# Magnetic resonance mass spectrometry profiling of myxobacterial extracts – higher resolution, deeper insights?

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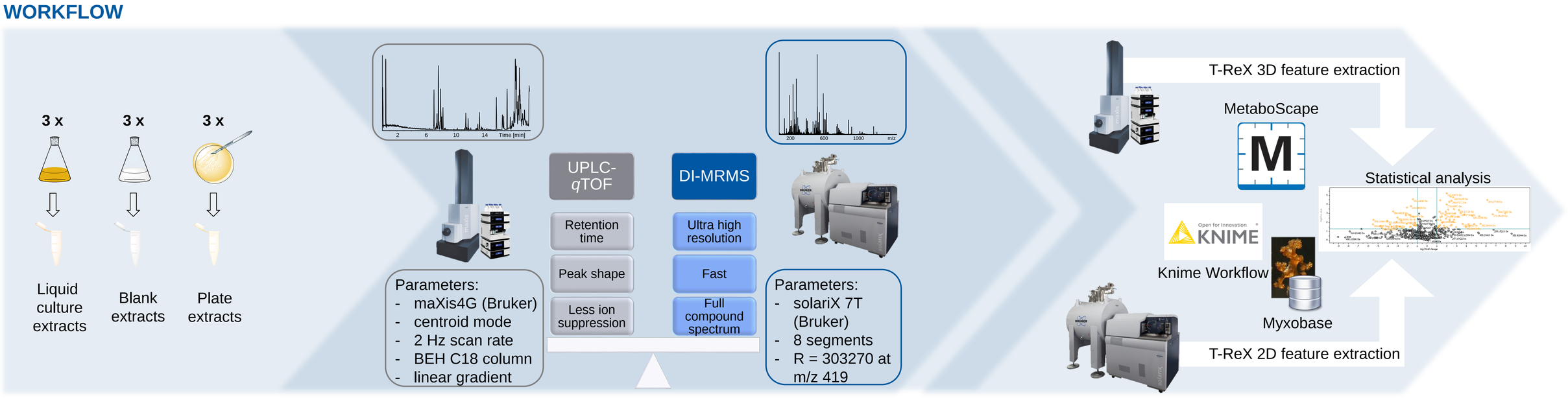
## INTRODUCTION

Currently, the discovery of myxobacterial secondary metabolites is based mostly on crude extract screening approaches using UPLC-*hr*MS and subsequent dereplication. [1] *Myxococcus xanthus* DK1622 is a well-described myxobacterial model organism where several different compound families have already been isolated from and correlated to biosynthetic gene clusters. [2,3] Nevertheless, bioinformatic prediction shows an even bigger biosynthetic potential of this strain on the genetic level.

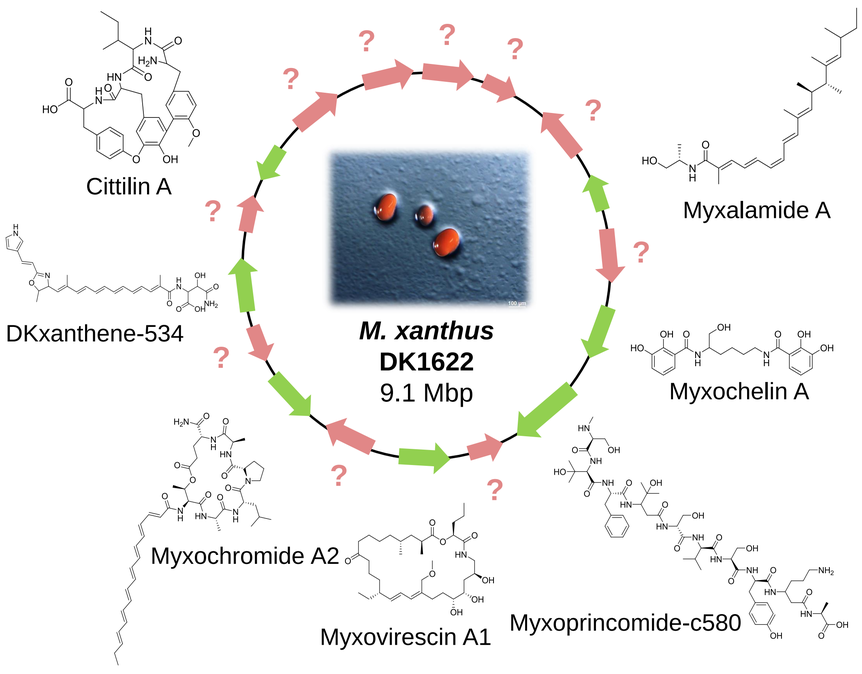
To create an in-depth insight into the secondary metabolome of *M. xanthus* DK1622 and to evaluate the potential of DI-MRMS for expanding the detection range of myxobacterial metabolites, we compared MS data of DK1622 after cultivation in liquid culture and on plate using our standard UPLC-*q*TOF platform as well as DI-MRMS. Here we present the results from this comparison and discuss implications for future natural product discovery.

