

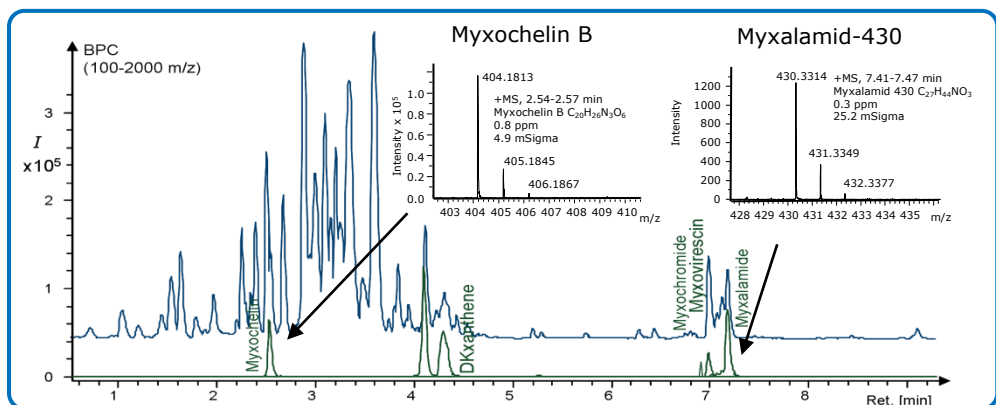
# Uncovering hidden secondary metabolites from myxobacteria using high-resolution chromatography and mass spectrometry

Daniel Krug<sup>1</sup>, Gabriela Zurek<sup>2</sup>, Niña Cortina<sup>1</sup>, Aiko Barsch<sup>2</sup>, Sandy Yates<sup>3</sup> and Rolf Müller<sup>1</sup>

<sup>1</sup>Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS) and Universität des Saarlandes, Saarbrücken, Germany; <sup>2</sup>Bruker Daltonik, Bremen, Germany; <sup>3</sup>BrukerDaltonics, Fremont, CA

## Introduction

Secondary metabolites from myxobacteria are an important source of biologically active natural products with considerable promise for human therapy. The large genetic potential of many myxobacterial species for secondary metabolite biosynthesis has hardly been accessed to date [1,2]. More than 100 core structures from myxobacteria have been characterized. However, the number of compound classes reported from individual strains clearly falls short of the genetic capabilities. Thus, the discovery of novel secondary metabolites which are presumably only produced in small quantities from genetically proficient myxobacterial producers currently constitutes a substantial bottleneck in the discovery process of novel lead structures.[3,4]



**Fig. 1:** High-resolution LC-MS analysis of an *M. xanthus* DK1622 extract. Targeted screening reveals known compounds with high confidence, even if they constitute only minor components in the complex mixture. Example mass spectra of two known metabolites are presented. Signals potentially representing novel metabolites span a high dynamic range.

