

Establishment of a standardized procedure for identification of microorganisms by MALDI-TOF MS

ASMS 2008, Poster # 260

Thomas Wenzel¹, Carrie L. Seachord², Thorsten Mieruch¹, Thomas W. Fuller³, Thomas Maier¹, Richard R. Drake³, Markus Kostrzewa¹
¹Bruker Daltonik GmbH, Leipzig, GERMANY
²Children's Hospital of The King's Daughters
³Eastern Virginia Medical School, Norfolk VA

Introduction

MALDI-TOF MS profiling has been shown to be a fast and reliable method for the classification and identification of microorganisms. Thereby, it is a promising tool for clinical diagnostic, environmental and taxonomical research, or food processing quality control. For routine utilization of the method, high quality databases as well as accurate and reproducible microorganism profile spectra measurements are mandatory. Therefore, standardized measurements on different mass spectrometers have to be established. Here, we present an optimization and standardization procedure using a dedicated standard that enables the tuning and precise calibration of MALDI-TOF mass spectrometers for microorganism identification using a dedicated reference database.

Methods

An adopted standard was developed containing an extract of *E. coli* and two additional proteins, covering the mass range from 4 to 17 kDa. Different MALDI-TOF instruments were used using this microorganism standard to reach minimum values for different parameters, i.e. mass accuracy, resolution, relative intensity, and signal intensity (counts

per shot) in repetitive measurements of all spots. Measurements were done with HCCA matrix in the positive linear mode. Calibration was performed based on a set of eight characteristic peaks of the complex standard. Further, 48 sample spots across the target were analyzed to control the success of parameter optimization. Subsequently, microorganisms from strain collections and clinical routine were identified by pattern matching against a reference database.

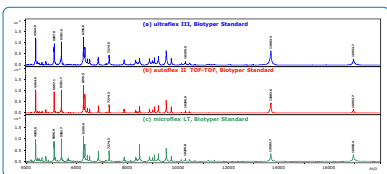


Fig. 1. MALDI-TOF MS spectra of a dedicated bacterial standard; measured on (a) ultraflex III, (b) autoflex II TOF/TOF and (c) microflex LT

| Protein | Molecular mass (kDa) | ultraflex III | | autoflex II TOF/TOF | | microflex LT | |
|---------|----------------------|----------------|------|---------------------|------|----------------|------|
| | | rel. intensity | res. | rel. intensity | res. | rel. intensity | res. |
| 4.36 | 4366.2 Da | 4549.6 | 406 | 4549.6 | 406 | 4057.0 | 416 |
| 4.522 | 5081.8 Da | 5087.0 | 444 | 5087.0 | 438 | 5086.9 | 407 |
| 7.234 | 7234.2 Da | 7234.5 | 504 | 7234.5 | 484 | 7234.7 | 484 |
| 10.336 | 10336.4 Da | 10336.5 | 710 | 10336.5 | 678 | 10336.5 | 710 |
| 14.29 | 14294.5 Da | 14294.5 | 938 | 14294.5 | 905 | 14294.5 | 892 |
| 16.618 | 16618.1 Da | 16618.6 | 1160 | 16618.6 | 1147 | 16618.6 | 1158 |
| 17.048 | 17048.1 Da | 17048.1 | 1160 | 17048.1 | 1147 | 17048.1 | 1157 |
| 17.048 | 17048.1 Da | 17048.1 | 1160 | 17048.1 | 1147 | 17048.1 | 1158 |

Table 1. Molecular masses and resolutions of eight characteristic peaks of the dedicated bacterial standard measured on ultraflex III, autoflex II TOF/TOF and microflex LT (spectra see Fig. 1).

Results

Several studies containing analyses of 48 samples across the target revealed mass accuracies of each protein of at least ±600 ppm. In all cases, the resolution of each protein was higher than 250 (examples, see Fig. 1 and Tab. 1). Next, mass spectra of two different microorganisms (i.e. *Proteus myxofaciens*, *Halomonas cupida*) were acquired on microflex LT and ultraflex III, respectively. The spectra were analyzed using MALDI Biotyper software and a reference database containing >1500 different microbial species. Both microorganisms could be unambiguously identified independent from the choice of mass spectrometer (Fig. 2).

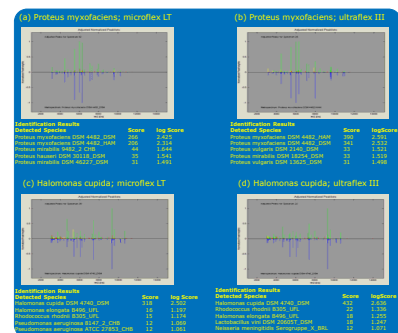


Fig. 2. Identification of *Proteus myxofaciens* (a, b) and *Halomonas cupida* (c, d) using the standard pattern matching method; spectra were acquired on microflex LT (a, c) or on ultraflex III (b, d).