## WORKFLOW

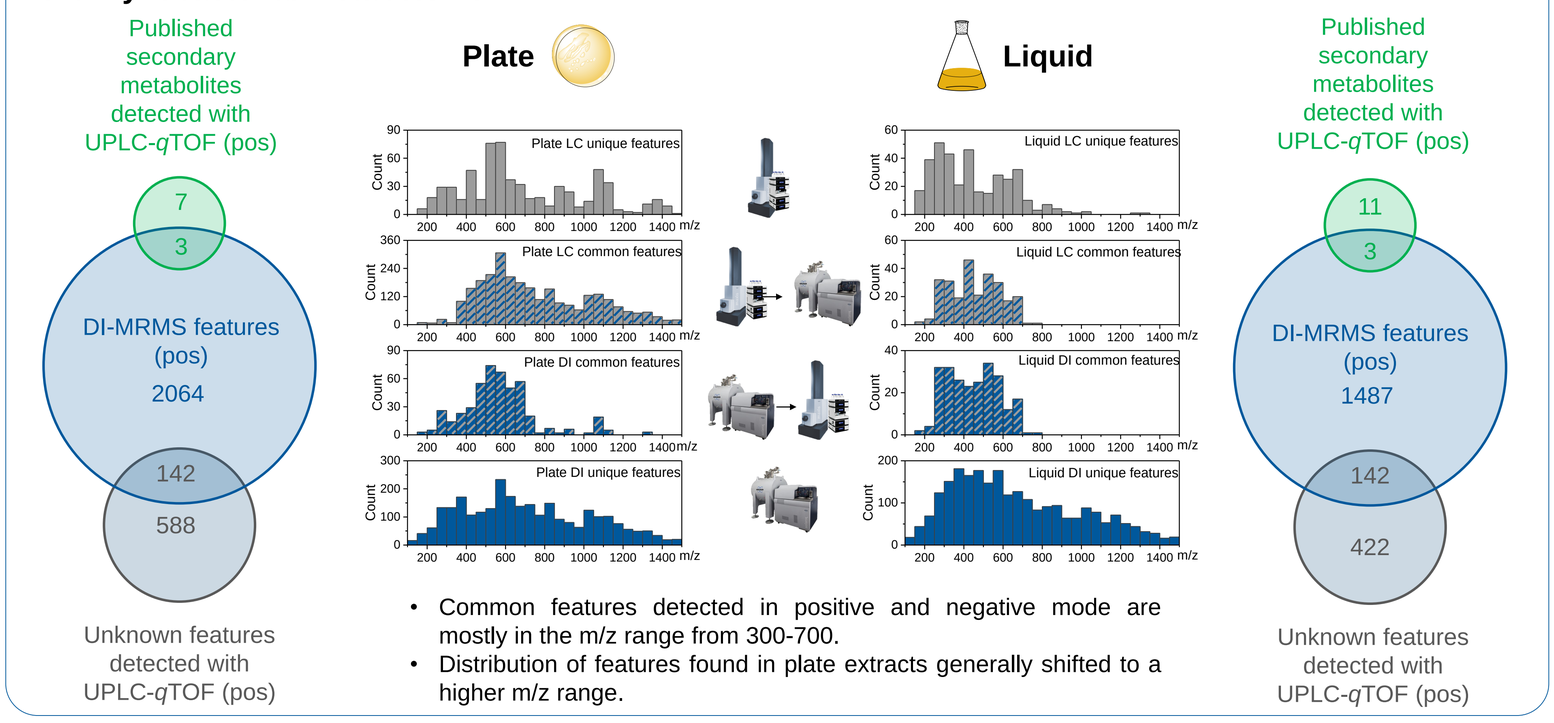



## RESULTS

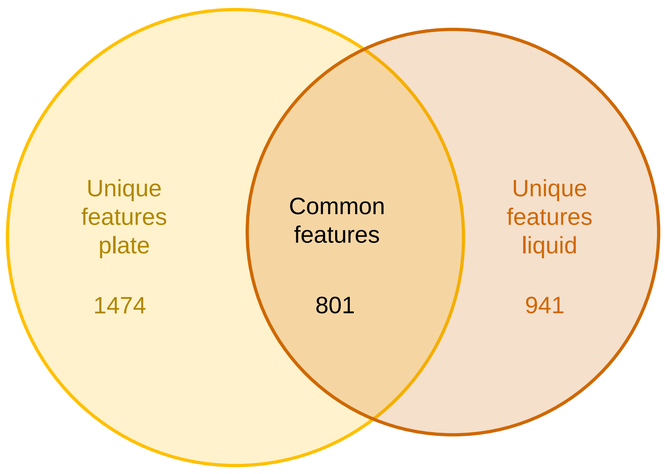
### *Myxococcus xanthus* DK1622 secondary metabolome




### DI-MRMS and UPLC-*q*TOF MS as complementary methods enabeling a comprehensive overview of the myxobacterial metabolome

