ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Metabolomics: Global Scenario and Explored Avenues.

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ABSTRACT

Metabolomics, study of small metabolites, represents the final outcome of all genetic and environmental influences in microbes to humans. It finds application in health and disease management, mutation studies, growth condition optimisation, environmental effects etc. Its workflow includes sample collection & preservation, data acquisition & processing, data analysis and metabolite identification. Recent past has witnessed an exponential growth in metabolomics due to development of cost effective/high throughput cutting edge technologies in instrumentation and data acquisition-analyses-interpretation. Its ability to simultaneously capture multiple metabolites, quantitatively and qualitatively, supplants individual metabolite specific assays making it a cost and time effective approach. Identification of key metabolites furthers understanding of biochemical pathways underlying several long/short term patho-physiological changes. These metabolites can be biomarkers for early disease diagnosis, progression monitoring and treatment compliances. Metabolomics has extensively contributed to existing knowledge-base at molecular level in crop and breeding science, embryogenesis, health and diseases, environmental stress and pollutants, herbal medicines etc. Several literature based databases and reference libraries have been created and continuously updated that complement further studies. This review summarises global and Indian picture of metabolomic studies across diverse fields along with a brief account of technical developments and methodologies involved and its path forward.

Keywords: metabolomics, India, methodologies, databases, applications

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ISSN: 0975-8585

CONCEPTUAL BASIS

Metabolomics represent a rapidly growing application driven science of system biology offering a direct and holistic view on the phenotype and physiology of any biological system. The word "metabolomics" represents the comprehensive analysis of all low molecular weight endogenous and exogenous metabolites (<1500 Da) at any given point of time [1-5]. These metabolites are indicators of metabolic pathways occurring during normal cell function and include processes like anabolism, catabolism etc.[6]. The complexity and magnitude of the metabolome depends on the organism as well as biological sample type such as blood, urine, cerebral spinal fluid (CSF) or tissue [7]. Recently volatile organic compounds (VOCs), which denote a wide range of stable chemicals / metabolites volatile at ambient temperature detectable in breathe out air / urine / faeces and sweat, are shown to offer understanding of healthy and disease specific metabolic variations [3, 4, 8-11].

Proximity of metabolomics to physiology, comprehending and reflecting biological effects of a cell or organism at the molecular level, facilitates its high applicability to various fields. Metabolome denotes the final expression of genes in a biological system and yields key information for a more cognitive understanding of systems biology. Phenotypic perturbations are reflected as amplified downstream metabolic adaptations, hence, metabolomics can serve as a tool to further enhance our knowledge on various functions and diseases of organisms [2, 12-15]. Its frequently used synonym, metabonomics has been defined by Nicholson et al.[16] as the quantitative measurement of the time linked multi-parametric response at metabolic level in living systems to pathological / physiological stimuli, drugs, toxins or alterations of genes. In practice, no difference prevails between both metabonomics and metabolomics and are often used interchangeably [17].

Methodologies in metabolomics can be untargeted and targeted. The former comprehensively analyses all the quantifiable analytes including chemical unknowns in a sample, while the latter measures designated groups of chemically and biochemically defined metabolites. Quantitative investigation of metabolites in a designated metabolic pathway or a specific category of compounds encompassing the analysis of a few metabolites is "metabolic profiling". "Metabolic fingerprinting" presents the equitable, global metabolite patterns or fingerprints that can identify differential metabolites in samples which may be a response to disease, environmental or genetic variations. Extracellular metabolites' fingerprint analysis in a cell culture medium reflecting cellular excretion or uptake of metabolites is "metabolic foot-printing" [3, 18, 19]. Study of the metabolome has been going on from decades with early applications in the field of toxicology, inborn metabolic errors and nutrition. In spite of this, metabolomics has received much lesser attention as against, transcriptomics, genomics and proteomics. Latest developments in analytical tools like Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy (MS) have led the extension of metabolomics in many arenas, including medicine, synthetic biology, predictive modelling of plant, animal and microbial systems.

