

## AP MALDI-MS<sup>n</sup> for rapid characterization of cyclic lipopeptides

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### Introduction

Cyclic lipopeptides belong to a prominent class of bioactive peptides isolated from bacteria. These compounds such as surfactin, fengycin, mycosubtilin, or bacillomycin, isolated from *Bacillus subtilis*<sup>1</sup> are amphiphilic, membrane active surfactants and peptide antibiotics with specific antimicrobial, antiviral and antitumor potential. Depending on the bacteria strain and nutritional and environmental conditions, variations in peptide ring size, sequence and in chain length of the fatty acid chain occur.

*B. pumilus* (strain IIBK9)<sup>2</sup> showed extensive activation against the apple scab pathogen *Venturia inaequalis*. With butanolic extracts of *B. pumilus* culture supernatants the cyclic lipopeptide pumilacidin was identified.

For structure elucidation of individual components even from crude extracts an atmospheric pressure MALDI quadrupole ion trap mass analyzer is well suited, since it combines the ruggedness of MALDI with the possibility of sequential fragmentation (MS<sup>n</sup>).

### Methods

An Agilent ion trap mass spectrometer (SL 1100 Series) equipped with an AP-MALDI source was used for analysis. 1  $\mu$ L of sample solution was mixed with 1  $\mu$ L of matrix solution. 1  $\mu$ L was spotted on a "dull finish" target. (HCCA saturated in acetonitril / water 1:1 or 2,5-DHB, 50 g/L dissolved in acetonitril / water 1:2) and dried at room temperature. All mass spectra were accumulated over 2 minutes. In the positive ion mode a potential of -3 kV was applied between sample target and inlet capillary. A stream of dry nitrogen was applied and the heating gas temperature was set to 300° C. For MS<sup>2</sup> and MS<sup>3</sup> the fragmentation amplitude was set between 1.0 and 1.8 V.

Surfactins were produced by *B. Subtilis* OKB 105, pumilacidins by *B. Pumilus* IIBK 9 and analyzed as butanol extracts. Surfactin isoforms were HCl-precipitated, MeOH-solubilized and isolated by HPLC. All samples are dissolved in acetonitril / water 1:2 with a concentration of 0.1 to 1 mg/ml.

### Results

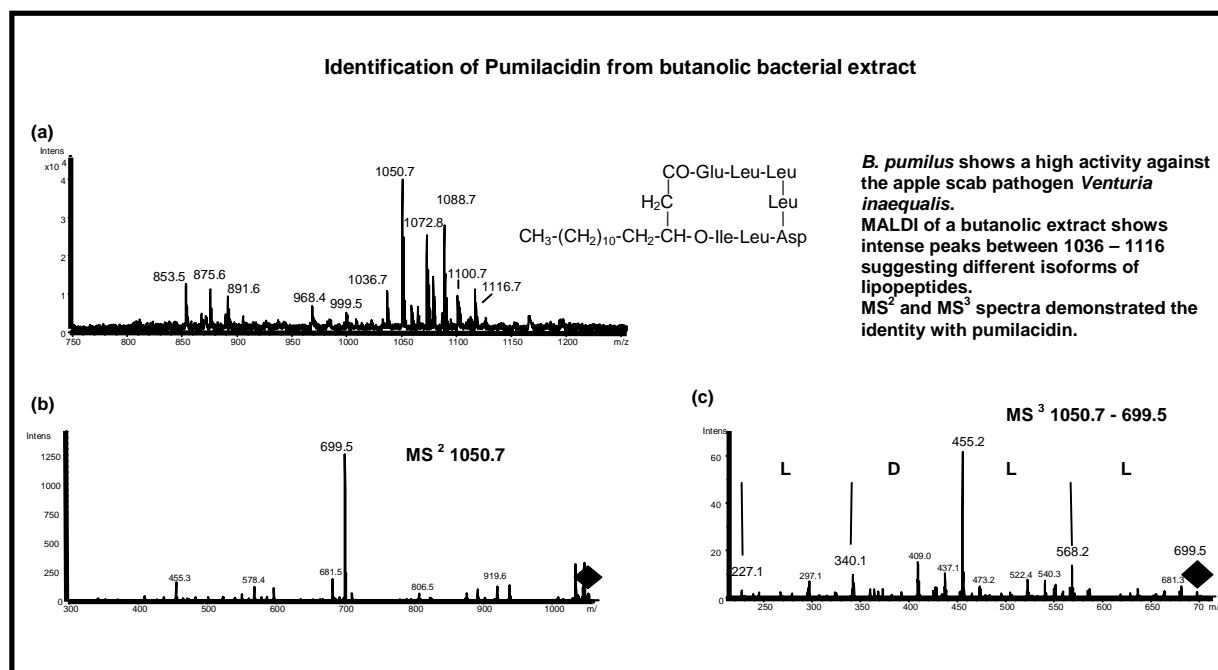
The compounds under investigation consist of a heptapeptide bound to a fatty acid via an ester linkage at the C-terminus and a peptide bond at the N-terminus. Surfactin compounds contain 7 amino acids, including a Glu and an Asp residue. The amino acids can vary at certain positions and the  $\beta$ -hydroxy fatty acid has a chain length of 13-15 carbon atoms. Moreover due to sample treatment with methanol, the carboxylic acid groups of the acidic amino acids can be fully or partially methylated. In MS/MS spectra, all investigated cyclic lipopeptides show the protonated peptide chain as base peak from concurrent cleavage of the ester bond and the peptide bond. MS<sup>3</sup> spectra of the

released peptide deliver the peptide sequence and the degree and position of methylation. The length of the fatty acid chain can be determined. The described fragmentation pattern can be used for a rapid screening for the presence of cyclic lipopeptides in raw bacterial extracts. This is shown for surfactins and pumilacidins.

For analyses HCCA and 2,5-DHB were tested as matrices. Compared to vacuum MALDI (on a Voyager STR, Applied Biosystems) HCCA spectra obtained with the AP MALDI instrument showed much higher abundant  $[M+H]^+$ - than  $[M+Na]^+$ -species. For 2,5-DHB spectra from the crystalline rim were comparable for both methods. HCCA was chosen as matrix for all analyses shown because of its higher reproducibility from shot to shot.

### Conclusion

The results show that multiple MS/MS steps are necessary to unambiguously identify cyclic lipopeptides. The advantage of using AP-MALDI on an ion trap is obvious when analyzing crude extracts. For rapid screening of these extracts no time consuming HPLC separations are necessary.



<sup>1</sup> Martin Kowall, Joachim Vater, Britta Kluge, Torsten Stein, Peter Franke, and Dieter Ziessow JOURNAL OF COLLOID AND INTERFACE SCIENCE **204**, 1-8 (1998).

<sup>2</sup> Georg Auling, Torsten Stein, unpublished