjabat et al., 2016)
G0624	*Pseudomonas oryzihabitans*	*Pseudomonas oryzihabitans*	Australia	
PA15	*Klebsiella aerogenes*	*Klebsiella aerogenes*	Australia	
RB11	*Salmonella enterica*	*Salmonella enterica* subsp. *enterica*	Australia	
ATCC 29212	*Enterococcus faecalis*	*Enterococcus faecalis*	United States	Reference strain

[a] Direct streak

[b] Direct streak with formic acid. All other isolates were extracted for the TOF/TOF 5800 - Mabritec identification method.

Table 2
Isolates with differing species identification results.

Isolate	VITEK® MS ID	TOF/TOF 5800 – MABRITEC ID	Gene sequencing
102145	*Aeromonas hydrophilia / caviae*	*Aeromonas hydrophilia*	*Aeromonas hydrophlilia subsp. hydrophilia*[a]
102149	*Aeromonas sobria / veronii*	*Aeromonas veronii*	*Aeromonas veronii*[a]
IMP 110	*Enterobacter cloacae*	*Enterobacter hormaechei subsp. steigerwaltii*	*Enterobacter cloacae / hormachei*[b]
IMP 11	*Enterobacter cloacae*	*Enterobacter asburiae*	*Enterobacter cloacae/ asburiae*[b]
C0619	*Staphylococcus pasteuri*	*Staphylococcus warneri*	*Staphylococcus pasteuri*[a]

[a] 16s rRNA sequencing.

[b] 16s rRNA, rpoB and hsp60 sequencing.

Declaration of Competing Interest

Valentin Pflüger is an employee of Mabritec Switzerland.

References

De Carolis, E., Vella, A., Vaccaro, L., Torelli, R., Spanu, T., Fiori, B., Posteraro, B., Sanguinetti, M., De Carolis, E., Vella, A., Vaccaro, L., Torelli, R., Spanu, T., Fiori, B., Posteraro, B., Sanguinetti, M., 2014. Review article application of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. J Infect Dev Ctries. https://doi.org/10.3855/jidc.3623.

Deak, E., Charlton, C.L., Bobenchik, A.M., Miller, S.A., Pollett, S., McHardy, I.H., Wu, M.T., Garner, O.B., 2015. Comparison of the Vitek MS and Bruker Microflex LT MALDI-TOF MS platforms for routine identification of commonly isolated bacteria and yeast in the clinical microbiology laboratory. Diagn. Microbiol. Infect. Dis. https://doi.org/10.1016/j.diagmicrobio.2014.09.018.

García-González, T., Sáenz-Hidalgo, H.K., Silva-Rojas, H.V., Morales-Nieto, C., Vancheva, T., Koebnik, R., Ávila-Quezada, G.D., 2018. Enterobacter cloacae, an emerging plant-pathogenic bacterium affecting chili pepper seedlings. Plant Pathol. J. https://doi.org/10.5423/PPJ.OA.06.2017.0128.

Gibb, S., Strimmer, K., 2012. Maldiquant: a versatile R package for the analysis of mass spectrometry data. Bioinformatics. https://doi.org/10.1093/bioinformatics/bts447.

Hoffmann, H., Roggenkamp, A., 2003. Population genetics of the nomenspecies Enterobacter cloacae. Appl. Environ. Microbiol. https://doi.org/10.1128/AEM.69.9.5306-5318.2003.

Kafka, A.P., Kleffmann, T., Rades, T., McDowell, A., 2011. The application of MALDI TOF MS in biopharmaceutical research. Int. J. Pharm. https://doi.org/10.1016/j.ijpharm.2010.12.010.

Paauw, A., Caspers, M.P.M., Schuren, F.H.J., Leverstein-van Hall, M.A., Delétoile, A., Montijn, R.C., Verhoef, J., Fluit, A.C., 2008. Genomic diversity within the enterobacter cloacae complex. PLoS One. https://doi.org/10.1371/journal.pone.0003018.

Picaud, V., Giovannelli, J.F., Truntzer, C., Charrier, J.P., Giremus, A., Grangeat, P., Mercier, C., 2018. Linear MALDI-ToF simultaneous spectrum deconvolution and baseline removal. BMC Bioinformatics. https://doi.org/10.1186/s12859-018-2116-3.

Rychert, J., Burnham, C.A.D., Bythrow, M., Garner, O.B., Ginocchio, C.C., Jennemann, R., Lewinski, M.A., Manji, R., Mochon, A.B., Procop, G.W., Richter, S.S., Sercia, L., Westblade, L.F., Ferraro, M.J., Branda, J.A., 2013. Multicenter evaluation of the vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system for identification of gram-positive aerobic bacteria. J. Clin. Microbiol. https://doi.org/10.1128/JCM.00682-13.

Sidjabat, H.E., Townell, N., Nimmo, G.R., George, N.M., Robson, J., Vohra, R., Davis, L., Heney, C., Patersona, D.L., 2015. Dominance of IMP-4-producing enterobacter cloacae among carbapenemase-producing Enterobacteriaceae in Australia. Antimicrob. Agents Chemother. https://doi.org/10.1128/AAC.04378-14.

Sidjabat, H., Cottrell, K., Flohr, G., Kitipornchai, L., Hawarden, B., Cervin, A., 2016. Diverse bacterial species in the microbiome of upper respiratory tract of patients with chronic rhinosinusitis and healthy participants. 2016. Abstract No 359. In: Australian Society for Microbiology Annual Scientific Meeting, https://doi.org/10.13140/RG.2.2.10059.39207. (Perth, Australia, 3–6 July 2016).

Singhal, N., Kumar, M., Kanaujia, P.K., Virdi, J.S., 2015. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Front. Microbiol. https://doi.org/10.3389/fmicb.2015.00791.

Toh, B.E.W., Zowawi, H.M., Krizova, L., Paterson, D.L., Kamolvit, W., Peleg, A.Y., Sidjabat, H., Nemec, A., Pflüger, V., Huber, C.A., 2015. Differentiation of acinetobacter genomic species 13BJ/14TU from acinetobacter haemolyticus by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDITOF MS). J. Clin. Microbiol. https://doi.org/10.1128/JCM.03468-14.

Zowawi, H.M., Sartor, A.L., Balkhy, H.H., Walsh, T.R., Johani, S.M.A., AlJindan, R.Y., Alfaresi, M., Ibrahim, E., Al-Jardani, A., Al-Abri, S., Al Salman, J., Dashti, A.A., Kutbi, A.H., Schlebusch, S., Sidjabat, H.E., Paterson, D.L., 2014. Molecular characterization of carbapenemase-producing escherichia coli and klebsiella pneumoniae in the countries of the gulf cooperation council: dominance of OXA-48 and NDM producers. Antimicrob. Agents Chemother. https://doi.org/10.1128/AAC.02050-13.

Zowawi, H.M., Sartor, A.L., Sidjabat, H.E., Balkhy, H.H., Walsh, T.R., Johani, S.M.A., Aljindan, R.Y., Alfaresi, M., Ibrahim, E., Al-Jardani, A., Al Salman, J., Dashti, A.A., Johani, K., Paterson, D.L., 2015. Molecular epidemiology of carbapenem-resistant acinetobacter baumannii isolates in the gulf Cooperation Council States: dominance of OXA-23-type producers. J. Clin. Microbiol. https://doi.org/10.1128/JCM.02784-14.