Metabolome analysis - mass spectrometry and microbial primary metabolites

While metabolite profiling has been carried out for decades, the scope for metabolite analysis has recently been broadened to aim at all metabolites in a living organism – also referred to as the metabolome. This is a great challenge, which requires versatile analytical technologies that are highly sensitive and specific, and to undertake this challenge mass spectrometry (MS) is among the best candidates. Along with analysis of the metabolome, the research area of metabolomics combines metabolite profiles, data mining and biochemistry and aims at understanding the interplay between metabolites. In this thesis, different topics have been addressed and discussed with the aim of advancing metabolomics to explore the concept in a physiological context. The metabolome comprises a wide variety of chemical compounds that act differently upon sample preparation, and therefore sample preparation is critical for metabolome analysis. The three major steps in sample preparation for metabolite analysis are sampling, extraction, and concentration. These three steps were evaluated for the yeast Saccharomyces cerevisiae with primary focus on analysis of a large number of metabolites by one method. The results highlighted that there were discrepancies between different methods. To increase the throughput of cultivation, S. cerevisiae was grown in microtiter plates (MTPs), and the growth was found to be comparable with cultivations in shake flasks. The carbon source was either glucose, galactose or ethanol, and metabolic footprinting by mass spectrometry was used to study the influence of carbon source on the extracellular metabolites. The results showed that footprints clustered according to the carbon source. Advances in technologies for analytical chemistry have mediated increased amounts of data generated in high resolution. One major limitation though is the digestion of data converting the information into a format that can be interpreted in a biological context and take metabolomics beyond the principle of guilt-by-association. To analyze the data there is a general need for databases that contain metabolite specific information, which will speed up the identification of profiled metabolites. To address the capabilities of electrospray ionization (ESI)-MS in detecting the metabolome of S. cerevisiae, the in silico metabolome of this organism was used as a template to present a theoretical metabolome. This showed that in combination with the specificity of MS up to 84% of the metabolites can be identified in a high-accuracy ESI-spectrum. A total of 66 metabolites were systematically analyzed by positive and negative ESI-MS/MS with the aim of initiating a spectral library for ESI of microbial metabolites. This systematic analysis gave insight into the ionization and fragmentation characteristics of the different metabolites. With this insight, a small study of metabolic footprinting with ESI-MS demonstrated that biological information can be extracted from footprinting spectra. Statistical analysis of the footprinting data revealed discriminating ions, which could be assigned using the in silico metabolome. By this approach metabolic footprinting can advance from a classification method that is used to derive biological information based on guilt-by-association, to a tool for extraction of metabolic differences, which can guide new targeted biological experiments.

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