Untargeted metabolomics based on segmented flow ms.

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Abstract

Dimorphic yeast called Hyphozyma roseonigra is employed as a biocatalyst to change the plant triterpenoid sclareol into ambrosial. The latter is a step in the synthesis of ambrafuran, a highly valuable chemical used in the fragrance sector. Unfortunately, little is understood about the microorganism's fundamental biochemistry. In this study, the integration of multi-platform metabolomics was employed to gain a deeper biochemical understanding of H roseonigra. Using untargeted LC-MS and NMR techniques, the focus on metabolomics alterations during growth and development was achieved. Every second day, cells from the early, log, and stationary phases were extracted using extraction techniques that were platform-compatible from cell suspensions that had been cultivated in batch culture. The detected selective ions annotated from the endo and exometabolomes (metabo-fingerprinting and metabo-footprinting) were found to fall primarily in the primary metabolism class after chemometric analysis of LC-MS and NMR data gathered from both intra and extracellular extracts. Insights into the active metabolic pathways during growth and development were gained with the help of pathway mapping and feature-based network correlation analysis, which did not identify terpene production. This study sheds new light on the fundamental metabolic skills of H. roseonigra and suggests that the detoxification of a hydrophobic xenobiotic molecule occurs during the metabolism of sclareol.

Keywords: Biocatalysts, Filamentous yeast, Hyphozyma roseonigra, Liquid chromatography, Mass spectrometry, Metabolomics, Nuclear magnetic resonance, Pathway mapping.

Introduction

Central nervous system (CNS) illnesses, such Alzheimer's and Parkinson's disease, are on the rise as the population ages. More study is required to identify innovative pharmacological targets and treatments because the underlying pathogenic mechanisms of many neurological illnesses are still poorly understood. Studies on brain metabolomics may shed light on the causes of certain CNS illnesses. In such small sample sizes, it is particularly harder to identify low metabolite concentrations [1]. Due to the increased demand for sensitive procedures, appropriate sample preparation and preconcentration steps are required. Solid-phase micro extraction (SPME) and liquid-phase micro extraction (LPME) are two new micro extraction techniques for sample pre-treatment. When these methods are coupled online with miniature separation methods, sample throughput can be increased. Small-volume sample analysis is appropriate for capillary electrophoresis (CE), chip-based systems, and Nano and micro-liquid chromatography (LC) [2]. Gas chromatography is another frequently used technique in metabolomics for small sample sizes (GC). Along with LC, on-line coupling with extraction methods is also conceivable with GC, and on-line derivatization is also a possibility. More specifically, derivatization with isotopes is gaining popularity when mass spectrometry (MS) is used as a detection technique to account for metabolite variation brought on by volume mismatch, sample loss, and matrix effects. Because of its superior detection sensitivity compared to nuclear magnetic resonance (NMR), which is also frequently used in metabolomics, MS is in fact a very ideal detection technology for brain metabolomics. Sensitive analyses of metabolites in brain samples have also been performed using electrochemical and fluorescence-based detection methods. However, fluorescence detection frequently necessitates a derivatization step in order to incorporate a fluorophore, in contrast to electrochemical detection, which is restricted to electroactive compounds. Therefore, these detection methods are better suitable for tailored metabolomics studies (when combined with LC or CE) [3].

The difficulties in analysing various small-volume matrices used in in-vivo brain metabolomics are the main topic of this paper. The difficulties in linking miniaturised separation techniques to MS detection are also taken into consideration, along with the difficulties that arise during sampling, sample preparation, and preconcentration. Because of this, unique miniaturised workflows are highlighted while typical brain metabolomics workflows are left out of the discussion. The debate also excludes in-vitro and ex-vivo samples such cell lines, organoids, and tissue samples. The following recent

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reviews provide more details about the difficulties associated with sample collection, preparation, analysis, and data normalisation for tissue samples as well as for single cells [4].

ECF and CSF, the brain's two fluids, are of particular importance in neurological and behavioural problems. Clinical neurology has found CSF collection, frequently carried out via lumbar puncture in the brain subarachnoid space, to be an essential tool. CSF is still a poorly represented matrix in animal model studies due to inadequate accessibility, intrusive sampling, and small sample sizes. The existence of non-specific binding of polar and/or bigger molecules to the sample catheter presents additional difficulties associated with lumbar puncture. Using catheterization of the subarachnoid space, a minimally invasive technique was suggested to enable serial CSF collection in conscious minipigs. In order to increase the amount and quality of the CSF samples obtained in post-mortem mice, Sakic described a modified cisternal puncture technique [5].

Conclusion

The study of brain metabolomics has become increasingly popular in recent years. By minimising the possibility of metabolite degradation that occurs when analysing post-mortem brain samples, in-vivo metabolomics profiling offers crucial real-time information about the biological status of the brain. However, it is still discovered that in-vivo neuromessenger analysis presents a significant analytical difficulty, mostly because brain fluids are difficult to obtain, sampling is quite invasive, and neuromessenger concentrations vary widely and rapidly.

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