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Metabolomic profiling reveals bacterial metabolic adaptation strategies and new metabolites

Hua Wang[1] and Luiz Pedro S. de Carvalho[2,3]

Abstract

How has metabolomics helped our understanding of infectious diseases? With the threat of antimicrobial resistance to human health around the world, metabolomics has emerged as a powerful tool to comprehensively characterize metabolic pathways to identify new drug targets. However, its output is constrained to known metabolites and their metabolic pathways. Recent advances in instrumentation, methodologies, and computational mass spectrometry have accelerated the use of metabolomics to understand pathogen–host metabolic interactions. This short review discusses a selection of recent publications using metabolomics in infectious/bacterial diseases. These studies unravel the links between metabolic adaptations to environments and host metabolic responses. Moreover, they highlight the importance of enzyme function and metabolite characterization in identifying new drug targets and biomarkers, as well as precision medicine in monitoring therapeutics and diagnosing diseases.

Addresses

[1] Pigments of Life Research Laboratory, School of Infection & Immunity, University of Glasgow, Glasgow G12 8TA, United Kingdom
[2] Mycobacterial Metabolism and Antibiotic Research Laboratory, The Francis Crick Institute, London NW1 1AT, United Kingdom
[3] Department of Chemistry, The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology, Jupiter 33458, United States

Corresponding authors: de Carvalho, Luiz Pedro S (Luiz.Carvalho@crick.ac.uk), (soriodecarval.lp@ufl.edu); Wang, Hua (Hua.Wang.2@glasgow.ac.uk)

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Bacteria, Metabolites, Metabolomics, Metabolic adaptation.

Introduction

Metabolomics has evolved over the years from the concept of "metabolic pattern" introduced in the late 1940s by Roger Williams using paper chromatography to the quantitative measurement of metabolites in the late 1960s from the advancement of gas chromatography (GC) and liquid chromatography (LC) coupled with mass spectrometry (MS) [1]. In 1971, Horning et al. reported the use of GC/MS [2] to collect the "metabolic profile" of human metabolic products, including drug metabolism [3]. "Metabolome" was later introduced by Oliver et al. [4] in 1998, using Fourier-transform infrared spectroscopy to measure the relative change in the concentration of metabolites from the consequence of gene deletion or overexpression in yeast. A year later, Nicholson et al. [5] reported the use of nuclear magnetic resonance for metabolomics as a quantitative method to complement the genomic and proteomic methods to provide a "quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification." In 2005, Smith et al. [6] created the first metabolomic database, METLIN, with over 5000 molecular standards. In 2020, it has over 860,000 molecular standards [7]. In 2009, Xia et al. [8] launched MetaboAnalyst, a freely accessible web-based server for comprehensive metabolomic data analyses, including statistical analysis, metabolite identification, and pathway mapping. To accelerate discoveries and consolidate the vast amount of MS data generated from research, Wang et al. [9] built the Global Natural Products Social Molecular Networking (GNPS), an open-access crowdsourced web-based platform, to share raw, processed, or identified MS data. Hence, metabolomics has emerged as a powerful tool with a wide range of applications in infectious diseases, human health, drug discovery, precision medicine, biotechnology, and agriculture.

This short review focuses on the progress of metabolomics over the last two years to investigate metabolic adaptations of bacterial pathogens that are of global health threat due to antimicrobial resistance (AMR) declared by the World Health Organization, such as *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* [10]. In 2019, AMR contributed to an estimated 4.95 million deaths, including 1.27 million attributed deaths, worldwide [11]. By 2050, AMR is predicted to cause 10 million deaths per year as the leading cause of death worldwide [12]. The current treatment strategy for infection mainly relies on the use of antibiotics to disrupt

essential bacterial cellular processes, such as cell envelope biosynthesis, protein production, and DNA replication. However, AMR bacteria can limit the uptake or actively expel drugs, or modify drugs or drug targets, effectively making antibiotics useless. Metabolomics can not only identify new metabolites and drug targets but also unravel the dynamic metabolic network within an organism and the pathogen–host interactions in response to antibiotics to improve diagnoses and treatment strategies. For example, what and where drugs can work in sync with our immune responses to fight off infections, and when certain drugs are most effective respective of disease stage. The studies presented here further showcase new enzymes, pathways, and natural products identified by metabolomic profiling and combinatory methods as functionally relevant for further study, thereby expanding the metabolome database in facilitating therapeutic target development and diagnosis of bacterial diseases.

