Single cell MALDI-TOF based identification of strains obtained from hospitalized patients


1 University of Applied Sciences Leiden, Leiden, Netherlands
2 Biosparq, Leiden, Netherlands
3 Maasstad hospital, Rotterdam, Netherlands

Objectives
Single cell MALDI-TOF is a newly developed platform, able to identify strains without previous culturing. This technique is capable of presenting bacterial cells individually to the ionization unit of the mass-spectrometer. Thus each cell produces a classifiable mass spectrum, enabling a quantitative analysis of a sample even if this contains a mix of microorganisms.

In this study we compared two typing methods to determine whether ten Serratia marcescens strains, collected from patients hospitalized at the Bronovo hospital in the Netherlands, were involved in an outbreak. The typing methods were: 1) AFLP, a frequently used molecular method for typing used by microbiology departments in hospitals, 2) The single cell MALDI-TOF technique (SC-MALDI-TOF). We determined whether the rapid SC-MALDI-TOF technique was able to type ten S. marcescens strains as accurate compared to the molecular technique AFLP.

Methods
In total, ten S. marcescens strains were cultured from infected wounds (n= 5), sputum (n=3), from a positive blood (n=1) culture and from a puncture of unknown source (n=1). They were recultured on bloodagar plates for identification with AFLP (Maasstad hospital, Rotterdam). For typing with SC-MALDI-TOF, strains were recultured on TSA plates. These plates were used to prepare a liquid culture (TSB) and cultured for 24 h at 37 °C. Bacterial pellets were created by centrifugation and washing (with sterile MQ-water) 250 μL of the dense liquid culture. These pellets were resuspended in 1 mL matrix that is especially composed for this type of MALDI. This mixture was subjected to the SC-MALDI-TOF.

Results
Data of both methods were analysed. AFLP with Bionumerics and SC-MALDI-TOF with Matlab. A dendrogram was calculated using the Pearson's correlation coefficient.

The AFLP data showed two identical strains, >90%. The (biological) similarities between the other 8 strains were below the 90% and were considered not to be involved in the outbreak. All the 10 strains were completely different from the control sample. The control sample was an ESBL 2149, Serratia fonticola.

The clustering results of the SC-MALDI-TOF were similar to the results of AFLP. The control sample in this case was a patient related E. coli strain.

Conclusion
NG-SC-MALDI-TOF is a new platform. The technique is promising and it is a rapid technique. Identification of strains can be done without culturing/isolation, so the “time-to-ID” is limited and it requires minimum lab infrastructure.

The golden standard method, AFLP and the SC-MALDI-TOF show, that the same two strains were considered identical. This is the first experiment that indicates that SC-MALDI-TOF is as accurate for typing when compared to AFLP.