## Methods

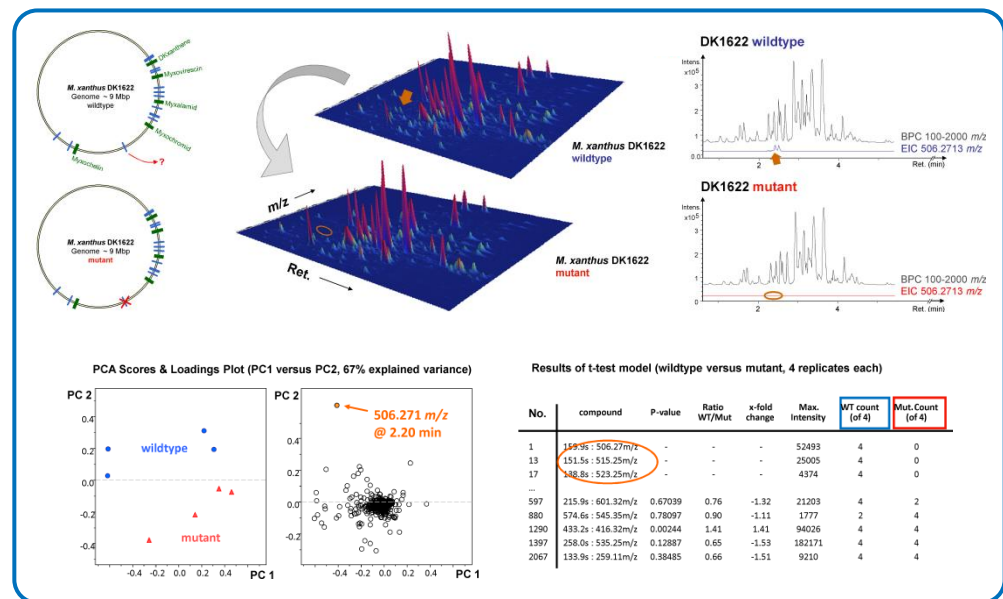
- *Myxococcus xanthus* extracts were separated under UPLC conditions using a RP C18 column (50 x 2 mm, 1.7 μm particle size).
- ESI-MS measurements were performed using positive ionization on the maXis UHR-TOF (Bruker Daltonik; *m/z* range: 100-2000 *m/z*, repetition rate: 3 Hz).
- Targeted screening was carried out using precise EICs and isotope pattern evaluation via SigmaFit™ with TargetAnalysis™ (Bruker Daltonik).
- Statistical interpretation using PCA or Student's t-test including all data preprocessing was performed with ProfileAnalysis™ 2.0 (Bruker Daltonik).

**Fig. 2:** Target screening results for an *M. xanthus* extract.

## Results

The possibility to apply both targeted queries and unbiased statistical treatment to the same dataset is a major advantage of high-resolution LC-MS measurements in metabolomics. Extracts derived from cultivations of *Myxococcus xanthus* DK1622 were subjected to targeted analysis, confirming the presence of 6 known secondary metabolite classes. Fig. 1 depicts the basepeak chromatogram of an *M. xanthus* extract with example mass spectra of a high and low abundant metabolite. Mass deviations significantly below 1 ppm and low mSigma-Fit values (< 30), in combination with retention time matching, enable the library-based identification of known compounds with high confidence (Fig. 2). Screening results are scored according to the observed

retention time, mass accuracy and mSigma-value. Multiple detections refer to isomers of the same metabolite e.g. DKxanthen-518. The secondary metabolomes of DK1622 mutants obtained by the inactivation of biosynthetic pathways were compared to wildtype metabolite profiles, in order to identify molecular features missing in the mutant extracts. Such experiments enable the discovery of previously unrecognized secondary metabolites and allow at the same time their assignment to a specific biosynthetic pathway encoded in the myxobacterial genome (Fig. 3). The evaluation with principle component analysis (PCA) and t-test highlighted 506.2713 *m/z*@2.2min as novel metabolite, with 515.250 *m/z* and 523.250 *m/z* being putative derivatives.



**Fig. 3:** Identification of a novel secondary metabolite (506.2713 *m/z* @ 2.20 min) from *M. xanthus* DK1622 by comprehensive analysis of metabolite profiles from the wildtype strain and a genetically engineered "knockout" mutant. Such novel compounds may be represented by subtle signals relative to the abundance of known metabolites and matrix substances (orange arrows). *m/z* range: 200-1200 *m/z*; Ret. time 1.40 – 4.40 min.

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## Summary

The hyphenation of high resolution chromatography to high-resolution mass spectrometry and statistical data evaluation, can significantly facilitate the process of uncovering "hidden" bacterial secondary metabolomes. In the present work, *M. xanthus* DK1622 could be shown to produce several novel secondary metabolites, in addition to the previously known natural product classes. Future efforts using the analytical approach presented here aim to correlate novel compounds to additional uncharacterized biosynthetic gene clusters found in the genomes of many other myxobacteria.

## References

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## Conclusions

- Efficient detection of molecular features in high-resolution LC-MS datasets is a crucial prerequisite for the subsequent mining of complex myxobacterial secondary metabolomes using statistical tools.
- Data acquired on the UPLC-ESI UHR-TOF platform are well suited for mining the secondary metabolome due to high mass accuracy and resolution combined with a fast scan rate.