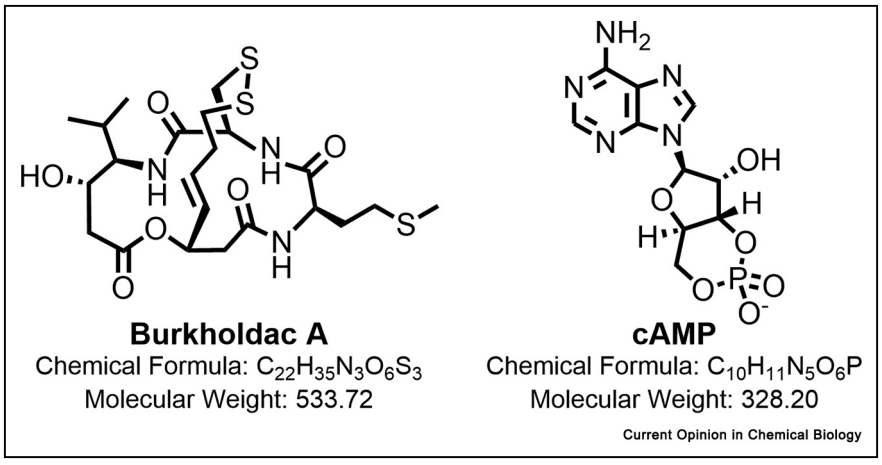
Bacterial metabolism plays a crucial role in nutrient preferences and adaptation to antibiotics and ecological niches

Tuberculosis (TB) is caused by *M. tuberculosis* and closely related species that are collectively called the *Mycobacterium tuberculosis* complex (MTBC) [13]. MTBC has limited genetic diversity, but infection outcomes vary, suggesting environmental and host factors. However, Øyås et al. [14] used non-targeted MS to profile exometabolome changes and derived nutrient uptake and by-product secretion rates and found metabolic phenotypes among 18 MTBC clinical strains to suggest genetic factors. They used flux-based analysis in combination with genomics to correlate the metabolic phenotypes to single-nucleotide polymorphisms (SNPs) effects on amino acid metabolism, the tricarboxylic acid cycle, pyruvate metabolism, glycolysis, and antibiotic sensitivity. They presented a validation of their SNP functionality prediction for the glycolytic enzyme pyruvate kinase, predicting the E220D substitution as functional, which was previously reported to abolish enzyme activity in mainly animal-adapted MTBC strains, inducing pyruvate requirement and glucose toxicity. They showed that clinical MTBC strains with E220D substitution can grow in glucose medium to support the prediction, which is different from animal-adapted strains. Their method can be applied to other closely related bacteria to explore metabolic phenotypes. Such as the work by Chang et al. [15], which used metabolomic profiling and found a correlation between carbon source metabolisms and ecological niches for the genotypically similar *Bacillus* species *B. subtilis*, *B. anthracis*, *B. cereus*, and *S. aureus*. For example, *B. anthracis* showed a decrease in carbohydrate and an increase in amino acid utilization in serum, and both *B. anthracis* and *S. aureus* that thrive in mammalian hosts share similar utilization of nutrients. The two studies applied metabolomics and found a correlation between metabolism and ecological niches.

