Metabolomics: A Promising Tool to Study Disease Biomarkers and Host-Pathogen Interactions

22

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Abstract

Metabolomics is a comprehensive analysis of small-molecule metabolite profiles of cellular processes using mass spectrometry combined with advanced techniques of gas/liquid chromatography and nuclear magnetic resonance spectrometry. Whenever microbial pathogens invade the host cellular system, the host immune system responds via several innate and adaptive mechanisms to eliminate the pathogens. As a result, both host and microbial pathogens elicit adaptive responses, leading to altered metabolic pathways such as glycolysis, fatty acid, amino acid biosynthesis, and thereby cellular metabolites. Finally, either host cells or pathogens survive. These unique metabolites are analyzed by metabolomics and used as biomarkers for disease and pathogen detection, differentiating between microbial pathogens, prediction of altered metabolic pathways during infection, and development of drug resistance. Metabolomics completes the loop of central dogma beyond the proteins and provides the information on molecular phenotyping. Metabolomics has emerged as a promising tool to aid better understanding of metabolic pathways and new drug targets, rapid disease diagnosis, and development of effective, shorter, personalized therapy. Herein, the application of metabolomics in clinical setting for the study of infectious diseases, viz., pathogen and disease detection, differentiating between microbial pathogens, alterations in associated biochemical pathways during infection, and drug resistance, is discussed. This chapter highlights the analytical techniques used for metabolite profiling.

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22.1 Introduction

Living cell is itself a perfect machine, where coded information on DNA is transcribed into mRNA and then translated to proteins, which further initiate controlled and functional metabolic processes of the cell. Omics is the systematic study of biological interaction in cells such as genes, transcripts, protein, and metabolites. Genomics, transcriptomics, and proteomics mostly provide the information on genome and its product but convey very limited information about phenotype. Interestingly, compounds with low molecular weight (less than 1 KDa) or metabolites of cellular processes are closely linked to molecular phenotype, where metabolites are the functional representation of cellular state and their abundance is often related to homeostasis dysregulation or disease occurrence [1]. The entire set of metabolites within biofluids, cells, tissues, and organisms are termed as metabolome, and their identification and quantification is called metabolomics. Metabolomics is a powerful tool for clinical diagnosis of diseases, to measure chemical phenotypes, and to provide highly integrated profiles of biological status of cells [1]. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are highly reproducible, quantitative techniques which are routinely used for the study of metabolites. However, other techniques such as capillary electrophoresis, infrared spectroscopy, and Raman spectroscopy are also used to study several metabolic disorders [2].

A recent metabolomics study reported the differential abundance of 373 and 204 metabolites within COVID-19 patients, indicating the disease severity of the COVID-19 pandemic [3]. The use of metabolomics in screening of infectious diseases has shown accurate and noninvasive diagnosis of metabolic biomarkers associated with microbial infections and diseases [4]. This technique has been widely used for the extensive study of several common diseases like *Mycobacterium tuberculosis* (*M. tuberculosis*), human immunodeficiency virus infection (HIV), hepatitis C, etc. [4]. Metabolomics can be used for detection/identification of disease and pathogen, to differentiate between microbial strains, to provide the information on virulence factors, for adaptation features of microbes in host environment, and to identify the novel compounds produced by microbes. The comprehensive characterization of metabolic phenotypes can progress precision medicine to various levels, including the characterization of metabolic derangements underlying the disease, discovery of new therapeutic targets, and discovery of biomarkers that may be used for either disease diagnosis or monitoring activity of therapeutics. Herein this

chapter, we have summarized the metabolomics techniques and their applications to study disease biomarkers and host-microbial pathogen interactions.

22.2 Analytical Techniques for Profiling of Metabolites

MS and NMR spectroscopy are complementary analytical techniques used for qualitative and quantitative analysis of metabolites extracted from various biological samples or biofluids (plasma, urine, blood, breath) of healthy and diseased individuals. These are highly sensitive, powerful, high-throughput techniques, capable of identifying several hundred metabolites in a single measurement and helping to understand the differences in the biological pathways of healthy and diseased individuals. Figure 22.1 depicts the general workflow for metabolomics.

22.2.1 Mass Spectrometry (MS)

MS measures the mass-to-charge (m/z) ratio of different molecules within a sample and calculates the exact molecular weight of components present in the sample. MS quantifies both known and unknown compounds within a sample via molecular weight determination and helps in elucidation of the chemical structure and, thus, chemical properties of different molecules. It is an ultrasensitive technique, which can detect molecules with high resolution ($\sim 10^3$–10^4), at even very low concentrations of femtomolar (10^{-15} MolesL^{-1}) to attomolar (10^{-18} MolesL^{-1}), and routinely used to analyze hundreds of compounds in a single sample [5]. MS is successfully used in metabolomics and provides new perspective to understand the

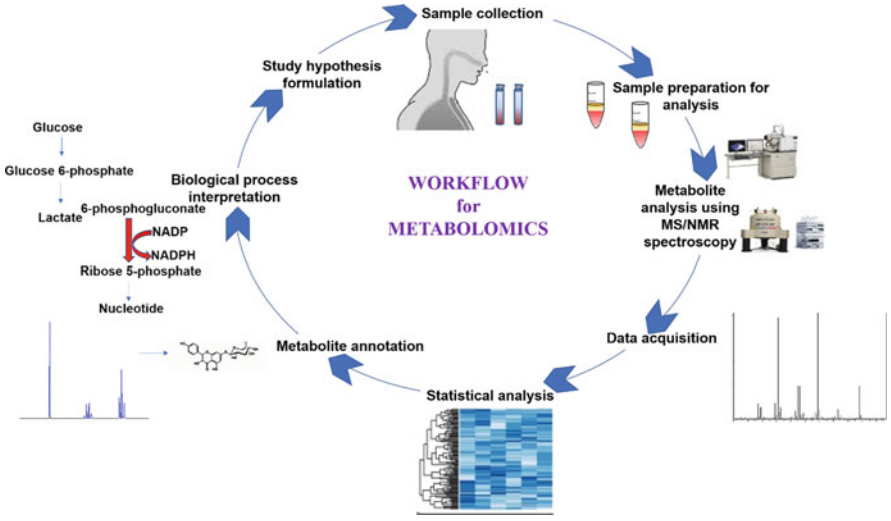


Fig. 22.1 General workflow for metabolomics

cellular processes, alterations in biochemical pathways due to host-pathogen interactions, and identification of disease biomarkers [6, 7]. MS typically consists of three functional units, namely, ionization source, mass analyzer, and ion detection system. The ionization source is used to ionize the analyte to gas phase ions, while the mass analyzer further sorts and separates the ions based on mass-to-charge ratio. After that, the separated ions are measured by the detector, and mass spectrum is generated based on m/z ratio of ions in sample and their intensities. Electrospray ionization (ESI) and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) are two ionization techniques, majorly used in metabolomics because of their soft nature of ionization, which prevents the fragmentation of molecular ions [5]. MALDI-TOF is routinely used for the identification of microbial strains. In MALDI-TOF, the cultured microbial colony from the collected sample is first transferred onto a MALDI target spot and then embedded in a matrix, and subsequently a laser is aimed at the target spot [8]. The spectrum generated requires further data processing before comparing with the reference database of known microorganisms. The National Institute of Standards and Technology (NIST) MS Data Center develops and makes available the database of reference GC/LC-MS libraries [9] and related software tools for identification of compounds by comparison with reference mass spectra. This makes it convenient for detection of microbial strains and also helps in elucidation of mechanisms involved in microbial pathogenesis [10, 11]. The Bruker Biotyper and Vitek MS bioMérieux are FDA-approved platforms for rapid, cost-effective, and accurate identification of microbes [12].