Table 1 presents a representative compilation of some of the recently published reviews on metabolomics studies from diverse fields of study.

Table 1: Reviews on metabolomics

SL. NO	Title of review paper	Ref
1.	Current Challenges in Plant Eco-Metabolomics	[20]
2.	Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies	[21]
3.	Vitamin C in Cancer: A Metabolomics Perspective	[22]
4.	Citrate usage in the leading causes of blindness: new possibilities for the old metabolite	[23]
5.	Microbial markers in colorectal cancer detection and/or prognosis	[24]
6.	Integration of genomics and metabolomics for prioritization of rare disease variants: a 2018 literature review	[25]
7.	Understanding and Designing the Strategies for the Microbe-Mediated Remediation of Environmental Contaminants Using Omics Approaches	[26]
8.	Unraveling Nutritional Regulation of Tacrolimus Biosynthesis in Streptomyces	[27]

	tsukubaensis through omic Approaches	
9.	Functional Genomics Approaches to Studying Symbioses between Legumes and Nitrogen-Fixing Rhizobia	[28]
10.	Concepts and Methods to Access Novel Antibiotics from Actinomycetes	[29]
11.	Using Omics to Understand and Treat Pulmonary Vascular Disease	[30]
12.	Biomarkers for primary biliary cholangitis: current perspectives	[31]
13.	Gene Discovery of Characteristic Metabolic Pathways in the Tea Plant (Camellia sinensis) Using 'Omics'-Based Network Approaches: A Future Perspective	[32]
14.	Metabolomics in Radiation-Induced Biological Dosimetry: A Mini-Review and a Polyamine Study	[33]

METABOLOMIC METHODOLOGY

Sampling and Sample Preparation

There exist multiple approaches for sampling and sample preparation for plants, animals and microbes. Plant sample preparation is chosen depending upon the analytical tools and metabolites of interest. Microbial metabolomics requires appropriate sample-preparation steps such as quenching of enzymatic activities, separation of exo- and endo-metabolomes and a thorough extraction of metabolites [34]. Biological sampling from humans or animals includes both invasive and non-invasive techniques. Biofluids are analysed usually without any extraction steps [35, 36]. Extensively studied biological fluids include blood and urine. Recently saliva and VOCs are attracting wide attention due to its non-invasive nature along with speedy and in-expensive disease screening procedures. Metabolic profiles are highly sensitive to external and internal factors such as hormones, genetic drift, age, rate of metabolism, diet, physical activities, xenobiotics etc. Therefore details concerning diet, physical activities and other validations are to be noted during sample collection. Animal / human samples procured for metabolic analysis need to be cautiously handled (maintaining low temperature and consistent sample extraction). Collected samples are to be preserved at appropriate conditions to prevent

For analysis, protein rich samples such as plasma may require prior extraction and/or filtration. Saliva samples may be treated with acetonitrile (for UPLC-QTOF-MS) or mixed with buffer and D_2O (for NMR). Generally urine needs minimal sample preparation, however due to effect of pH and the ionic strength variation on proton, lyophilisation is performed [37-39]. Frozen samples of urine and faeces for VOCs analyses are thawed at 60°C in a water bath. Urine samples may be treated with base/salt/acid to increase the concentration of VOCs available for analysis. Carboxen/polydimethylsiloxane solid phase micro extraction fibres are used to collect the VOCs from the faeces/urine vial headspaces and inject into the Chromatography (GC) port for GC/ GC-MS analysis [10, 11]. Breathe processing involves collection of the alveolar breath using portable breath collection condensers with filters for separation of saliva / dead space air. The subjects wear a nose clip while inspiring and expiring for sample collection. Collected samples may be stored at -80 °C till, GC-MS or NMR based, analyses are carried out [40-43].