Another example of metabolic responses is from *Burkholderia* spp., such as *B. cenocepacia*, which causes life-threatening infection in immunocompromised and cystic fibrosis (CF) individuals [16]. Jaiyesimi et al. [17] employed untargeted metabolomics on three *B. cenocepacia* strains grown in synthetic CF medium (SCFM), LB medium, and in the presence and absence of the antibiotic trimethoprim of liquid and solid-phase extracts. They discovered the presence of C13-acyl-homoserine lactone, a quorum-sensing molecule that has not been previously found in *B. cenocepacia*, and pyochelin-type siderophores only when grown in SCFM with trimethoprim. Other *Burkholderia* spp., such as *B. pseudomallei*, causes melioidosis, an infectious disease with a 30%–35% fatality rate [18]. It can be treated with β-lactams, but it is also intrinsically resistant to many antibiotics, and there is no vaccine. Aiosa et al. [19] employed metabolomics, transcriptomics, proteomics, and feature-based molecular networking, a recently developed analysis method in GNPS for MS data analysis [20] to investigate secondary metabolites, such as siderophores and quorum-sensing molecules, from a *B. pseudomallei* surrogate *B. thailandensis* mono- and co-culture with epithelial (LA-4) and macrophage (RAW 264.7) cell lines. They carried out the liquid and solid-phase extraction for untargeted metabolomic profiling and found distinct metabolic features of the mono- and co-cultures between the two cell types. Examples are burkholdac A (Figure 1), burkholdac B, and spiruchostain C, which are potent inhibitors of histone deacetylase, detected in both co-cultures but N-acyl-anthranilic acids, an inhibitor of histone acetyltransferases, were only detected in *B. thailandensis* + RAW 264.7 cells. Both studies demonstrate the importance of environment-dependent production of secondary metabolites, such as in the host infection site versus artificial media, and pathogen metabolic responses as consideration for drug mechanisms and therapeutic intervention strategies.

In another study, Dunphy et al. [21] carried out untargeted metabolomic profiling of *P. aeruginosa* and *S. aureus* monocultures in SCFM. *P. aeruginosa* and *S. aureus* also commonly infect the lungs of CF patients [22]. They found species-specific metabolites and shared nutrients to suggest nutrient competition: *P. aeruginosa* produced 131 metabolites and consumed 49 metabolites, *S. aureus*-specific produced 54 metabolites and consumed 19 metabolites, consisting of amino acids, lactic acid, and glucose. Both consumed amino acids and glucose and, therefore, have shared catabolites, whereas *P. aeruginosa* has unique metabolites that include tryptophan-derived catabolites and

Figure 1



Chemical structure, formula, and molecular weight of burkholdac A and cAMP.

quinoline, from the quinoline signaling system, as potential biomarkers.

Similarly, Jo et al. [23] applied targeted metabolomic profiling on isogenic clinical strains, except for the SCC*mec* type IV gene that confers resistance to oxacillin, of methicillin-susceptible and methicillin-resistant *S. aureus* (MSSA and MRSA) metabolic response to oxacillin, a β-lactam that inhibits peptidoglycan biosynthesis. They found lower levels of intracellular metabolites involved in peptidoglycan biosynthesis, such as UTP, glutamine, lysine, and acetyl-CoA, and up-regulation of proteins related to peptidoglycan biosynthesis in MRSA compared to MSSA. They speculated that MRSA tolerates oxacillin by maintaining cell wall integrity *via* the up-regulation of the peptidoglycan biosynthetic pathway, thereby using the intracellular metabolites involved in peptidoglycan biosynthesis. Furthermore, metabolomic studies with ^{13}C isotope tracing by Liu et al. [24] found that *M. tuberculosis* secondary messenger metabolite cyclic adenosine 3′,5′-monophosphate (cAMP, Figure 1) regulates nitrogen metabolism and peptidoglycan biosynthesis. This was explored by Thomson et al. [25], which functionally characterized a novel mycobacterial phosphodiesterase that lowers cAMP, resulting in a decreased tolerance to cell wall antibiotics, such as ethambutol, D-cycloserine, and vancomycin. These studies show that metabolites that affect cell wall biosynthesis are potentially important to consider in administering antibiotics and developing alternative therapeutic strategies.

