

# Software routine allowing identification of extracellular biofilm proteins involved in cell adhesion based on data from QCM-D/MALDI experiments

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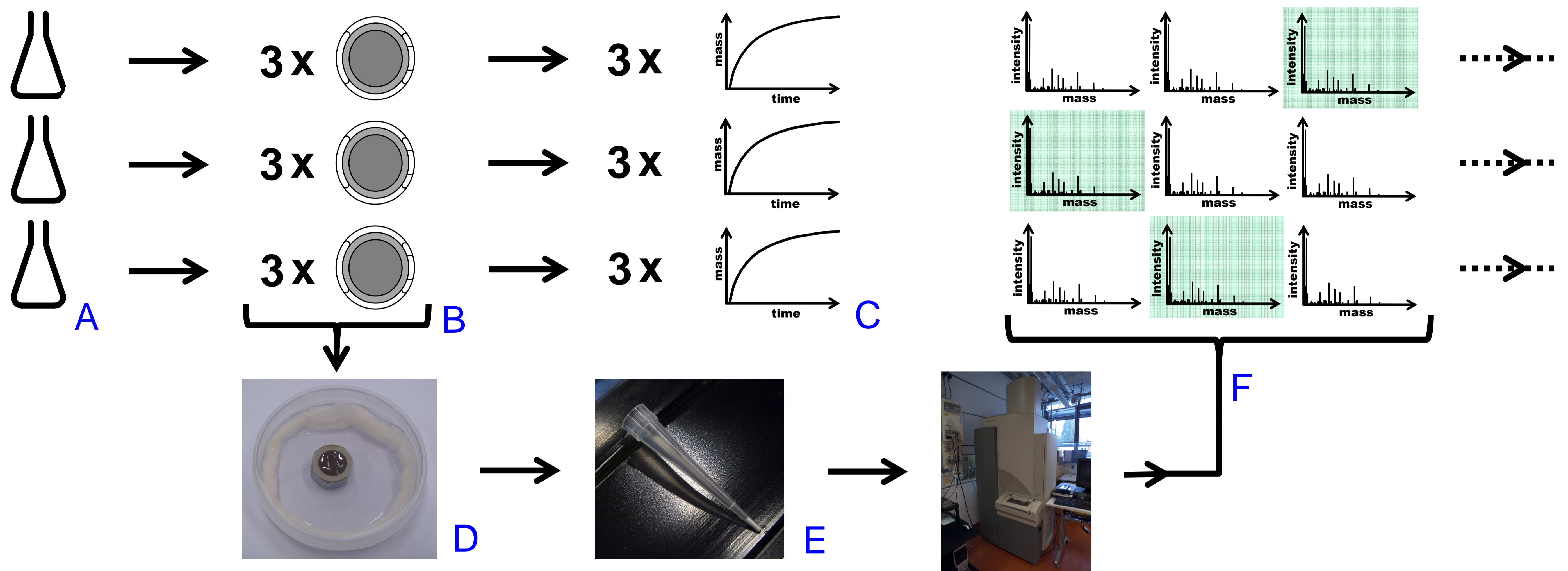
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## INTRODUCTION

The first step in the settlement of planktonic bacterial from a liquid medium onto a solid surface is the adsorption of substances contained in the medium onto the surface. The layer of adsorbed substances, e.g. proteins, polysaccharides, etc. is called conditioning film.

## AIM OF THE STUDY

The aim of the study was to determine which kind of proteins from cell free *Pseudomonas aeruginosa* PAO1 supernatant adsorb onto our model surface TiO<sub>2</sub>, which is often used as material for transplants.



## MATERIALS AND METHODS

Supernatant of one and three days cultured *P. aeruginosa* (A) was adsorbed onto quartz-crystal-microbalance (QCM-D) chips (B) and the adsorbed mass was monitored (C).

The adsorbed layer was digested by trypsin (D) and concentrated using chromatographic material (E) contained in a pipette tip (ZipTip®).

After concentration the sample was spotted onto a MALDI target plate and mass spectra of the contained peptides (F) were generated.

Using algorithms implemented in PHP the peak lists generated from the mass spectra were analysed and matched against theoretical tryptical digests of all proteins encoded in the genome of PAO1 generated by scripts and stored in an xml based data base system (G).

From the matched proteins identified in three independent experiments of different bacterial cultures (H), selected peaks were further analysed using MALDI-ToF/MSMS (I).

The obtained MS-MS spectra were matched against theoretical fragmentations of the peptide sequences (J) to ensure the identity of the protein. A scoring system (K) was used to sort the found proteins by their probability of identification.

## RESULTS AND DISCUSSION

We were able to identify several intracellular as well as extracellular proteins including adhesion proteins to be involved in conditioning film formation in all three supernatants of *P. aeruginosa* PAO1 by MALDI-ToF/MS and to ensure their correct identification by MALDI-ToF/MSMS.

## CONCLUSIONS

As demonstrated by the experiments the coupling of QCM-D with high resolution mass spectrometry (MALDI-ToF/MS) is a powerful tool for analyzing conditioning films of supernatant of *P. aeruginosa*.