Analytical Techniques

MS and NMR platforms dominate the metabolomic literature, each with its own advantages and limitations [44]. One of the main advantages of using NMR for metabolomics is that most metabolites exhibit characteristic spectra. However the limitations include its higher detection levels (1–5 µM) that require larger sample volume [19, 43]. Recent advances in NMR instrumentation have greatly enhanced its sensitivity. MS offers exceptional selectivity and sensitivity, and is based on mass-to-charge ratio of molecules. There are many existing variations for the ionization as well as analysis of the fragments [3, 43, 45-47]. Although it is destructive to sample, costly and labour intense, MS offers greater sensitivity for metabolite detection. Generally MS systems are coupled to GC, LC or capillary electrophoresis (CE) for the separation of metabolites. GC supports robust separation of volatile and non-volatile metabolites after derivatization. Through LC, separation of different categories of metabolites based on the column and eluent can be achieved. CE has an added advantage of separating polar metabolites in reduced sample volumes. Advancements in MS techniques as well as fast, high-resolution separation systems like capillary HPLC and Ultra-Performance Liquid Chromatography (UPLC) have significantly improved specificity and sensitivity of these techniques for

metabolite analysis [12, 13]. Other techniques used for metabolomics include Infrared and Raman spectroscopy which involve rapid high-throughput global metabolic analysis to discriminate samples [48]. Selection and suitability of these technologies rely on the study objectives and the requisite sensitivity and selectivity.

Processing of raw data and data analysis

The complex spectra from MS / NMR containing signals relating to several metabolites is processed and analysed to obtain relevant data (Figure 1). Data extraction is followed by chemometric analysis using computer assisted statistical interpretation strategies based on the data magnitude and intricacy. A series of data obtained by the use of different techniques such as NMR, MS, UV–Vis and IR spectroscopy enhance the accuracy of metabolite identification. Addition of pure reference compound for confirmation of metabolite identification has been found to be useful [43, 49-56].