However, MS most often requires pure sample for identification; therefore combining MS with chromatographic separation techniques maximize the ability of MS for accurate identification of compounds. Gas chromatography (GC) and liquid chromatography (LC) are pre-MS separation methods, wherein GC is used for the separation of volatile compounds, while thermally unstable and nonvolatile molecules are separated by LC. More polar biomolecules such as organic acids, nucleosides, nucleotides, ionic species, polyamines, organic amines, etc. are analyzed by LC-MS, where mobile phase is liquid and no derivatization process is required [10, 11]. GC-MS is widely used for analysis of alcohols, esters, alkaloids, amino acids, sugars, drugs, toxins, fatty acids, etc. [10, 11]. For GC, prior to analysis, sample is chemically derivatized to convert the nonvolatile compounds to volatile form. Derivatization improves sample volatility, selectivity, detectability, separation, and thermal stability in chromatographic applications, increases retention time, enlarges substrate spectrum, and removes tailing. GC-MS is a robust analytical technique with high sensitivity, selectivity, and reproducibility. GC-MS overcomes the limitations of matrix effect and ion suppression associated with LC-MS by co-eluting the compounds, therefore resulting in greater chromatographic resolution [10, 11]. Interestingly, studies have demonstrated the successful use of GC-MS-based metabolomics for the identification of microbial strains and biomarkers associated with pathogenicity and infectious diseases [13]. In recent years, mass spectrometry imaging (MSI) has emerged as a promising technique which enables the in situ detection of several compounds, ranging from metabolites to proteins, and can also simultaneously provide spatiotemporal distribution of molecular species in

a variety of samples [10, 11, 14]. It has the capability to image thousands of molecules, such as metabolites, lipids, peptides, proteins, and glycans, in a single experiment without labeling. The combination of information gained from mass spectrometry (MS) and visualization of spatial distributions in thin sample sections makes this a valuable chemical analysis tool for biological specimen characterization. MSI is increasingly used as an analytical technique both as complement and replacement to other imaging methods. By improving sample preparation protocols, instrument throughput, resolution capabilities, streamlined data analysis, and quantitation, it is anticipated that MSI will be routinely utilized in clinical settings. Advances in MS has certainly improved the selectivity, sensitivity for detection at lower concentration, compatibility with separation techniques, and ability for qualitative and quantitative analysis, thus making MS an ideal analytical technique for profiling of metabolites in clinical settings.

22.2.2 Nuclear Magnetic Resonance (NMR)-Based Spectroscopy

NMR is one of the principal analytical techniques used in metabolomics, preferably for long-term or large-scale metabolite profiling, when it is not restricted to analysis of only biofluid or tissue extract. Being a nondestructive analytical technique, NMR is more advantageous to use for metabolomics. Moreover, NMR spectroscopy has relatively high reproducibility and high throughput and requires almost no separation techniques. The principle of NMR is based on the magnetic properties, referred to as "spin," of certain atomic nuclei to provide information about their immediate environment. Each metabolite is made up of atom consisting of nuclei, and when magnetic field is applied, specific NMR signal is obtained by the radio-frequency pulses that interact with the nuclei of an atom and characterize the resonate frequency of that nuclei based on the environment and chemical surroundings [15]. Thus, the environment of that nucleus, the presence of electrons and protons on neighboring atoms, and their interactions affect the magnetic field, and thus, the energy required to flip the nucleus. Metabolites such as alcohols, sugars, highly polar compounds, organic acids, etc. are detected and characterized by NMR spectroscopy [15]. Moreover, different classes of metabolites such as nitrogen-containing, phosphorous-containing, and protein-bound metabolites (lipoprotein particles) and certain inorganic metabolites (H^+ ions and metal ions) can be identified either separately or simultaneously [15, 16]. MS-based analytical methods being destructive, both LC-MS and GC-MS are unsuitable for analyzing living samples, but with NMR spectroscopy, the metabolite profiling of living cell in real time is possible [17, 18]. Milner et al. used ^{1}H NMR for the analysis of metabolites in urine and feces samples of healthy and influenza virus-infected mice. Significantly elevated levels of certain metabolites such as acetylcarnitine, ascorbate, glucose, and 3-hydroxybutyrate were found in urine sample of obese mice indicating alterations in metabolic pathways associated with kidney [19]. Similarly, discriminant metabolites such as acetate and trimethylamine were rapidly identified by ^{1}H NMR spectroscopy during urinary tract infection caused by *Escherichia coli*

Table 22.1 Comparison between MS and NMR

Mass spectrometry	NMR spectroscopy
Needs derivatized pre-sample preparation for GC-MS analysis	Easy or minimal sample preparation with no need of derivatization step and has high reproducibility
MS is not suitable for profiling of living cell metabolites	Suitable for profiling of living cell metabolites [22]
For maximization of metabolites detection, MS requires different ionization methods	Rapid analysis, all metabolites can be observed in a single measurement
Highly sensitive and selective method. It is suitable for targeted analysis, can operate for sample even at concentration of 10^{-6} M, and can be efficient up to 10^{-18} M [23]	Almost 10–100 times less sensitive than GC-MS/LC-MS but suitable for both targeted and untargeted analysis and able to measure sample between concentration 10^{-4} to 10^{-5} M [23]
Ionization is an important factor for resulting data. MS line intensity is not directly correlated to metabolite concentration [15]	Easy quantitative method, signal intensity is directly proportional to number of nuclei in the molecule and to metabolite concentration [15]
MS could be used for metabolic flux analysis but destructive nature of MS-based methods resulted in more limitations than NMR-based fluxomics	Suitable for in vivo and in vitro metabolic flux analysis [24, 25]

(*E. coli*) [20]. With simultaneous use of magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI), NMR can be a versatile tool in biomedical field for metabolite imaging and analysis of living samples [21]. Table 22.1 gives a comparison between MS and NMR.

22.3 Metabolomics for Infectious Diseases

Human beings are under continuous exposure to various pathogenic and nonpathogenic microorganisms such as viruses, bacteria, parasites, and other extrinsic factors. The human gut itself harbors a complex and dynamic population of microorganisms called the gut microbiota, comprising beneficial, commensal, and opportunistic microbes, which exert a remarkable influence on the host during homeostasis and disease. The gut microflora plays a significant role in host nutrient metabolism, xenobiotic and drug metabolism, maintenance of structural integrity of the gut mucosal barrier, immunomodulation, and protection against pathogens. Although the microorganisms which are part of gut microflora are well adapted to the human body, under certain unfavorable and immunocompromised conditions, few commensal microorganisms often turn pathogenic to result in disease such as candidiasis caused by the opportunistic fungus, *Candida albicans*. On the other hand, there are many other microbes which are not part of the human microbiota and can cause several diseases. Some common examples of infectious diseases are urinary tract infections, common cold, tuberculosis, HIV/AIDS, influenza, viral hepatitis,

measles, typhoid, chicken pox, diphtheria, dengue, chikungunya, etc.; however, the list remains exhaustive.

Infectious diseases are major causes of mortality and morbidity worldwide. They are transmissible and caused by various pathogens, including bacteria, fungi, viruses, and parasites. Infections by these pathogens result in illness and disease. Signs and symptoms and treatment of infectious diseases depend on the host and the pathogen. The invasion of host by the microbial pathogens and pathogenicity have been widely studied since decades, and several vaccines or therapeutics have been developed to limit the microbial infections. While vaccines are available as protective measures for many diseases but for others, vaccines are still not available. Treatments for diseases are dependent on therapeutic targets that are already available or still not known yet. Even in this era of ongoing COVID-19 pandemic which has severely impacted several millions of the world population and resulted in millions of deaths in 2020 (https://www.worldometers.info/coronavirus/), there is still no vaccine or medicine currently available for COVID-19. However, many of the symptoms can be treated, and getting early care from a healthcare provider can make the disease less dangerous. Undoubtedly, COVID-19 has led to a drastic change in the social structure and lifestyle of humans globally. Therefore, in the light of such background, it is essential to identify disease at early stage and also to find therapeutic targets to treat infectious diseases. The design and development of vaccines and therapeutic drugs depend on many factors including the host immune system and type of the pathogen.