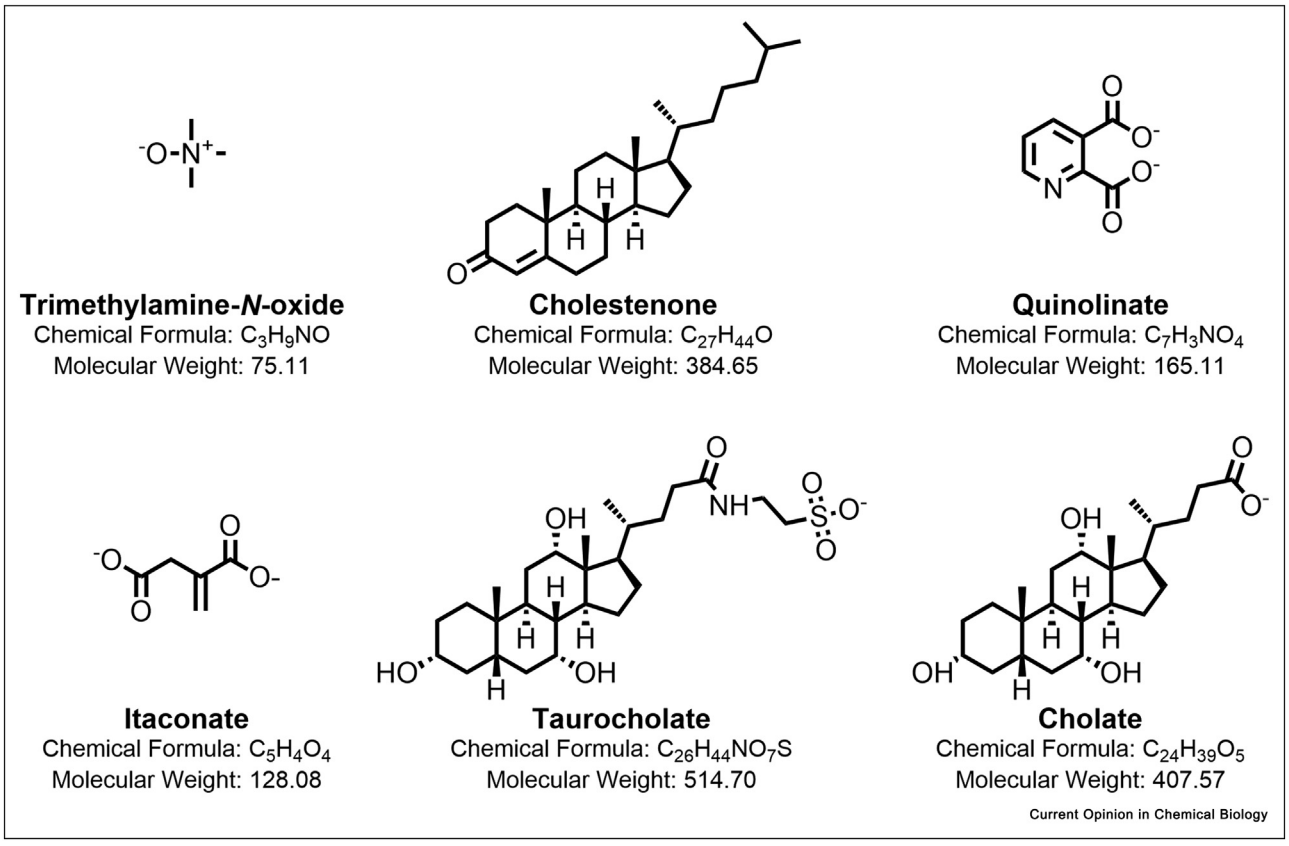
The studies presented here using metabolomic profiling reveal metabolic profiles and metabolic responses between pathogens, host, and drugs are important factors for disease diagnosis and therapeutic intervention strategies and developments.