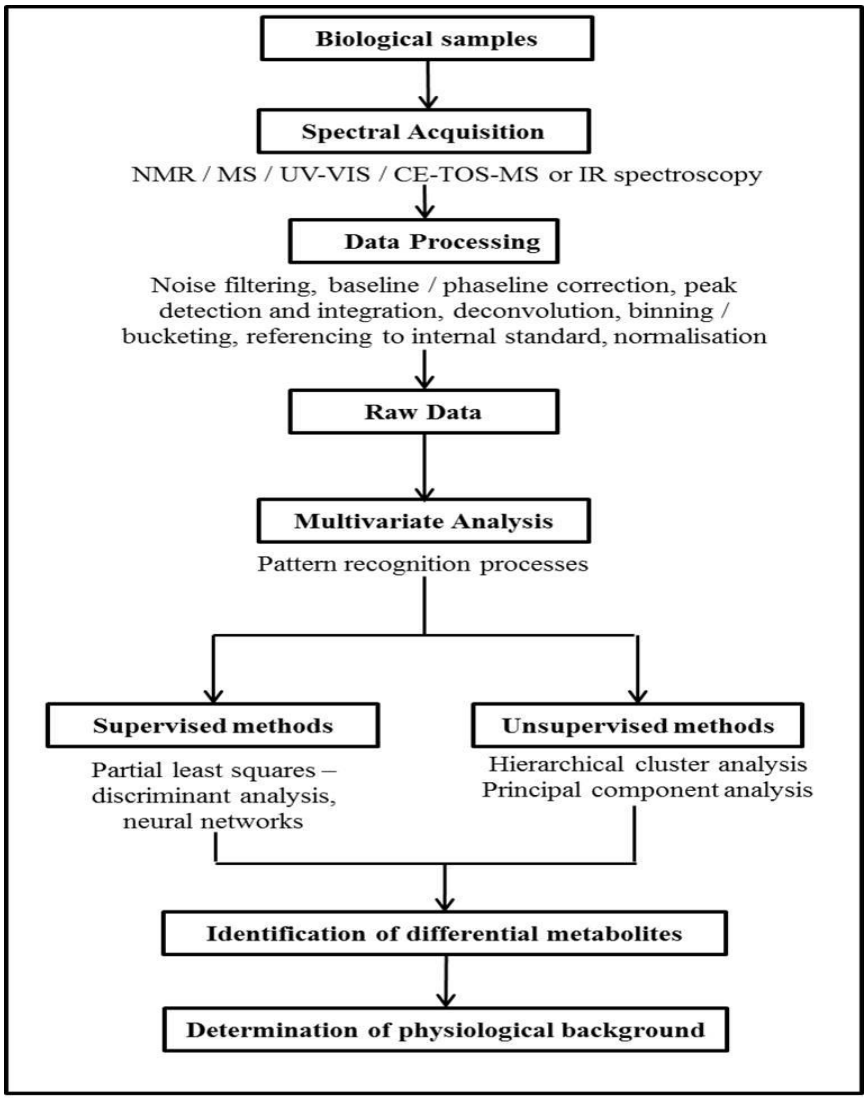

Figure 1: Data Processing Outline

POTENTIAL APPLICATIONS

Microbial Metabolomics

Metabolomics has applications in various microbiological fields such as metabolic pathway identification, microbial engineering, phytopathogen studies, mutant screening, functional gene research and

foodomics. Metabolome analysis at the level of microorganisms plays an important role for developing ways for studying higher organisms [34]. Microbial metabolomic phenotyping experiments can distinguish silent gene knockout mutants leading to identification of specific gene function [57]. Extensive studies have been carried out on yeast metabolomics [58-60]. These include phenotyping of aging-related genes, *in vivo* metabolite protein interactions and foot-printing to distinguish yeast mutants for functional genomics [2, 61-63]. Yeast has been reported to have an estimated 1100 metabolites [64]. Metabolomic studies, on the collaborative dynamics between *E. coli* O157:H7 and *Bifidobacterium longum* representing pathogenic - commensal bacterial gut model, demonstrated that aspartate and serine from *B. longum* are utilized by *E. coli*. This revealed the establishment of a producer - consumer like relationship between them [65]. Similar symbiotic relationship between two isolated colonies of *Bacillus megaterium* and *Ketogulonicigenium vulgare* via metabolites exchange in their ecosystem has also been studied [66].

Metabolomic studies on phytopathogens delineated several facets of these microbes such as identification of gene functions and involvement in transport mechanism and cellular stress, effect of mutant pathogens and identification of fungal diseases on infected hosts [67, 68]. Cao, et al. [69] could characterize a symbiotic relationship between endophytic fungus *Neotyphodiumlolii* and rye grass by metabolomic analysis of three infected and uninfected host tissues. Via ^{1}H-NMR, Keon, et al. [70] have reported significant increase in apoplastic metabolites during infection of *Septoriatritici* (wheat) by *Mycosphaerella graminicola*. Traditionally, most investigations on the interaction of entomopathogens and hosts have been focused at the levels of genome and transcriptome and only recently metabolomics approach has been explored [71-73].

Gut microbes play an important integral part in the intestinal ecosystem and modulate various processes at the systemic level. Thus alterations in commensal microflora were found to influence human health and disease. Studies revealed the impact of gut microbiota in modulating metabotype expression at gut, other organs and biofluids [74].Choline, bile acids and fatty acids were the main chemical classes involved in the microbiome-mammalian metabolism [75, 76].

Apart from metabolites, initial draft of the human metabolome has also reported ≈1200 drugs and ≈3500 food components [6].''Foodomics'' is a discipline that applies diverse omics technologies to examine the connection of food components and diet with health and disease for improved health. Some of the wide applications of metabolomics in this domain include (i) detecting/profiling of toxins/volatile metabolites related to a particular microbial food contamination, (ii) genetically-modified (GM) crops quality analyses, (iii) elucidating variation between harvests from different seasons, (iv) exploring gut microbiota-host metabolic communications with a specific attention on the co-metabolism of food composition and (v) to study the metabolic phenotypic shift in response to diverse weaning diets. Toxins and sub-products in food degraded by micro-organisms are relevant aspects of food safety [12, 77-84]. Representative website resources for microbial metabolomics are presented in table 2.