Clinical isolates derived from Children's Hospital of The King's Daughters were measured on a fourth instrument (ultraflex III) optimized according to the described procedure and analyzed using MALDI Biotyper (examples in Table 2). A secure identification could be performed. For Achromobacter, only the genus could be determined (logScore lower than 2.0).

| Organism (best match) | logScore | Organism (second best match) | logScore |
|--------------------------------------|----------|------------------------------------|----------|
| <i>Proteus myxofaciens</i> DSM 18202 | 2.120 | <i>Proteus mirabilis</i> DSM 4181 | 1.816 |
| <i>Proteus mirabilis</i> DSM 4181 | 2.088 | <i>Proteus mirabilis</i> DSM 4181 | 2.078 |
| <i>Proteus mirabilis</i> DSM 17506 | 2.088 | <i>Proteus mirabilis</i> DSM 18202 | 1.981 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.078 | <i>Proteus mirabilis</i> DSM 18202 | 2.073 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.073 | <i>Proteus mirabilis</i> DSM 18202 | 2.068 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.068 | <i>Proteus mirabilis</i> DSM 18202 | 2.063 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.063 | <i>Proteus mirabilis</i> DSM 18202 | 2.058 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.058 | <i>Proteus mirabilis</i> DSM 18202 | 2.053 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.053 | <i>Proteus mirabilis</i> DSM 18202 | 2.048 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.048 | <i>Proteus mirabilis</i> DSM 18202 | 2.043 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.043 | <i>Proteus mirabilis</i> DSM 18202 | 2.038 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.038 | <i>Proteus mirabilis</i> DSM 18202 | 2.033 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.033 | <i>Proteus mirabilis</i> DSM 18202 | 2.028 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.028 | <i>Proteus mirabilis</i> DSM 18202 | 2.023 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.023 | <i>Proteus mirabilis</i> DSM 18202 | 2.018 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.018 | <i>Proteus mirabilis</i> DSM 18202 | 2.013 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.013 | <i>Proteus mirabilis</i> DSM 18202 | 2.008 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.008 | <i>Proteus mirabilis</i> DSM 18202 | 2.003 |
| <i>Proteus mirabilis</i> DSM 18202 | 2.003 | <i>Proteus mirabilis</i> DSM 18202 | 1.998 |
| <i>Proteus mirabilis</i> DSM 18202 | 1.998 | <i>Proteus mirabilis</i> DSM 18202 | 1.993 |
| <i>Proteus mirabilis</i> DSM 18202 | 1.993 | <i>Proteus mirabilis</i> DSM 18202 | 1.988 |
| <i>Proteus mirabilis</i> DSM 18202 | 1.988 | <i>Proteus mirabilis</i> DSM 18202 | 1.983 |
| <i>Proteus mirabilis</i> DSM 18202 | 1.983 | <i>Proteus mirabilis</i> DSM 18202 | 1.978 |
| <i>Proteus mirabilis</i> DSM 18202 | 1.978 | <i>Proteus mirabilis</i> DSM 18202 | 1.973 |
| <i>Proteus mirabilis</i> DSM 18202 | 1.973 | <i>Proteus mirabilis</i> DSM 18202 | 1.968 |
| <i>Proteus mirabilis</i> DSM 18202 | 1.968 | <i>Proteus mirabilis</i> DSM 18202 | 1.963 |
| <i>Proteus mirabilis</i> DSM 18202 | 1.963 | <i>Proteus mirabilis</i> DSM 18202 | 1.958 |
| <i>Proteus mirabilis</i> DSM 18202 | 1.958 | <i>Proteus mirabilis</i> DSM 18202 | 1.953 |
| <i>Proteus mirabilis</i> DSM 18202 | 1.953 | <i>Proteus mirabilis</i> DSM 18202 | 1.948 |
| <i>Proteus mirabilis</i> DSM 18202 | 1.948 | <i>Proteus mirabilis</i> DSM 18202 | 1.943 |
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| <i>Proteus mirabilis</i> DSM 18202 | 1.898 | <i>Proteus mirabilis</i> DSM 18202 | 1.893 |
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| <i>Proteus mirabilis</i> DSM 18202 | 1.888 | <i>Proteus mirabilis</i> DSM 18202 | 1.883 |
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