As a part of adaptation to the surrounding environment and protection from microbial pathogens, the immune system plays a vital role in providing protection to the human host. Human immune system consists of innate and adaptive immune systems. Soon after infecting a human host, the microbial pathogen tries to hijack the host cellular system for its survival. Existing literature supports the fact that the host metabolic pathways get perturbed during infection, and in order to protect against infection, integrins, cytokines, and tissue-specific immune cells such as monocytes, macrophages, B cells, and T cells are activated to destroy the microbial pathogen. Therefore, it is essential to identify the metabolites, and the holistic approach of systems biology used for metabolite profiling is called "metabolomics." Systems biology integrates omics technologies such as genomics, transcriptomics, proteomics, and metabolomics. Of these, metabolomics is relatively a novel approach and offers a robust platform for researchers to understand the influence of different factors in the metabolic pathway of an infected cell. From a clinical standpoint, metabolomics can detect the pattern of metabolites that are associated with particular disease or pathogen, may detect an unknown metabolite indicating new pathophysiological conditions, and also trace the effectiveness or toxicity of a drug in disease. This would certainly aid in early detection of pathogen and disease, facilitate timely disease prevention and treatment, identify new drug targets, and improve therapeutic interventions.

Identification of microbial species is a crucial bottleneck for clinical diagnosis of infectious diseases. Quick and reliable identification is a key factor to provide suitable antimicrobial therapies and avoid the development of multiple-drug

resistance. The conventional methods for identification of microbial pathogens are very tedious and time-consuming and require laboratory skills and proper clinical setup. This delays the identification of pathogen and early disease diagnosis and thus, impedes timely treatment of disease. However, application of metabolomics for clinical disease diagnosis and identification of microbial pathogens certainly opens new prospects, and chemical analysis of microbial metabolites can facilitate rapid detection of the pathogens and help differentiate between them.

22.3.1 Identifying and Differentiating Between Microbial Pathogens

Volatile organic compounds (VOCs) are low molecular weight and carbon-based organic molecules with vapor pressure of $\geq$0.01 kPa at 20 °C and are naturally volatile in ambient temperature [26]. In 1964, coliform bacteria were detected via profiling of VOCs which lead to identification of indole, acetoin, pyruvate, and 2,3-butanediol in culture media [27]. VOCs are produced by resident microbes and various other microbes as primary (e.g., ethanol, acetone, acetic acid) or secondary metabolites (signaling molecules) (Table 22.2) [26]. These VOCs can diffuse into breath, urine, feces, and sweat; therefore, analysis of VOCs involves an inexpensive, noninvasive method for collection of samples. Profiling of VOCs can help fingerprint metabolites produced by the infecting pathogen or reflect pathogen-induced host responses or a combination of both (Fig. 22.2). Testing for volatile biomarkers in clinical samples offers an option for developing rapid and potentially inexpensive disease screening tools. The testing of volatiles can be performed frequently in follow-up studies, which may indicate disease progression and be helpful in monitoring therapeutic intervention. The direct link of diffused VOCs to microbial infection in host was well demonstrated by specific VOCs released by the resident pathogen during host-pathogen interactions [34], e.g., indole metabolite found in *E. coli* infections [35], 2-aminoacetophenone compound reported in infection by the respiratory tract pathogen, *Pseudomonas aeruginosa* (*P. aeruginosa*) [36], and high level of *p*-menth-1-en-8-ol metabolite detected in fecal samples of cholera patients infected with *Vibrio cholerae* (*V. cholerae*) (Table 22.2) [32]. VOCs collected from exhaled breath require noninvasive, less strenuous sampling and are widely diagnosed by GC-MS and NMR spectroscopy. However, ion-molecule reaction-mass spectrometry (IMR-MS), field asymmetric ion mobility spectrometry (FAIMS), and selected ion flow tube-mass spectrometry (SIFT-MS) are also used for profilingVOCs [37]. The profiling of VOCs showed 68% sensitivity and 100% specificity for the detection of *P. aeruginosa* in sputum and cough swab samples of cystic fibrosis patients [38], by detecting volatile hydrogen cyanide (HCN) using SIFT-MS [38, 39]. HCN may be a specific indicator of *P. aeruginosa* infection in vivo and offers promise as a biomarker for noninvasive detection of *P. aeruginosa* infection by breath analysis. In another study, it was possible to discriminate between healthy controls and cystic fibrosis patients with or without *P. aeruginosa* colonization based on detection of C5–C16 hydrocarbons and *N*-methyl-2-methylpropylamine, using gas chromatography-time of flight-MS [40]. Active

Table 22.2 Specific VOCs produced by various microbial pathogens

VOCs	Pathogen	Method	Source	Disease	Reference
Naphthalene, 1-methyl-cyclohexane, hexyl-heptane, 2,2,4,6,6-pentamethyl-benzene, 1,3,5-trimethyl-1-hexene, 4-methyl	*M. tuberculosis*	GC-MS	Breath	Active TB	[28]
HCN	*P. aeruginosa*	SIFT-MS	Breath	Cystic fibrosis	[29]
2-Aminoacetophenone		GC-MS			[30]
2,3-Butanediol, [R-(R*,R*)]-, hexadecane; and undecane, 3,8-dimethyl metabolites, *N*,*N*-dimethylacetamide; phosphonic acid, (*p*-hydroxyphenyl); 3,5-decadien-7-yne, 6-t-butyl-2,2,9,9-tetramethyl; 1,6-dioxacyclododecane-7, 12-dione; caprolactam; 5,7-octadien-2-one, 3-acetyl; nonanal; and 5-hepten-2-one, 6-methyl-	*Helicobacter pylori*	GC-MS	Breath	Gastric cancer	[31]
Dimethyl disulfide	*Vibrio cholerae*	GC-MS	Feces	Cholera	[32]
p-Menth-1-en-8-ol					[32]
3-Methyl-2-butanone and styrene	*C. albicans*	GC-MS	Breath	Oral candidiasis	[7]
Combination of p-xylene, 2-octanone, 2-heptanone, n-butyl acetate	*C. krusei*	GC-MS			
1-Hexanol	*C. tropicalis*	GC-MS			
α-Trans-bergamotene, β-trans-bergamotene, a β-vatirenene-like sesquiterpene, or trans-geranylacetone	*A. fumigatus*	GC-MS	Breath	Invasive aspergillosis	[33]

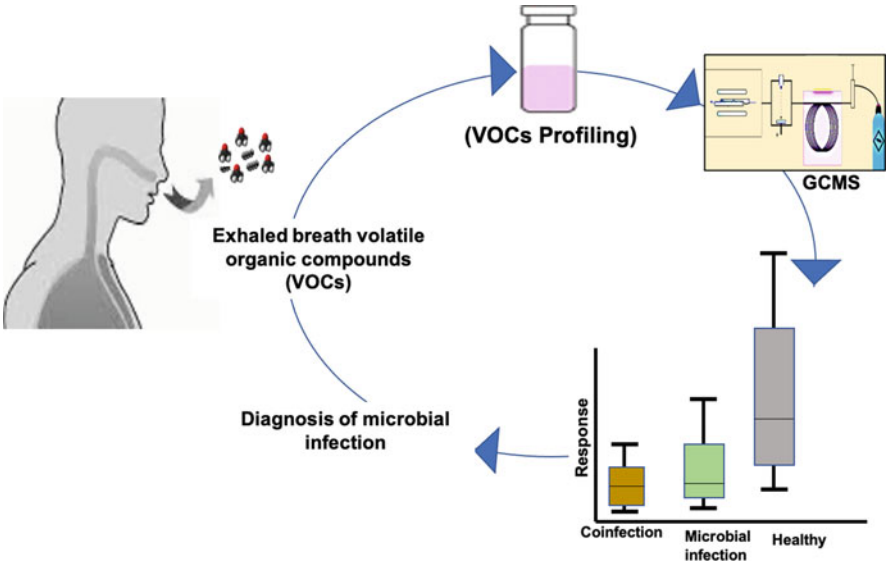


Fig. 22.2 Illustration for profiling of VOCs for identification of microbial pathogens and disease diagnosis