Table 2: Database Resources for Metabolomics

Table 2a: Databases for human, plant and microbial metabolomics

Studies based on human samples	
Name	**Website**
Human Metabolome Database	http://www.hmdb.ca/ [6, 85, 86]
LIPID Metabolites and Pathways Strategy	http://www.lipidmaps.org/ [87]
Madison-Qingdao Metabolomics Consortium Database	http://mmcd.nmrfam.wisc.edu/ [88]
Small Molecule Pathway Database	http://smpdb.ca/ [89]
Studies based on plant samples	
KaPPA-View	http://kpv.kazusa.or.jp/ [90]
The Metabolome Tomato Database	http://www.ab.wur.nl/moto/

	[91]
KOMICS The Kazusa Metabolomics Portal	http://www.kazusa.or.jp/komics/en/ [90]
Studies based on microbes	
Encyclopaedia of *Escherichia coli* K-12 Genes and Metabolism	http://www.ecocyc.org/ http://www.ecmdb.ca [92]
The Yeast Metabolome Database	http://www.ymdb.ca [93]
SYSTOMONAS	http://www.systomonas.de [94]
Microbial Natural Products Database	http://archive.is/9ZKLI
University of Minnesota Biocatalysis/Biodegradation Database	http://umbbd.ethz.ch/ [95]
BioCyc Pathway/Genome Databases and Pathway Tools Software	http://biocyc.org/ [96]

Table 2 b: General databases for metabolomics

Name	Website
Biochemically, Genetically and Genomically structured	http://bigg.ucsd.edu. [97]
DOME	http://www.comp-sys-bio.org/DOME/domeschema.html
KEGG COMPOUND	http://www.genome.jp/kegg/compound/ http://www.genome.jp/kegg/kegg1a.html [98]
PubChem	http://pubchem.ncbi.nlm.nih.gov/about.html
MetaCyc Metabolic Pathway Database	http://metacyc.org/ [99]
Biological Magnetic Resonance Data Bank/	http://www.bmrb.wisc.edu/metabolomics [100]
MetaboLights	http://www.ebi.ac.uk/metabolights [101]
KNApSAcK Family Databases/	http://kanaya.naist.jp/KNApSAcK_Family [102]
NMRShiftDB	http://nmrshiftdb.nmr.uni-koeln.de/ [103]
MapMan	http://mapman.gabipd.org/web/guest/mapman [104]
Magnetic Resonance Metabolomics Database	http://www.liu.se/hu/mdl/main/ [105]
MassBank	http://massbank.ufz.de/MassBank/index.html
ACD Databases	http://www.acdlabs.com/products/dbs/nmr_db/

ISSN: 0975-8585

Table 2 c: Miscellaneous databases

Name	Website
FooDB	http://foodb.ca/
USDA Database for the Flavonoid Content of Selected Foods	http://www.ars.usda.gov/News/docs.htm?docid=6231
National Microbiological Database	http://www.foodsafety.govt.nz/industry/general/nmd/
DrugBank	http://www.drugbank.ca/ [106]
The Toxin and Toxin Target Database	http://www.t3db.ca/ [107]
Spectral Database for Organic Compounds	http://sdbs.db.aist.go.jp/sdbs/cgi-bin/cre_index.cgi

Table 2 d: Reference Libraries

Name	Website
Golm Metabolome Database	http://gmd.mpimp-golm.mpg.de/ [108]
MS-based NIST/EPA/NIH Mass Spectral Library	http://www.nist.gov/srd/nist1a.cfm
Birmingham Metabolite Library	http://www.bml-nmr.org/ [109]
ChEBI	https://www.ebi.ac.uk/chebi/ [110]

