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Review

# Recent developments and applications of metabolomics in microbiological investigations

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ABSTRACT

Metabolomics is the comprehensive (qualitative and quantitative) analysis of all metabolites within an organism or a biological system. By studying endogenous metabolites produced from an organism in or around growing biosystems or cells at a given time during growth or the production cycle, metabolomics can potentially provide critical information to help understand the changes occurring in the relevant metabolic pathways. The emerging field of microbial metabolomics has received much attention in recent years, because it not only offers a broad picture of the altered pathways, but also elaborates the mechanisms of the interplay between microbe and host. This article reviews major issues in microbial metabolomics, and gives a comprehensive, critical overview of the current state of the art, future challenges and trends in microbal metabolomics, including systems microbiology and foodomics.



**Contents**



*Abbreviations:* CE, Capillary electrophoresis; ECVA, Extended canonical variates analysis; FT-ICR, Fourier transform-ion cyclotron resonance; GC, Gas chromatography; HCA, Hierarchical cluster analysis; LC, Liquid chromatography; LDA, Linear discriminant analysis; MS, Mass spectrometry; NMR, Nuclear magnetic resonance spectroscopy; PCA, Principal-components analysis; PCDA, Principal-component discriminant analysis; PLS-DA, Partial least squares discriminant analysis; QDA, Quadratic discriminant analysis; QIT, Quadruple ion trap; Q-TOF, Quadrupole time of flight; STOCSY, Statistical correlation spectroscopy.

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## 1. Introduction

To understand the biological mechanism on all different hierarchical levels, systems biology was introduced to examine the structure and the dynamics of cellular and organismal functions, rather than the characteristics of isolated parts of a cell or an organism [1]. Systems biology has now evolved as a broad research field that offers the prospect of assisting in solving fundamental problems [2]. The metabolome is one of the components in systems biology. It is the core in connection with many cellular changes and phenotypes [3]. Specifically, metabolism is regulated by gene expression and post-transcriptional and post-translational events, metabolites are functional entitles within cells and their amounts vary as a consequence of genetic or physiological changes, and can reflect the phenotype (Fig. 1A). Moreover, the metabolome, which refers to the global collection of small-molecule metabolites (such as metabolic intermediates, hormones and other signaling molecules, and secondary metabolites) found within a biological sample, is an amplified product of upstream molecular changes in the transcriptome and the proteome [4].

Progress in studies of the metabolome or metabolites has been aided by the recent advent of analytical technologies for comprehensive metabolic analysis, termed "metabolomics" [5]. Historically, there were two terms, "metabolomics" and "metabonomics", which were defined as the quantitative measurement of the dynamic multi-parametric metabolic response of living systems to pathophysiological stimuli or genetic modification [6]. The difference between the two terms is simply because of usages amongst different groups that have popularized them. In practice, the term "metabolomics" is often used interchangeably with "metabonomics" [7] (Fig. 1B). Metabolomics is the endpoint of the omics cascade, so it provides access to the biochemical phenotype. Integrative use of multiple approaches in systems biology may thus be a better strategy and can also further offer insight for better understanding of an organism [8].

Metabolomics has proved to be an acceptable, reproducible platform technology, capable of capturing key molecular signatures and characteristics of diseases at different stages and progression [9,10]. Considering the importance of microbes as model organisms, the application of metabolomics techniques in microbiology has recently received much attention. The first microbial metabolomics study was in 1992 by Elmroth et al. [11], who employed GC-MS combined with chemometrics to detect fatty acids, amino acids and sugars in evaluating bacterial contamination of cultures of *Leuconostoc mesenteroides*.

So far, microbial metabolomics has been widely applied in various microbiological fields, such as microorganism identification, mutant screening and functional gene research, metabolic pathway identification and microbial engineering. Compared to studying plants and animals, the main disadvantage of microbe-based metabolomics is that the microbial metabolites are generally complicated and thus difficult to identify. Moreover, the intracellular and extracellular metabolites in microbes are not easily separable. Within a system-wide framework, there are also distinct advantages in studying microbial systems over higher organisms. As microbes are less complex biological organisms, the majority of genome-sequenced data of microorganisms are readily available. Relevant information on gene regulation, metabolic network, and physiology of microbial cells is also easily accessible. Microbiology is therefore a research area that can greatly benefit from recent advances in metabolomics [12].

In the past few years, there have been several excellent reviews on microbial metabolomics [13–18]. However, there is growing awareness that many technical problems in microbial metabolomics have remained unsolved, especially with regards to sample preparation, biomarker identification and mechanism interpretation. There are no reviews providing a comprehensive, in-depth summary of current strategies of microbial metabolomics.

In this context, we seek to focus this review on research methodology and to provide an update on recent progresses and pitfalls of microbial metabolomics. In addition, we also discuss challenges associated with microbial metabolomics.

## 2. Analytical technologies

Numerous analytical procedures (e.g., sample preparation, signal acquisition, data processing, and data analysis) are involved in a metabolomics study and can directly influence the final results and the biological interpretations. Currently, there are no definitive or standardized operating procedures for microbial metabolomics. Analysis of data from identical samples using different analytical technologies will differentially affect the data and can potentially lead to incorrect findings and contradictory conclusions (Fig. 2A). Numerous analytical technologies were recently developed in several laboratories that promoted the formulation of in-house strategies for sample preparation, and analytical and bioinformatics tools for analysis of the vast amount of data generated [19,20]. Herein, we provide a step-wise description of general analytical technologies for microbial metabolomics.

### 2.1. Sample preparation

In order to obtain meaningful metabolomics data, microbial metabolomics requires appropriate sample-preparation steps, which include immediate quenching of enzymatic activities, separation of exo- and endo-metabolomes, and the thorough extraction of metabolites.

As intracellular metabolites in microbes may also undergo rapid metabolic responses during the sample-preparation steps (usually in 1–2 s), it is crucial to collect the samples quickly. Such a strategy prevents alterations in the microbial substrate concentrations and helps maintain the stability of the microbial metabolism. Specifically, this step is regarded as especially important for continuous cellular cultivation, as the medium-substrate concentration (such as, glucose) is generally low, so the physiological changes tend to be rapid. To date, some simple instrumentation for collecting samples has been improvised for microbial metabolomics. For example, Schaefer et al. [21] developed an automated sampling device, coupled to a stirred tank reactor, for monitoring intracellular metabolite dynamics with a sampling rate of 4.5 $s^{-1}$. For determination of rapid changes, Buziol et al. [22] developed a sampling technique based on a continuous stream of biosuspension, from the bioreactor, which was mixed with a glucose solution in a turbulent mixing chamber. In the same year, Visser et al. [23] presented a novel device, namely BioScope, that allowed elucidation of *in vivo* kinetics of microbial metabolism via perturbation experiments.

Generally, the separation of extracellular metabolites and cells should be kept at low temperatures to prevent side reactions. However, the intracellular metabolism is also altered after sampling, due to separation from the culture environment. As current metabolomics focuses on the metabolite *per se*, sample quenching is required to stop the metabolism at specific time intervals to obtain the real metabolic information at given times. The ideal quenching technology should therefore meet two basic criteria:

(1) rapid quenching of enzyme activity;
(2) maintaining the cell or organism intact at all times.