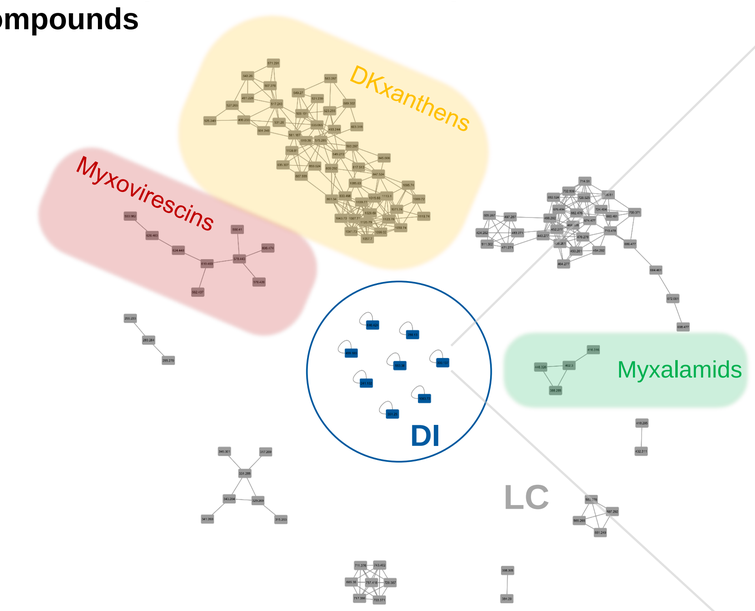



### Metabolite profiles observed on plate strongly differ from liquid cultivation



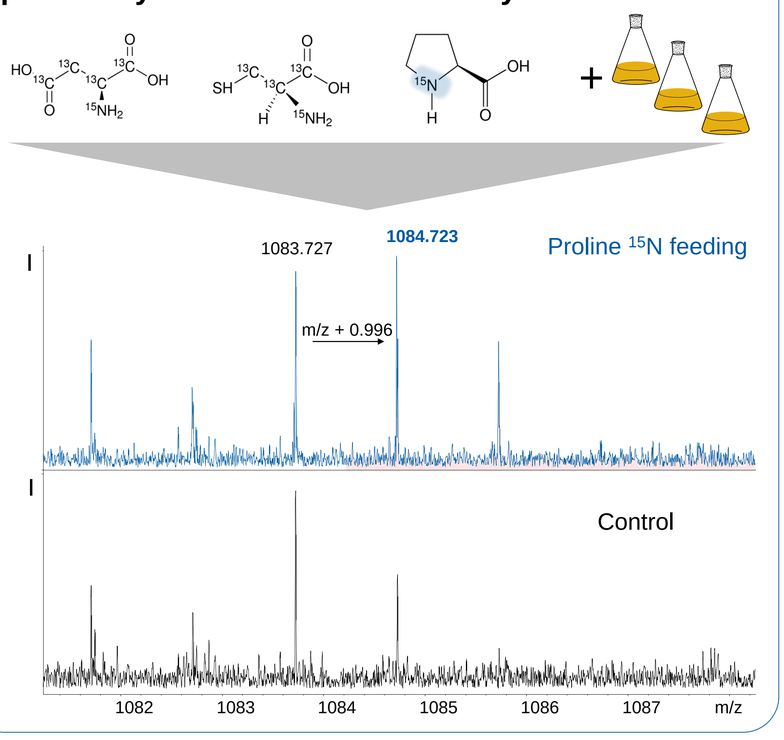

When cultivated on plate and in liquid culture, about 1/4 of the detectable features can be found in both extracts while the rest are exclusive to each cultivation method.

### DI-MRMS expands the chemical space of detectable compounds




Spectral networking of MS² features detected with UPLC-*q*TOF ( ) and eight most intense features found uniquely with DI-MRMS ( ) shows chemical novelty of DI-MRMS features.

### Feeding with isotope-labeled amino acids reveals previously undiscovered secondary metabolites




## Conclusion

- Statistical analysis of the *M. xanthus* DK1622 metabolome revealed major differences between DI-MRMS and UPLC-*q*TOF data sets.
- The number of features detected with MRMS is intriguingly high and they also seem to cover a different chemical space compared to UPLC-*q*TOF analysis.
- Cultivation of the strains in liquid and on solid medium shows a partly overlapping metabolome as well as a large number of features unique to each cultivation method.
- Feeding experiments can be used to gain further knowledge about the structure and biosynthesis of the newly discovered features.
- Although standardized and simplified workflows are essential for large scale screening and dereplication, our results strongly suggest implementing complementary methods and conditions to these workflows in order to drastically increase the scope of such analyses.

## References

[1] Krug, D., Müller, R., Nat. Prod. Rep. **2014,** 31 (6), 768–783.

[2] Cortina N.S., Krug D. *et al.*, Angew. Chem. Int. Ed. Engl. **2012**, 51 (3), 811–816.

[3] M. Hoffmann, D. Auerbach *et al.*, ACS Chem. Biol. **2018**, 13, 273-280.