Bacterial metabolic signatures and co-metabolism

The pathogen–host relationship involves co-metabolism that is potentially unique between pathogen species and their diseases. In this context, co-metabolism is the metabolism of chemicals between the bacteria and the host, similar to the human microbiome, resulting in particular co-metabolites and metabolic profiles. For example, Fernández-García et al. [26] carried out untargeted multiplatform MS (GC/MS, LC/MS, and capillary electrophoresis/MS) analyses to track lung metabolic changes upon *M. tuberculosis* infection in mice at the fourth and ninth week. They reported dynamic changes of 1215 co-metabolites, with 456 putatively annotated, from both lung tissues and *M. tuberculosis*. Examples include a decrease in carbohydrates in the fourth week of infection, a decrease in triacylglycerols, and an increase in amino acids and proteolysis-metabolites throughout the infection, high levels of itaconate, a host immuno-metabolite, in the ninth week, and an increase in oxido-reductive metabolites such as glutathione and related compounds and ergothioneine. Interestingly, they found high levels of trimethylamine-*N*-oxide (TMAO, Figure 2) in the fourth week, presumably produced by the host flavin monooxygenase 3 degradation of trimethylamine, a gut bacterial metabolite, potentially from *M. tuberculosis*. The characterization of TMAO-trimethylamine co-metabolism and metabolic pathways could further help to understand the *M. tuberculosis* adaptation strategies and pathogenesis.

Another co-metabolite of interest is cholestenone (Figure 2), reported by Chandra et al. [27]. The authors carried out global metabolomic profiling of *M. tuberculosis*-infected macrophages and found over 500 metabolites. The infected macrophages had an accumulation of

Figure 2



Chemical structure, formula, and molecular weight of trimethylamine-*N*-oxide, cholestenone, quinolinate, itaconate, taurocholate, and cholate.

itaconate, and interestingly, cholestenone, an oxidized product of cholesterol by *M. tuberculosis*. While the authors did not look for *M. tuberculosis* cholesterol catabolic intermediates, the authors identified the *M. tuberculosis* 3β-hydroxysteroid dehydrogenase (3β-Hsd, Rv1106c) from mutant studies to be responsible for generating cholestenone during *M. tuberculosis* infection but not required for cholesterol utilization. Moreover, cholestenone was detected in TB patient sputum samples. However, other sources of cholestenone, potentially by other cholesterol oxidases from other bacteria, are not ruled out, as low levels of cholestenone are present in the plasma of patients without TB.

Currently, there are limited data on pediatric TB and child-specific biomarkers. Dutta et al. [28] combined metabolomics and transcriptomics and found three metabolites, N-acetylneuraminate, quinolinate, and pyridoxate, as candidate biomarkers for TB disease activity in children, and the combination of 4 metabolites (γ-glutamylalanine, γ-glutamylglycine, glutamine, and pyridoxate) markers to accurately identified treatment response. Similarly, Magdalena et al. [29] performed targeted metabolomic analysis of serum in pediatric patients with active and latent TB patients and found elevated levels of leucine and kynurenine and reduced levels of citrulline and glutamine in blood and serum samples. Interestingly, quinolinate (Figure 2) reported by Dutta et al. is a by-product of the kynurenine pathway, the primary route for tryptophan catabolism that Collins et al. [30] found to be important in monitoring treatment and indicator of bacterial clearance in adult TB patients.

A major host immuno-metabolite is itaconate (Figure 2), which has multiple biological functions, including bactericidal activities, inflammation modulation, and protein modification [31–33]. Interestingly, pathogens, such as *Y. pestis*, *P. aeruginosa*, and *M. tuberculosis*, can assimilate itaconate as a potential carbon source [34,35]. Riquelme et al. [36] applied targeted metabolomics and found itaconate-adapted *P. aeruginosa* replaces its lipopolysaccharides, a major surface immunostimulatory component, with extracellular polysaccharides that protect the bacteria from oxidative stress but also induce myeloid cells to produce itaconate.