pulmonary tuberculosis (TB) was distinguished from nonactive TB by breath VOC patterns wherein 1,3,5-trimethylbenzene was identified in active pulmonary TB and 1,2,3,4-tetramethylbenzene in the nonactive stage [41]. Profiling VOCs in urine samples of TB patients and healthy controls using GC-MS coupled to a headspace sampler revealed a panel of five selected biomarkers, namely, alpha-xylene, isopropyl acetate, 3-pentanol, dimethylstyrene, and cymol, which enabled discrimination between TB-infected and healthy individuals with an accuracy of 98.8% (area under the curve [AUC] of 0.988) [41]. VOCs which were principally derivatives of naphthalene, benzene, and alkanes and metabolic products of *M. tuberculosis* were identified in breath samples of TB patients as biomarkers of pulmonary TB [28]. *Helicobacter pylori* (*H. pylori*) is the main etiological factor of gastritis and associated with duodenal ulcer and gastric cancer. Its diagnosis involves invasive method of endoscopy with gastric biopsies. Specific VOC profiles were detected for *H. pylori* by noninvasive expired air method, which is a low-cost method with good patient compliance and can be used for gastric cancer diagnosis [31]. Fungal pathogens were also identified based on signature volatiles such as 3-methyl-2-butanone and styrene and were detected as characteristic VOCs of *C. albicans* [7]. However, Koo et al. clearly distinguished invasive aspergillosis from pneumonia with 94% sensitivity and 93% specificity, wherein α-trans-bergamotene, β-trans-bergamotene, a β-vatirenene-like sesquiterpene, or trans-geranylacetone VOCs (secondary metabolites) were identified in invasive aspergillosis patients (Table 22.2) [33]. Traxler et al. reported high concentrations of acetaldehyde, propanal, and n-propyl acetate in the breath of pigs which indicated coinfection by both bacteria and influenza A virus and an interaction between the pathogens (Table 22.2)

[42]. Electronic nose sensor (gas sensor array) is a rapid, accurate sensing technique, popular for microbial screening. Fend et al. used electronic nose sensor to detect *M. tuberculosis* in both culture and spiked sputum samples and achieved the detection limit of 1×10^4 mycobacteria ml^{-1} with specificity of 91% and sensitivity of 89% (Table 22.2) [43]. The ability of an electronic nose (e-nose) to detect *M. tuberculosis* in clinical specimens opens the way to developing this method as a rapid, automated system for early diagnosis of respiratory infections. Although e-nose sensors are much simpler and cost-effective, metabolomics techniques are able to provide details of pathophysiology of patient. However, VOCs collected from exhaled breath could be influenced by environment or activity before or during sampling; therefore, lack of consistency of proposed breath biomarkers is a certain limitation associated with profiling of VOCs [44]. Nevertheless, profiling VOCs still is a promising, novel, noninvasive, pathogen-specific approach for precise identification and monitoring of disease and discrimination of diseased person from healthy.

22.3.2 Fingerprinting Metabolic Differences in Biochemical Pathways of Host and Pathogen Associated with Host-Pathogen Interactions, Disease, Pathogenicity, and Drug Resistance

Fingerprinting metabolic differences between differentially regulated biochemical pathways of host and pathogen can facilitate the discovery of potential biomarkers associated with human dysbiosis or microbial diseases and provide insights into the host metabolites, microbial metabolites, their potential functions, impact on host-pathogen interactions, disease, pathogenesis, and drug resistance (Fig. 22.3).

Upon invasion of the host cells by the microbial pathogens (bacteria, virus, fungi, and protozoa), the pathogen-associated molecular patterns (PAMPs) present on microbes are recognized in host immune cells by pathogen recognition receptors (PRR) such as retinoic acid-inducible gene I-like receptors, C-type lectin receptors, and nucleotide-binding oligomerization domain-like receptors. Subsequently, host-defense mechanism is activated against the pathogen resulting in the production of antiviral agents, proinflammatory cytokines/chemokines and antibodies [45]. The host-pathogen interactions result in altered metabolic products (glucose, amino acid, lipids, and nucleotides) and synthesis of metabolites or several virulence factors (e.g., adhesins, modulins, toxins, etc.) in the microbial pathogens, which allow them to successfully replicate in host cell by evading host immune system [46]. Targeted metabolomics of the stationary phase growth arrested epimastigotes, and exponentially growing *Trypanosoma cruzi* parasites, known to cause Chagas disease, revealed the adaptive metabolic changes that epimastigotes undergo before they get into the metacyclic trypomastigote stage [47]. This finely tuned adaptive metabolic mechanism enables switch from highly reduced, energy-rich metabolites such as glucose to oxidized energy-poorer nutrients such as amino acids, found abundantly in the stationary phase of *T. cruzi* epimastigote. This metabolic plasticity might be crucial for the survival of the parasites under different environmental conditions. Knowledge of metabolic capabilities during their life cycle can reveal

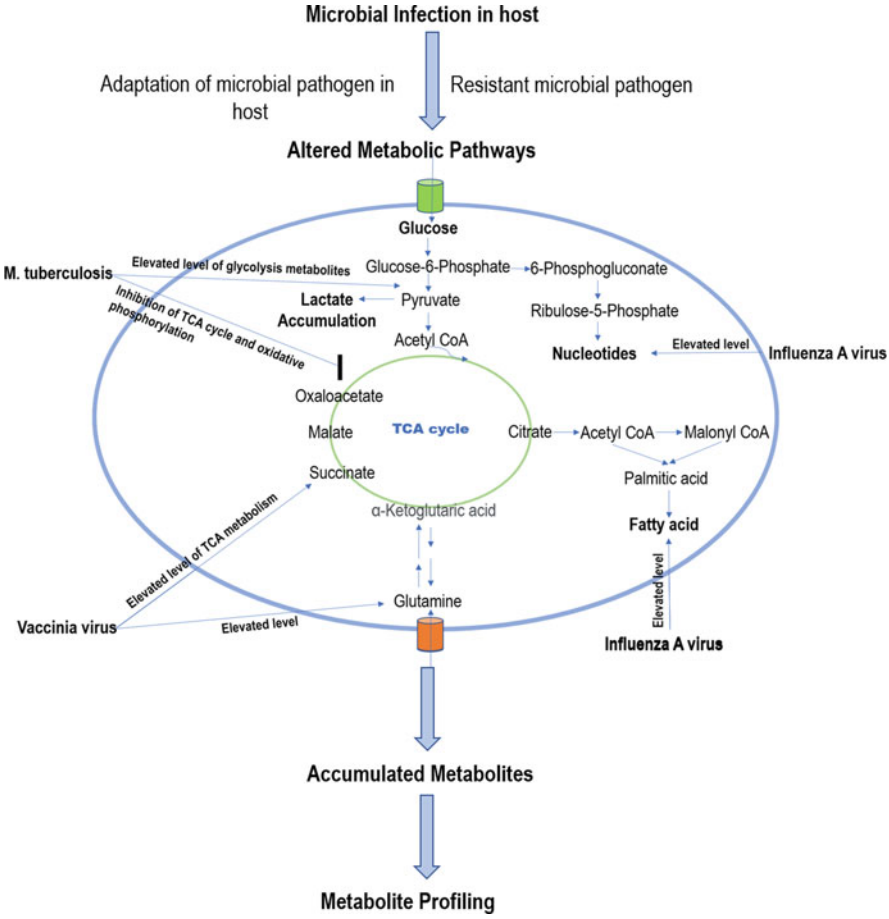


Fig. 22.3 Schematic representation for alterations in metabolic pathways, intermediates, and metabolites due to host-pathogen interactions

metabolic checkpoints as novel targets for designing therapeutic interventions and disease control. Triatomine is a vector for *T. cruzi* and during the replication and passage of *T. cruzi* through its intestinal tube, the vector immune system produces significant amount of oxidants [6]. It is suggested that triatomine recognition of PAMPs trigger innate and humoral immunity and cellular protection.

The Warburg-like metabolism (increased uptake of glucose and increased production of lactate in presence of oxygen) observed during infection by *Mycobacterium tuberculosis* is also an example of switching of bioenergetic metabolic pathways (switch from oxidative phosphorylation to glycolysis) [48]. Metabolic changes revealed that during infection, pyruvate is converted to lactate instead of entering into tricarboxylic acid (TCA) cycle and results in accumulation of intermediates of glycolysis and lactate, which are further used as an additional energy source for growth of bacteria (Fig. 22.3). Remarkably, the ability to use

glucose-6-phosphate and/or amino acids instead of glucose favors the activation of certain virulence factors or genes or metabolites, which are essential for the cytosolic lifestyle of the pathogen and help in the adaptation of bacteria to harsh environments such as the urinary tract, the blood stream, and the meninges [49]. There exists links between metabolism and expression of those genes whose products are required for entry, proliferation, protection, and persistence in the preferred infection niches for extra- and intracellular pathogenic bacteria. Abundance of virulence factors are yet another response of pathogen for evading host immune system and adapting to host physiological conditions, e.g., pertussis toxin (PTX) secretion by *Bordetella pertussis* (*B. pertussis*) induces hyperinsulinemia and hypoglycemia conditions in infected host [50]. PTX is involved in catalysis of ADP-ribosylation of alpha subunits $G\alpha_{i/o}$ (Gi/o) protein family and interferes in intracellular signaling, and the increased insulin release causes hypoglycemia in host [50]. Microbial infections also alter the production of inflammatory cytokines such as tumor necrosis factor (TNFα levels), which activates the sympathetic nervous system and, thereby, reduces the blood glucose level (hypoglycemia), which is a significant observation as biomarker during severe malaria and cerebral malaria [51].

