Karlsruhe Institute of Technology

Institute of functional Interfaces

Impact of surface properties on biofilm formation of *Pseudomonas aeruginosa* - Mass profiling using MALDI-TOF/MS and multivariate data Analysis

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Introduction

It has been well-documented that when examined by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI TOF-MS), bacterial cells give unique spectra and distinctive marker molecules that enable characterization of the species.[1,2] The premise is that there are specific surface molecules which are differentially expressed in different cell types that can enable the characterization of cell genus, species, and even mutants.[3] This study applies this principle to determine the influence of surface properties on biofilm formation of a single species, *Pseudomonas aeruginosa* SG81.
The main objective in this study is the characterization of the 'mass fingerprint' given from the surface of these cells. The different molecules on the cell surface give reproducible mass-to-charge peaks or groups of peaks which have been found to be characteristic.

Material and Methods

Samples of the different surfaces (copper, stainless steel, PVC and glass) were covered with cultivation broth which was inoculated with *Pseudomonas aeruginosa*. The biofilm cultures of *Pseudomonas aeruginosa* have been grown for 24h using a protocol which is established in our working group.

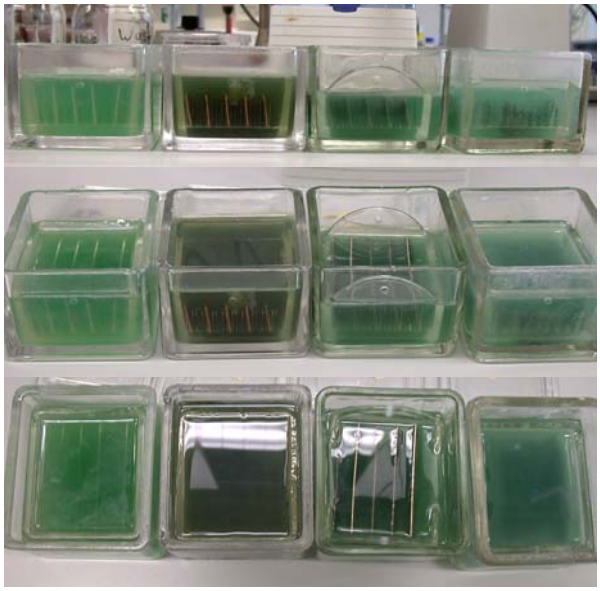

Fig 1: biofilm growth on different surfaces (from left: glass, copper, stainless steel, PVC)

The MALDI-Tof analysis were carried out on an Applied Biosystems 4800 MALDI-ToF instrument. The harvested cells of bacteria were spotted on a MALDI target. After drying, α-Cyano-4-hydroxycinnamic acid as matrix was overlaid.

MALDI-Tof - instrument settings:

Polarity:	linear positive mode
Accelerating Voltage:	20 kV
Grid Voltage:	91% of acc. Voltage
Delay Time:	200 ns
Mass range:	5 – 25 kDa
Low mass gate:	5000 Da
Fixed Laser intensity	

The data set was translated to JCAMP file format and loaded into Bruker OPUS™ Software (V 6.5). Prior to multivariate data analysis, the data was normalized by vector standardisation.
For Cluster Analysis (CA) and principle component analysis (PCA) 60 spectra were used.

Results

Figure 2 shows the MALDI-Tof mass spectra of biofilms of *Pseudomonas aeruginosa* grown on different surfaces. The visual comparison of the spectra shows that several peaks occur in all samples and some are different.

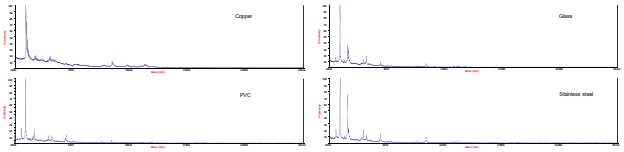

Fig 2: MALDI-ToF MS of biofilms on different surfaces

In Figure 2a plot of the principle component analysis is shown. It can be clearly seen that it is possible to differentiate between all the sample types.

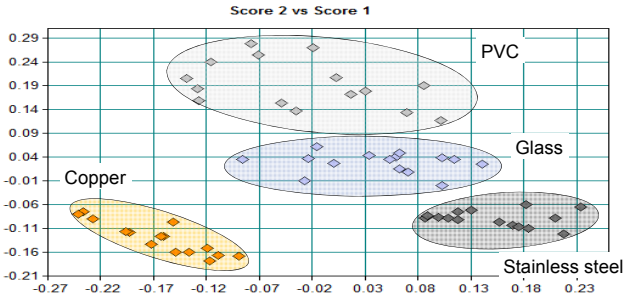

Fig 2a: PCA plot of the MALDI-ToF mass spectra from *P. aeruginosa*

These results were validated with a cluster analysis (Fig 2b). Again here it can be seen that a differentiation with a good heterogeneity is possible.

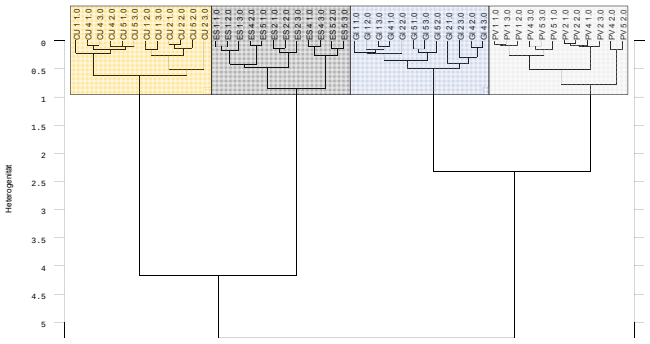

Fig 2b: CA plot of the MALDI-ToF mass spectra from *P. aeruginosa* (from left: copper (CU), stainless steel (ES), glass (GL), PVC (PV))

Conclusion

This method enables a rapid screening for the impact of surface properties on biofilm formation. To improve the method a modified MALDI target is in development which enables the direct mass spectrometric measurement on the sample coupon without harvesting the bacteria.

Literature

[1] Haag, A.M, et al., Rapid identification and speciation of Haemophilus bacteria by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, J. Mass Spectrom. 33, 750-756
[2] Rulle, V. B, et al., Rapid identification of environmental bacterial strains by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, Rapid Comm. In Mass Spec. 18: 2013-19
[3] Claydon, M.A., et al, The rapid identification of intact microorganisms using mass spectrometry, Nature Biotechnology 14, 1584-1586