In another *P. aeruginosa* study, Mahmud et al. [37] used targeted metabolomics to study *P. aeruginosa* FadE2, an acyl-CoA dehydrogenase (isobutyryl-CoA and isovaleryl-CoA, from valine and leucine catabolism), which mediates the suppression of host energy metabolism and mitochondrial unfolded protein response (UPR), and therefore reduces host survival. The loss of FadE2 restores host energy metabolism, increases ROS generation, and activates UPR. *In vitro*, FadE2 has a preference for isobutyryl-CoA. However, the exact mechanism of how FadE2 modulates UPR is unclear.

An interesting study by Alavi et al. [38] found *Blautia obeum*, a key microbiome bacterium that can reduce *Vibrio cholerae*, a human diarrheal pathogen, by degrading the bile salt taurocholate, which activates the expression of *V. cholerae* virulence genes. They identified the *B. obeum* bile salt hydrolase degrades taurocholate to cholate (Figure 2) using *E. coli* as a heterologous system that constitutively expressed the *B. obeum* bile salt hydrolase and used targeted MS to show a reduction of taurocholate in intestinal homogenates. Furthermore, they showed that the bile salt hydrolase levels in the human gut microbiome are correlated to *V. cholerae* infection outcome.

While the pathogen–host interaction is often viewed as a tug-of-war on nutrients, the studies presented here show that pathogenic or commensal bacteria can utilize host nutrients and affect the host or their environment. Importantly, bacterial and pathogen–host metabolic signatures from co-metabolism can offer insights into the link between bacterial diseases and metabolism.

Outlook: opportunities in discovery

Metabolomics is no longer a "patchwork of results" [39] limited to a dataset from a specific method or sample. The advancement of computational MS and continual additions of new metabolites in databases are truly making metabolomics a powerful tool to comprehensively and quantitatively characterize metabolic pathways. However, it should be noted that metabolomics builds from understanding what are the metabolites, enzymes, and transporters responsible for metabolism. Thus, the incorporation of enzyme functional characterization adds value to the output of metabolomic data, such as the work by Liu et al. [24], Thomson et al. [25], Chandra et al. [27], Riquelme et al. [36], Mahmud et al. [37], and Alavi et al. [38] mentioned above. Other examples include Tian et al. [40] using transcriptomics and metabolomics to characterize SlyA, a transcriptional regulator in *Salmonella typhimurium*, and Wang-Kan et al. [41] applying untargeted metabolomics and found an accumulation of oxidized fatty acids in the endometabolomes of *E. coli* and *Salmonella enterica Typhimurium acrB* mutants, suggesting these oxidized fatty acids are native substrates for AcrB, a pump protein of the multidrug resistance efflux complex AcrAB-TolC. The discovery of potential native substrates offers molecular evidence for substrate–enzyme-transporter-metabolism links and responses.

Besides the applications of metabolomics to understand infectious diseases, Männle et al. [42] offered an exciting outlook from a comparative genomic survey and metabolomic profiling of *Nocardia*, an actinobacterial genus that includes opportunistic pathogenic species. The authors showed *Nocardia* has far more metabolic diversity and biosynthetic gene clusters than that of *Streptomyces* and *Amycolatopsis*, therefore, an important and overlooked source of natural products. An example is their characterization of new mycobactin- and carboxymycobactin-like siderophores formobactin, nocardimicins, and brasilibactin.

In summary, metabolomics, with MS databases and community-based platforms, such as MetaboAnalyst and GNPS, has greatly expanded our understanding of bacterial metabolic responses to their environment and served as a powerful tool in basic research to facilitate discovery science.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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