Amino acids are another metabolite product which is associated with interactions between host and pathogen and influences outcome of infection. At the infection site, both host and pathogen share similar nutritional substrates and generate common metabolic products; therefore, cross talk between their metabolic pathways could affect the pathogenesis of infection. The alteration in leucine/isoleucine, arginine, tryptophan, glutamine, and proline amino acids might influence the outcome of infection and regulation of host immune system [10, 11]. L-Glutamine induces expression of *Listeria monocytogenes* virulence gene factors [52]. *Vaccinia virus* alters the metabolic pathways of the host for efficient replication and fully relies on glutamine but not glucose to anaplerotically maintain TCA cycle (Fig. 22.3) [53]. During infection, the host imposes manganese and zinc starvation on invading pathogens, but altering cellular metabolism contributes to the ability of pathogens to resist manganese starvation and that ArlRS a global staphylococcal virulence regulator enables *Staphylococcus aureus* to overcome nutritional immunity by facilitating this adaptation. *S. aureus* adapts to the impaired glycolysis by switching its dependency for energy from glycolysis to amino acid metabolism pathway and reduces demands of manganese and zinc for their growth [54]. As a response of host cell to restrict *Leishmania* infection, L-arginine is metabolized through oxygenation by inducible nitric oxide synthase (iNOS) to form nitric oxide (NO) [55]. In order to inhibit this antimicrobial response of host, the pathogen increases the arginase activity, arginine transport, and arginine deiminase pathway to compete with iNOS for NO production and, thus, depletes the arginine [55]. Tryptophan is required for the proliferation of T cell and boost the immune system against microbial infection [56]. Indoleamine 2,3-dioxygenase (IDO) is a rate-limiting enzyme in the breakdown of the essential amino acid tryptophan to kynurenine. Interestingly, the microbial pathogen depletes the tryptophan pool from the cell by inducing the immunomodulator IDO to form kynurenine, which, in turn, promotes apoptosis of neutrophils and inhibits reactive oxygen species (ROS). Thus, the accumulation of kynurenine could serve as an indicator of *Mycobacterium* infection (*M. avium* subsp.

paratuberculosis and other virulent mycobacteria) [56]. The probiotics such as *Lactobacillus acidophilus* W22, *Bifidobacterium lactis* W51, *Lactobacillus brevis* W63, *Bifidobacterium bifidum* W23, *Lactococcus lactis* W58, and *Enterococcus faecium* W54 can be used as a supplement of tryptophan to increase its concentrations in serum and reduce the infections related to the upper respiratory tract [10, 11]. Furthermore, elevated amino acid catabolism and folate and lipoic acid biosynthesis were observed in sputum of cystic fibrosis patients, which indicated the evolution of microbial pathogens against sulfonamide drugs which target folate biosynthesis [57]. Increased level of amino acids such as histidine, phenylalanine, glutamine, methionine, lysine, leucine, tryptophan, tyrosine, and glutamic acid were observed during white spot syndrome virus infection to evade the host immune system and facilitate increased viral replication and multiplication [58]. Microbial infections especially virus invasions in host induce synthesis of nucleotide, protein, fatty acid, and cholesterol for viral replication, and the virus remodels the host intracellular membrane for assembly of virus with host cell components [59, 60]. Elevated levels of fatty acid synthesis and glutaminolysis are found in cells infected with viruses like human cytomegalovirus, influenza A, hepatitis C, etc. and such modifications of carbon source utilization facilitate virus replication and virion production by increasing energy available (Fig. 22.3) [61]. In response, host immune system activates 5′-AMP-activated protein kinase (AMPK) to inhibit the fatty acid synthesis [62]. However, AMPK signaling could facilitate or inhibit intracellular viral replication depending on the microbial infection [62]. Alterations in lipid metabolism were found to be triggered by Zika virus infection, and infected patients showed increased levels of several phosphatidylethanolamine (PE) lipid species in serum, especially plasmenyl-phosphatidylethanolamine (pPE) (or plasmalogens) linked to polyunsaturated fatty acids, indicating increased viral replication and infectivity [63]. Biosynthesis of plasmalogens requires functional peroxisomes, which are important sites for viral replication. Influenza A virus promotes synthesis of prostaglandin E2 (PGE_2), an eicosanoid generated by cyclooxygenases, and inhibits recruitment and activity of macrophages; thus the virus suppresses both innate and adaptive immunity [64]. Analysis of COVID-19 patient samples exhibited plasma metabolome changes, indicating perturbed oxidative pathways of cellular energy production [65]. The levels of acylcarnitines (palmitoylcarnitine, oleoylcarnitine, and stearoylcarnitine) and metabolites associated with tricarboxylic acid (TCA) cycle were reduced, indicating attenuated entry of fatty acyls into the mitochondria for ß-oxidation. Lactate dehydrogenase level was increased with disease severity, whereas significantly reduced level of numerous amino acid (tryptophan, proline, valine, isoleucine, and citrulline) was found in mild and moderate COVID patients [65].

Metabolomics provides the comprehensive information on metabolites altered during infection and offers scope to explore through metabolite profiling the dynamics of adaptations, which contributed to the microbial death or survival to become resistant. Metabolite profiling of ampicillin-resistant *E. coli* revealed that recycling of anhydro-muropeptides plays an important role in mediating resistance to ampicillin [66, 67]. *E. coli* breaks down over 60% of the murein of its side wall and reuses the component amino acids to synthesize about 25% of the cell wall for the next

generation, and 1,6-anhydro-*N*-acetylmuramic acid (anhMurNAc) is returned to the biosynthetic pathway by conversion to *N*-acetylglucosamine-phosphate (GlcNAc-P) [66, 67]. A study reported the accumulation of dTDP-rhamnose (a deoxy sugar) in *E. coli* cells treated with quinolones (nalidixic acid and norfloxacin) and that increasing concentrations of dTDP-rhamnose upregulated the gyrase A transcription and helped the cells cope with antibiotic by sequestering the antibiotic and reducing drug-gyrase complex formation [68]. N-Acetyl-glutamate was accumulated in *E. coli* in response to kanamycin and spectinomycin antibiotics, which might be a hallmark of protein synthesis inhibition [68]. Multidrug-resistant (MDR) strains of *E. coli* with resistance to ciprofloxacin, ampicillin, gentamicin, and sulfamethoxazole showed increased levels of L-serine, glycine including L-cystine, L-cysteine-sulfinate, S-sulfo-L-cysteine, L-cystathionine, L-methionine sulfoxide, and L-methionine metabolites [69]. Altered pathways for amino acids, phenylpropanoids, and purine metabolism resulted in altered glucose, fatty acids, and ammonia biosynthesis which might help in acquiring drug resistance and survival under stress conditions [69]. Upregulation of cysteine and methionine amino acids involves redox reactions in bacteria, which prevent the entry of drugs into the cells and result in drug resistance [69]. MDR (fluconazole, ketoconazole, and miconazole) strains of *C. albicans* exhibited increased drug efflux; higher membrane fluidity; significantly reduced levels of N-methylnicotinate, glycerol, N-dimethylglycine, ribitol, proline, L-aspartate, valine, glutamine, and N-acetyl-aspartic acid metabolites; but increased levels of glycerophosphocholine, L-aspartate-4-semialdehyde, 2-oxoglutarate, adenine, lysophosphatidylcholine (16:1), phytosphingosine-1-P, Cer(d18:0/14:0), serine, spermine, lactate, inosine, citrate, 2-deoxyribose, and succinate, predicting high tolerance of *C. albicans* cells against drugs, oxidative stress, and temperature variations [70].

