

Characterization of myxobacterial secondary metabolite profiles by high resolution LC-ESI-TOF-MS data combined with statistical and targeted data evaluation

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Introduction

Bacteria producing secondary metabolites with biological activities are important sources of potential new drugs [1]. An example for natural products discovered from myxobacteria are the Epothilones being promising anti-cancer agents. Chromatographic separation of bacterial culture extracts combined with ESI-TOF-MS analysis is a powerful tool for the comparison of production patterns and the identification of compounds via their molecular formula. Manual data examination is time consuming due to high complexity of samples. Statistical methods can facilitate the extraction of relevant information. We describe here a metabolomics based approach for extracts from myxobacteria using ESI-TOF-MS data, principal component analysis (PCA) and a subsequent identification via the molecular formula from accurate mass data.

[1] Gerth K. J. Biotechnol., 106(2-3), 233-253 (2003)

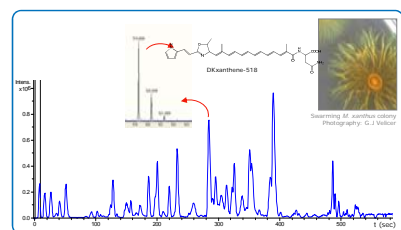


Fig. 1 UPLC chromatogram and high-resolution ESI-TOF-MS measurement from a *Myxococcus xanthus* extract.

Methods

Bacteria were fermented in a 50 mL scale using complex media. Cultures contained XAD 16 resin as absorber. Cells and absorber resin were harvested by centrifugation, extracted with methanol and diluted in acetonitrile/water prior to injection. LC-MS measurements were performed using a micrOTOF™ orthogonal ESI-TOF mass spectrometer (Bruker Daltonik, Bremen) coupled to a UPLC system (Waters, Milford) equipped with photodiode array detector. The separation was carried out with a binary acetonitrile-water gradient with 0.1 % formic acid and a reversed phase column (50x2 mm, 1.7 μm particles) (Fig. 1). Data was acquired in ESI positive and negative mode (Scan range: 150-1200 m/z). Sodium formate solution was injected as external calibration standard. Data was evaluated using ProfileAnalysis™ software for principal component analysis and Generate Molecular Formula™ (GMF) with SigmaFit [2,3].

[2] Krug D. LC-GC Europe, March 2007 Applications Book, 41-42 (2007)
[3] Ojanpera S. Rapid Commun. Mass Spectrom., 20 (7), 1161-1167 (2006)

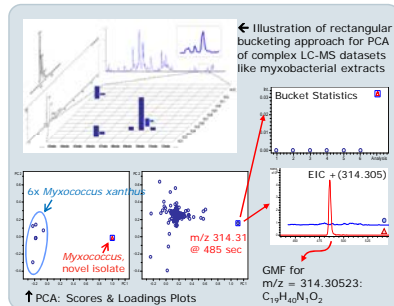


Fig. 2 PCA analysis of extracts from 6 *M. xanthus* strains plus one novel *Myxococcus* isolate facilitates comparative metabolite profiling and identification of compounds via their molecular formula.

Results

LC-MS data was prepared for PCA analysis in ProfileAnalysis using a bucketing approach (Fig. 2). The LC-MS was integrated from 0.2-7.5 min and 200-1200 m/z in time- and m/z-buckets of 0.2 min and 1 m/z. Each data set was normalized to the total intensity in an analysis. The variability of the instrumental analysis was regularly checked by two quality control (QC) samples. The first QC samples consisted of a standard mixture of compounds unrelated to the real samples in order to control retention time and mass calibration stability. The second QC sample was one of the bacterial extracts injected several times randomly within the sequence of measurements.

In order to assess the variation in the fermentation experiment, five closely related strains producing the same predominant compounds were fermented with six biological replicates each. The replicate extracts of the five strains revealed considerable deviation within replicate fermentations, but still

resulted in formation of groups indicative of subtle systematic differences between their metabolite profiles (Fig. 3). Compounds representing such differences were identified using the accurate mass data. Clustering of these analyses occurred in part due to a high degree of quantitative variation regarding production of DKxanthenes, a recently characterized family of compounds which are important for the complex lifecycle of *M. xanthus* [4].

In a second sequence, 110 bacterial isolates of the species *M. xanthus* covering largely variant spatial ranges (from centimeter scale to locations worldwide [5]) were cultured and analyzed under the above described conditions. Comparison of subsets of these extracts with samples from novel uncharacterized strains in a PCA model (Fig. 2) serves to quickly obtain an overview of their metabolite profiles and identify novel compounds.

[4] Meiser P. Proc. Natl. Acad. Sci. USA, 103 (50), 19128-19133 (2006)
[5] Vos M. Velicer G.J. Appl Environ Microbiol., 72(5), 3615-25 (2006)

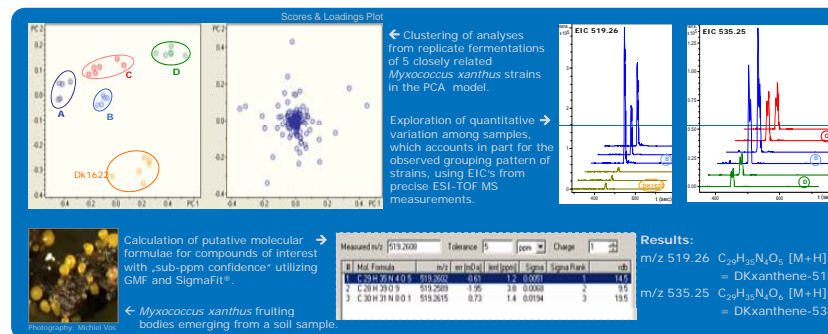


Fig. 3 PCA for extracts from 5 closely related *M. xanthus* strains reveals systematic differences between their metabolite profiles. UPLC-ESI-TOF MS data is used for GMF and allows to evaluate also quantitative variation among samples.

Summary

The exploration of myxobacterial metabolite profiles by LC-MS screening for the presence of new natural products is described. Extracts from fermentations of *Myxococcus* strains are analysed by UPLC-coupled ESI-TOF mass spectrometry and the obtained data are processed using principal component analysis (PCA). The generation of molecular formulae from accurate mass measurements facilitates rapid compound identification. PCA is used to find trends in the different secondary metabolite production patterns from *Myxococcus* strains which may reveal correlations regarding not only the geographical distribution of the samples, but also biological properties of the strains.

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Conclusions

- The differences in secondary metabolite production patterns of myxobacterial strains can be extracted by PCA, even considering significant biological variation within replicate fermentations.
- Accurate mass and isotopic pattern data from ESI-TOF-MS are the key to enable the identification of compounds according to their molecular formula.
- We project an application to the discovery of novel compounds produced by myxobacteria, by simultaneously enabling rapid de-replication and definition of putative target compounds for further studies.