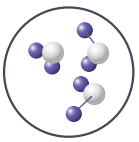
Yang et al., J Metabonomics Metabolites 2012, 1:2
http://dx.doi.org/10.4172/2325-9736.1000e109



Journal of
Metabonomics & Metabolites

Editorial A SCITECHNOL JOURNAL

Metabolite Profiling and Dynamic ^{13}C Metabolomics of One-Carbon Assimilation Pathways in Methylotrophic and Methanotrophic Bacteria

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Methylotrophic and methanotrophic bacteria (methylotrophs and methanotrophs) are a diverse group of microbes that can use reduced one-carbon (C1) sources, such as methanol and methane as a sole source for both energy generation and carbon assimilation. *Methylobacterium extorquens* AM1 is one of the best-characterized model organisms for the study of C1 metabolism in the methylotrophic bacteria. In *M. extorquens* AM1, the methanol is first oxidized to a key intermediate of formate through multiple reactions and then formate is assimilated via three overlapped cycles, the serine cycle, ethylmalonyl-CoA pathway and tricarboxylic acid cycle [1]. Methanotrophic bacteria form a highly specialized group of microbes utilizing greenhouse gas methane as a sole source of carbon and energy. It consists of type I and type II groups, which differ in intracellular membrane arrangement, pathways of carbon assimilation, and phospholipids fatty acid composition [2]. A number of novel methanotrophic phyla were isolated and described, including new members of the Alpha- and Gammaproteobacteria, and Verrucomicrobia [3,4]. Recently, the available genome sequences of methylotrophs and methanotrophs, such as *Methylobacterium extorquens* AM1, *Methylococcus capsulatus* Bath, *Methylomonas sp.* LW13, *Methylosinus trichosporium* OB3b and *Methylomicrobium alcaliphilum* 20Z, have further provided the ability to perform integrated metabolism studies by applying transcriptomics, proteomics, metabolomics and ^{13}C metabolomics [5-8].

Metabolomics is emerging as one of the most important of the "-omics" technologies, as cellular metabolism and its regulation often will more closely reflect the cell status in response to genetic or environmental perturbations, than measurements that are upstream of metabolic conversions such as transcriptomics or proteomics. The continued development of analytical platforms, database libraries, chemometric data analysis tools, and ^{13}C labeling techniques are allowing metabolomics studies to cover a broader range of metabolites, and are also providing key advancements for ^{13}C flux analysis. Mass spectrometry (MS) based metabolomics, in which a separation technique such as gas chromatography (GC), capillary electrophoresis (CE) or liquid chromatography (LC) is coupled to a mass spectrometer, has been widely applied to profile metabolomes or determine metabolite concentrations. Due to the versatile separation characteristics of LC, broader selectivity, and omission of derivatization steps, LC–MS is often the preferred technique for metabolomic analysis. Metabolites are typically moderately to highly polar small molecules, which are often too hydrophilic to be reliably retained and separated on common reversed-phase columns (RPLC). New chromatographic techniques, including hydrophilic interaction liquid chromatography (HILIC), ion-pairing reverse phase chromatography and hybrid phase chromatography are gaining popularity for metabolomics applications [9-11]. However, some metabolites with similar physicochemical properties have proven challenging for LC analyses. As a result, the combination of multiple LC-based and GC-based methods for the same sample was preferred to increase the coverage of metabolites. In a recent report, we have investigated the central metabolism of *M. extorquens* AM1 and *M. trichosporium* OB3b by using a combination of complementary separation techniques (RPLC, HILIC and comprehensive two-dimensional gas chromatography) with MS detection [10,12].

In addition to a good retention and separation of metabolites, introduction of internal standards (I.Ss.) to the samples prior to metabolite extraction is important for reliable quantification. When complex biological extracts are injected into an electrospray ionization source, the ionization efficiency of metabolites can be suppressed or enhanced due to the presence of less volatile and coeluting compounds [10]. By adding ^{13}C-labeled I.Ss., especially cell culture derived global ^{13}C-labeled I.Ss., to the samples, corrections can be made for the variations arising from instrumental analysis and sample preparation [10,13]. With the introduction of a global ^{13}C-labeled I.Ss., the concentrations of more than 40 metabolites have been well profiled in *M. extorquens* AM1 and *M. trichosporium* OB3b [10,14].

Many efforts have been made to develop efficient chemometric data analysis tools in our laboratory and elsewhere [15,16]. Parallel Factor Analysis (PARAFAC) is one of the mathematical tools for peak deconvolution that provides accurate quantification of metabolites of interest even in the presence of overlapping compounds. Recently, a novel PARAFAC method was reported for the analysis of nearly co-eluting ^{12}C and ^{13}C isotopically labeled metabolites on GC-MS and two-dimensional gas chromatography-time-of-flight mass spectrometry (GC x GC-TOFMS) data [15,17]. This methodology further forms the basis for dynamic ^{13}C flux analysis to determine the fate of interesting metabolites in *M. trichosporium* OB3b, via quantitative determination of ^{13}C-label uptake as a function of time [17]. More recently, by using ^{13}C-based metabolomics to track the dynamic labeling patterns of CoAs derivatives, novel metabolic pathways including the ethylmalonyl-CoA pathway have been demonstrated as important for glyoxylate regeneration in the central metabolism of *M. extorquens* AM1 and *M. trichosporium* OB3b [14,18,19].

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Received: November 20, 2012 **Accepted:** November 23, 2012 **Published:** November 26, 2012

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