Recently, using untargeted metabolomics, Diaz et al. studied differential metabolites between *M. tuberculosis* and live attenuated *M. tuberculosis* vaccine named MTBVAC and correlated the vaccine with its parental strain based on metabolite profiling [71]. Identification of some of the differential metabolites might be useful as potential vaccination biomarkers [71]. Studies clearly indicate that carbohydrate, amino acid, and fatty acid metabolism pathways are majorly affected during microbial infection, adaptation, and evolution. The altered metabolites of these cellular processes accumulate in host cell and may serve as a target for controlling disease severity and progression. Metabolite profiling certainly advances our understandings on host-microbial interactions, metabolic pathways, and targets which can be used for the development of effective therapeutics and treatments.

22.4 Conclusion

Microbial infections have become impossible to eradicate, and they are still causing epidemics and pandemics and affecting millions of lives. Metabolomics has emerged as a powerful approach, which directly represents the molecular phenotype and reflects the underlying cellular processes and alterations in infected or diseased

state. Metabolomics provides the information which can be translated into assays or technology for easy and rapid disease diagnosis, identification of microbial strains, and study of host-pathogen interactions to aid development of effective therapeutics and disease treatment.

22.5 Future Perspectives

High-sensitivity and high-throughput metabolomics have enabled comprehensive detection of thousands of small-molecule metabolites in host and microbial communities. In the last 20 years, advances in techniques for metabolomics, availability of databases for metabolites, and analytical software have contributed to establishing new methods for disease diagnosis, metabolic studies, improved treatment, and molecular phenotyping of cellular processes. Recent technique such as high-resolution metabolomics can simultaneously identify the metabolic pathways and associated inflammatory cytokines, which can save the time for the elucidation of host immune response upon microbial invasion. Importantly, metabolomics has allowed clinicians to ameliorate potential effects of the errors occurred during metabolism and adaptation of pathogen in host. Metabolomics data can be utilized by computational biologists to design models for microbial pathogenesis and to identify new drug targets, especially against MDR pathogens, so that personalized therapy can be developed (Fig. 22.4). Metabolomics offers new opportunities for biomarker discovery in complex diseases and may provide pathological understanding of diseases beyond traditional technologies.

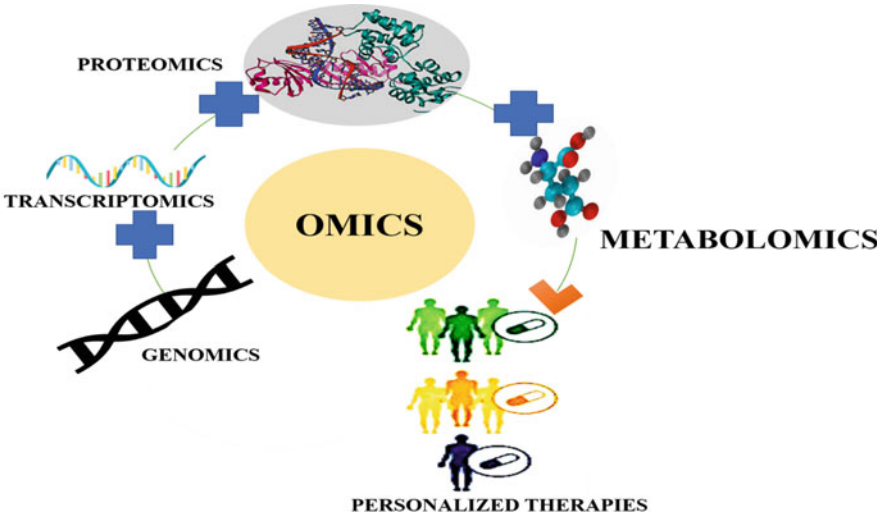
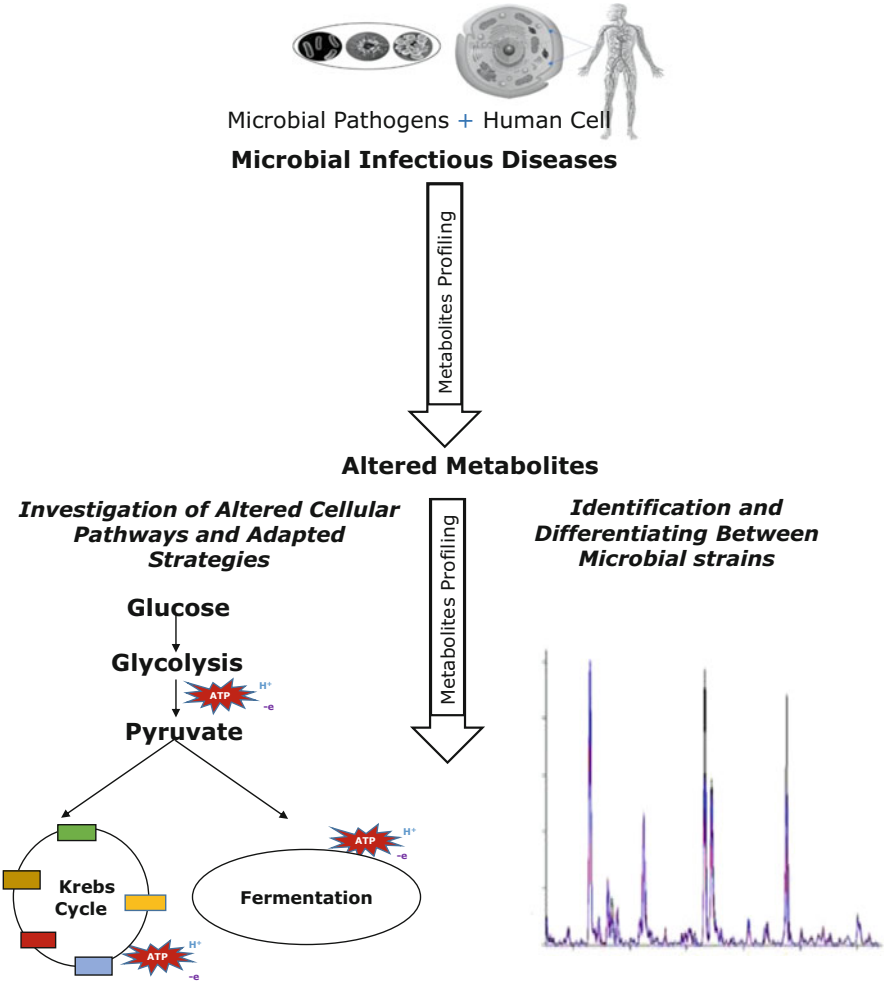


Fig. 22.4 Future perspectives of functional metabolomics



Scheme 22.1 Graphical representation of abstract

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References

1. Clish CB (2015) Metabolomics: an emerging but powerful tool for precision medicine. Cold Spring Harb Mol Case Stud 1(1):a000588. https://doi.org/10.1101/mcs.a000588

2. Kim SJ, Kim SH, Kim JH et al (2016) Understanding metabolomics in biomedical research. Endocrinol Metab (Seoul) 31(1):7–16. https://doi.org/10.3803/EnM.2016.31.1.7
3. Shen B, Yi X, Sun Y et al (2020) Proteomic and metabolomic characterization of COVID-19 patient sera. Cell 182(1):59–72.e15. https://doi.org/10.1016/j.cell.2020.05.032
4. Fernández-García M, Rojo D, Rey-Stolle F et al (2018) Metabolomic-based methods in diagnosis and monitoring infection progression. In: Silvestre R, Torrado E (eds) Metabolic interaction in infection. *Experientia Supplementum*, vol 109. Springer, Cham. https://doi.org/10.1007/978-3-319-74932-7_7
5. Marshall DD, Powers R (2017) Beyond the paradigm: combining mass spectrometry and nuclear magnetic resonance for metabolomics. Prog Nucl Magn Reson Spectrosc 100:1–6. https://doi.org/10.1016/j.pnmrs.2017.01.001
6. Mesías AC, Garg NJ, Zago MP (2019) Redox balance keepers and possible cell functions managed by redox homeostasis in *Trypanosoma cruzi*. Front Cell Infect Microbiol 9:435. https://doi.org/10.3389/fcimb.2019.00435
7. Hertel M, Hartwig S, Schütte E et al (2016) Identification of signature volatiles to discriminate *Candida albicans*, *glabrata*, *krusei* and *tropicalis* using gas chromatography and mass spectrometry. Mycoses 59(2):117–126. https://doi.org/10.1111/myc.12442
8. Rahi P, Vaishampayan P (2020) MALDI-TOF MS applications in microbial ecology studies. Front Microbiol 10:2954. https://doi.org/10.3389/fmicb.2019.02954
9. Introduction-of-NIST-17 (n.d.). https://www.americanlaboratory.com/913-Technical-Articles/340911-Introduction-of-NIST-17-A-Major-Update-of-Mass-Spectral-Libraries-and-Software-at-the-65th-ASMS-Conference-on-Mass-Spectrometry-and-Allied-Topics
10. Ren JL, Zhang AH, Kong L et al (2018a) Advances in mass spectrometry-based metabolomics for investigation of metabolites. RSC Adv 8(40):22335–22350. https://doi.org/10.1039/c8ra01574k
11. Ren W, Rajendran R, Zhao Y et al (2018b) Amino acids as mediators of metabolic cross talk between host and pathogen. Front Immunol 319(018):9. https://doi.org/10.3389/fimmu.2018.00319
12. Marko DC, Saffert RT, Cunningham SA et al (2012) Evaluation of the Bruker Biotyper and Vitek MS matrix-assisted laser desorption ionization–time of flight mass spectrometry systems for identification of nonfermenting gram-negative bacilli isolated from cultures from cystic fibrosis patients. Eur J Clin Microbiol 50(6):2034–2039. https://doi.org/10.1128/JCM.00330-12
13. Hou L, Wei X, Zhuo Y et al (2018) GC-MS-based metabolomics approach to diagnose depression in hepatitis B virus-infected patients with middle or old age. Aging (Albany NY) 10(9):2252. https://doi.org/10.18632/aging.101535
14. Miura D, Fujimura Y, Yamato M et al (2010) Ultrahighly sensitive in situ metabolomic imaging for visualizing spatiotemporal metabolic behaviors. Anal Chem 82(23):9789–9796. https://doi.org/10.1021/ac101998z
15. Emwas AH, Roy R, McKay RT et al (2019) NMR spectroscopy for metabolomics research. Meta 9(7):123. https://doi.org/10.3390/metabo9070123
16. Dona AC, Kyriakides M, Scott F et al (2016) A guide to the identification of metabolites in NMR-based metabonomics/metabolomics experiments. Comput Struct Biotechnol 14:135–153. https://doi.org/10.1016/j.csbj.2016.02.005
17. Jeong S, Eskandari R, Park SM et al (2017) Real-time quantitative analysis of metabolic flux in live cells using a hyperpolarized micromagnetic resonance spectrometer. Sci Adv 3:e1700341. https://doi.org/10.1126/sciadv.1700341
18. Motta A, Paris D, Melck D (2010) Monitoring real-time metabolism of living cells by fast two-dimensional NMR spectroscopy. Anal Chem 82(6):2405–2411. https://doi.org/10.1021/ac9026934
19. Milner JJ, Wang J, Sheridan PA et al (2014) ^{1}H NMR-based profiling reveals differential immune-metabolic networks during influenza virus infection in obese mice. PLoS One 9(5):97238. https://doi.org/10.1371/journal.pone.0097238

20. Lussu M, Camboni T, Piras C et al (2017) H NMR spectroscopy-based metabolomics analysis for the diagnosis of symptomatic E. coli-associated urinary tract infection (UTI). BMC Microbiol 17(1):201. https://doi.org/10.1186/s12866-017-1108-1
21. Ntziachristos V, Pleitez MA, Aime S et al (2019) Emerging technologies to image tissue metabolism. Cell Metab 29(3):518–538. https://doi.org/10.1016/j.cmet.2018.09.004
22. Freedberg DI, Selenko P (2014) Live cell NMR. Annu Rev Biophys 43:171. https://doi.org/10.1146/annurev-biophys-051013-023136
23. Tsedilin AM, Fakhrutdinov AN, Eremin DB et al (2015) How sensitive and accurate are routine NMR and MS measurements. Mendeleev Commun 25(6):454. https://doi.org/10.1016/j.mencom.2015.11.019
24. Giraudeau P (2020) NMR-based metabolomics and fluxomics: developments and future prospects. Analyst 145(7):2457–2472. https://doi.org/10.1039/D0AN00142B
25. Judge MT, Wu Y, Tayyari F et al (2019) Continuous in vivo metabolism by NMR. Front Mol Biosci 6:26. https://doi.org/10.1128/JVI.03134-13
26. Zhu J, Bean HD, Kuo YM et al (2010) Fast detection of volatile organic compounds from bacterial cultures by secondary electrospray ionization-mass spectrometry. J Clin Microbiol 49 (2):769. https://doi.org/10.1128/JCM.00392-10
27. Geldreich EE, Kenner BA, Kabler PW (1964) Occurrence of coliforms, fecal coliforms, and *Streptococci* on vegetation and insects. Appl Microbiol 12(1):63–69. https://doi.org/10.1128/aem.12.1.63-69.1964
28. Phillips M, Basa-Dalay V, Blais J et al (2012) Point-of-care breath test for biomarkers of active pulmonary tuberculosis. Tuberculosis 92(4):314–320. https://doi.org/10.1016/j.tube.2012.04.002
29. Enderby B, Smith D, Carroll W et al (2009) Hydrogen cyanide as a biomarker for *Pseudomonas aeruginosa* in the breath of children with cystic fibrosis. PediatrPulmonol 44(2):142–147. https://doi.org/10.1002/ppul.20963
30. Scott-Thomas AJ, Syhre M, Pattemore PK et al (2010) 2-Aminoacetophenone as a potential breath biomarker for *Pseudomonas aeruginosa* in the cystic fibrosis lung. BMC Pulm Med 10:56. https://doi.org/10.1186/1471-2466-10-56
31. Tong H, Wang Y, Li Y et al (2017) Volatile organic metabolites identify patients with gastric carcinoma, gastric ulcer, or gastritis and control patients. Cancer Cell Int 17(1):1–9. https://doi.org/10.1186/s12935-017-0475-x
32. Garner CE, Smith S, Bardhan PK et al (2009) A pilot study of faecal volatile organic compounds in faeces from cholera patients in Bangladesh to determine their utility in disease diagnosis. Trans R Soc Trop Med Hyg 103(11):1171–1173. https://doi.org/10.1016/j.trstmh.2009.02.004
33. Koo S, Thomas HR, Daniels SD et al (2014) A breath fungal secondary metabolite signature to diagnose invasive aspergillosis. Clin Infect Dis 59(12):1733–1740. https://doi.org/10.1093/cid/ciu725
34. Briard B, Heddergott C, Latg JP (2016) Volatile compounds emitted by *Pseudomonas aeruginosa* stimulate growth of the fungal pathogen *Aspergillus fumigatus*. MBio 7(2). https://doi.org/10.1128/mBio.00219-16
35. Wikoff WR, Anfora AT, Liu J et al (2009) Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci U S A 106(10):3698–3703. https://doi.org/10.1073/pnas.0812874106
36. Pabary R, Huang J, Kumar S et al (2016) Does mass spectrometric breath analysis detect *Pseudomonas aeruginosa* in cystic fibrosis? Eur Respir J 47(3):994–997. https://doi.org/10.1183/13993003.00944-2015
37. Miekisch W, Schubert JK, Noeldge-Schomburg GF (2004) Diagnostic potential of breath analysis–focus on volatile organic compounds. Clin Chim Acta 347(1–2):25–39. https://doi.org/10.1016/j.cccn.2004.04.023
38. Carroll W, Lenney W, Wang T et al (2005) Detection of volatile compounds emitted by *Pseudomonas aeruginosa* using selected ion flow tube mass spectrometry. Pediatr Pulmonol 39(5):452–456. https://doi.org/10.1002/ppul.20170

39. Gilchrist FJ, Belcher J, Jones AM et al (2015) Exhaled breath hydrogen cyanide as a marker of early *Pseudomonas aeruginosa* infection in children with cystic fibrosis. ERJ Open Res 1:00044–02015. https://doi.org/10.1183/23120541.00044-2015
40. Robroeks CM, van Berkel JJ, Dallinga JW et al (2010) Metabolomics of volatile organic compounds in cystic fibrosis patients and controls. Pediatri Res 68(1):75–80. https://doi.org/10.1203/PDR.0b013e3181df4ea0
41. Sethi S, Nanda R, Chakraborty T (2013) Clinical application of volatile organic compound analysis for detecting infectious diseases. Clin Microbiol Rev 26(3):462–475. https://doi.org/10.1128/CMR.00020-13
42. Traxler S, Barkowsky G, Saß R et al (2019) Volatile scents of *influenza A and S. pyogenes* (co-) infected cells. Sci Rep 9(1):18894. https://doi.org/10.1038/s41598-019-55334-0
43. Fend R, Kolk AH, Bessant C et al (2006) Prospects for clinical application of electronic-nose technology to early detection of *Mycobacterium tuberculosis* in culture and sputum. J Clin Microbiol 44(6):2039–2045. https://doi.org/10.1128/JCM.01591-05
44. Ahmed WM, Lawal O, Nijsen TM et al (2017) Exhaled volatile organic compounds of infection: a systematic review. ACS Infect Dis 3(10):695–710. https://doi.org/10.1021/acsinfecdis.7b00088
45. Amarante-Mendes GP, Adjemian S, Branco LM (2018) Pattern recognition receptors and the host cell death molecular machinery. Front Immunol 9:2379. https://doi.org/10.3389/fimmu.2018.02379
46. Eisenreich W, Rudel T, Heesemann J et al (2019) How viral and intracellular bacterial pathogens reprogram the metabolism of host cells to allow their intracellular replication. Front Cell Infect Microbiol 9:42. https://doi.org/10.3389/fcimb.2019.00042
47. Barisón MJ, Rapado LN, Merino EF et al (2017) Metabolomic profiling reveals a finely tuned, starvation-induced metabolic switch in *Trypanosoma cruzi*e pimastigotes. JBiolChem 292 (21):8964–8977. https://doi.org/10.1074/jbc.M117.778522
48. Cumming BM, Pacl HT, Steyn AJ (2020) Relevance of the Warburg effect in tuberculosis for host-directed therapy. Front Cell Infect Microbiol 10:506. https://doi.org/10.3389/fcimb.2020.576596
49. Fuchs TM, Eisenreich W, Heesemann J et al (2012) Metabolic adaptation of human pathogenic and related non-pathogenic bacteria to extra-and intracellular habitats. FEMS Microbiol Rev 36 (2):435–462. https://doi.org/10.1111/j.1574-6976.2011.00301.x
50. Melvin JA, Scheller EV, Miller JF et al (2014) *Bordetella pertussis* pathogenesis: current and future challenges. Nat Rev Microbiol 12(4):274–288. https://doi.org/10.1038/nrmicro3235
51. Mavondo GA, Mavondo J, Peresuh W et al (2019) Malaria Pathophysiology as a Syndrome: Focus on glucose homeostasis in severe malaria and phytotherapeutics management of the Disease. In Parasites and Parasitic Diseases. Intech Open. https://doi.org/10.5772/intechopen.79698
52. Haber A, Friedman S, Lobel L et al (2017) L-glutamine induces expression of *Listeria monocytogenes* virulence genes. PLoS Pathog 13(1):e1006161. https://doi.org/10.1371/journal.ppat.1006161
53. Fontaine KA, Camarda R, Lagunoff M (2014) Vaccinia virus requires glutamine but not glucose for efficient replication. J Virol 88(8):4366–4374. https://doi.org/10.1128/JVI.03134-13
54. Radin JN, Kelliher JL, Parraga Solorzano PK et al (2016) The two-component system ArlRS and alterations in metabolism enable *Staphylococcus aureus* to resist calprotectin-induced manganese starvation. PLoS Pathog 12(11):e1006040. https://doi.org/10.1371/journal.ppat.1006040
55. Olekhnovitch R, Bousso P (2015) Induction, propagation, and activity of host nitric oxide: lessons from *Leishmania* infection. Trends Parasitol 31(12):653–664. https://doi.org/10.1016/j.pt.2015.08.001

56. Plain KM, de Silva K, Earl J et al (2011) Indoleamine 2,3-dioxygenase, tryptophan catabolism, and *Mycobacterium avium* subsp. *paratuberculosis*: a model for chronic mycobacterial infections. Infect Immun 79(9):3821–3832. https://doi.org/10.1128/IAI.05204-11
57. Quinn RA, Lim YW, Maughan H et al (2014) Biogeochemical forces shape the composition and physiology of polymicrobial communities in the cystic fibrosis lung. MBio 5(2):e00956–e00913. https://doi.org/10.1128/mBio.00956-13
58. Fan W, Ye Y, Chen Z et al (2016) Metabolic product response profiles of *Cherax quadricarinatus* towards white spot syndrome virus infection. Dev Comp Immunol 61:236–241. https://doi.org/10.1016/j.dci.2016.04.006
59. Heaton NS, Randall G (2011) Multifaceted roles for lipids in viral infection. Trends Microbiol 19:368–375. https://doi.org/10.1016/j.tim.2011.03.007
60. Miller S, Krijnse-Locker J (2008) Modification of intracellular membrane structures for virus replication. Nat Rev Microbiol 6:363–374. https://doi.org/10.1038/nrmicro1890
61. Sanchez EL, Lagunoff M (2015) Viral activation of cellular metabolism. Virology 479-480:609–618. https://doi.org/10.1016/j.virol.2015.02.038
62. Silwal P, Kim JK, Yuk JM et al (2018) AMP-activated protein kinase and host defense against infection. Int J Mol Sci 19(11):3495. https://doi.org/10.3390/ijms19113495
63. Queiroz A, Pinto IFD, Lima M et al (2019) Lipidomic analysis reveals serum alteration of plasmalogens in patientsinfected with ZIKA virus. Front Microbiol 10:753. https://doi.org/10.3389/fmicb.2019.00753
64. Sander WJ, O'Neil HG, Pohl CH et al (2017) Prostaglandin E_2 as a modulator of viral infections. Front Physiol 8:89. https://doi.org/10.3389/fphys.2017.00089
65. Song JW, Lam SM, Fan X et al (2020) Omics-driven systems interrogation of metabolic dysregulation in COVID-19 pathogenesis. Cell Metab 32(2):188–202. https://doi.org/10.1016/j.cmet.2020.06.016
66. Johnson JW, Fisher JF, Mobashery S (2013) Bacterial cell-wall recycling. Ann N Y Acad Sci 1277(1):54. https://doi.org/10.1111/j.1749-6632.2012.06813.x
67. Zampieri M, Enke T, Chubukov V et al (2017a) Metabolic constraints on the evolution of antibiotic resistance. Mol Syst Biol 13(3):917. https://doi.org/10.15252/msb.20167028
68. Zampieri M, Zimmermann MC et al (2017b) Nontargeted metabolomics reveals the multilevel response to antibiotic perturbations. Cell Rep 19(6):1214–1228. https://doi.org/10.1016/j.celrep.2017.04.002
69. Lin Y, Li W, Sun L et al (2019) Comparative metabolomics shows the metabolic profiles fluctuate in multi-drug resistant *Escherichia coli* strains. J Proteome 207:103468. https://doi.org/10.1016/j.jprot.2019.103468
70. Li L, Liao Z, Yang Y et al (2018) Metabolomic profiling for the identification of potential biomarkers involved in a laboratory azole resistance in *Candida albicans*. PLoS One 13(2):e0192328. https://doi.org/10.1371/journal.pone.0192328
71. Díaz C, del Palacio JP, Valero-Guillén PL et al (2019) Comparative metabolomics between *Mycobacterium tuberculosis* and the MTBVAC vaccine candidate. ACS Infectious Diseases 5(8):1317–1326. https://doi.org/10.1021/acsinfecdis.9b00008