Plant Metabolomics

Metabolomics in plant science has played a key role to understand cellular systems and interpreting gene functions. Plant kingdom cataloguing reveals an approximate of 200,000 metabolites [18].This new conceptual approach is a favourable tool for finding bio-activities from medicinal plants and nutraceutical breeding [111, 112]. A strategy of combined study of the transcriptome, metabolome and metabolic pathway was utilised to understand the governing mechanisms between gene expression and subsequent metabolic phenotype [113]. Studies on the phytochemical diversity and core metabolic systems in *Arabidopsis thaliana* species showed involvement of a small number of key regulatory genes contributing to its functional differentiation. A simple transcript-to-metabolite mode of regulation has been suggested to bring about plant secondary metabolism related dynamics and diversity. Gene-to-metabolite link governing the metabolism against sulphur deficiency induced stress or pathogen infection has been examined for various plants [114]. Prediction of new gene function via exploration of association between expression of gene and metabolite build-up is yet another accomplishment of this integrated analysis. This approach in the model plant *Athaliana* was exhaustively done to construct the AtMetExpress development dataset [113].

Metabolomic approach finds its application in crop and breeding science as well. Genetics in conjunction with metabolomics may lead to the identification of new biomarkers for quality enhancing breeding programmes and can lead to the development of a hybrid cultivar rich in quality enhancing metabolites. It offers enormous potential for enhancing nutritive, pharmaceutical as well as nutraceuticals in plants [111, 115]. This approach has been used for differentiating four chemotypes of *Withaniasomnifera* [114, 116]. GM and metabolomics studies indicated that GM potatoes were similar in composition to the parent cultivar except for the metabolites originating from the introduced gene. Such an approach can largely contribute to the public approval of GM crops [117].

Application of metabolomics is attracting attention in the development of natural products in health care sector. Metabolic fingerprints of herbal medicine products allow rigorous quality control. Metabolome-refined herbal extracts can be combined with other desired biochemical components for developing wide spectrum therapeutic products. It is anticipated to provide crucial evidence-based scientific validations for developing modern phytomedicines and to overcome the bottlenecks in natural product research. Various

other applications of metabolomics include quantitative bioactivity prediction, bioavailability and fate of natural compounds and safety / toxicity assessment of herbal medicines [118-121].

Animal Metabolomics

Different animal models have been used to understand systems biology via metabolomic approach as they share numerous vital cellular/molecular structures and biological characteristics with advanced organisms like humans. *Danio rerio* (Zebra fish), *Caenorhabditis elegans, Drosophila melanogaster* and *Rattusnorvegicus* (rat) are some of such well explored vertebrate models due to their genomic similarity with humans, rapid life cycle, availability of many mutants and analysis amenability [50, 122-124].

Robust correlation between the types and quantities of metabolome and biological activities during embryogenesis of zebra fish showed the importance of metabolomics as a specific finger print during developmental process [123]. Embryos of wild-type and mutant zebra fish were visibly indistinguishable but had distinct metabolomes [122]. NMR- and UPLC-MS analysis of metabolites of wild and metallothionein deletion mutants of *C. elegans*, after exposure to cadmium, demonstrated enhanced production of phytochelatins and protective role of phytochelatin synthase against cadmium toxicity [125]. Metabolomic studies on mitochondrial mutants of *C. elegans*, generally used as models for primary mitochondrial disease, showed the alterations in energy yielding catabolic pathways [126]. Metabolomics has been used to examine the association between metabolites, breeding conditions and phenotype (temperature, stress etc.) in *D. melanogaster*. Changes in metabolite fingerprints were observed in inbred and outbred flies [124]. Perturbations in homeostasis in *D melanogaster*, in response to environmental factors, demonstrated the suitability of this platform for study of environmental stressors on organisms [127-130].

Rat model has been used to study the effects of exposure to diverse environmental stress and pollutants. Neerathilingam, et al. [50] demonstrated that exposure of rats to environmental chemicals such as organophosphates led to presence of signature metabolites in urine. These metabolite variations may serve as markers to screen water safety, nuclear processing and environmental monitoring. They further identified a group of important metabolites for differentiating chronic and acute exposure to tributyl phosphate or triphenyl phosphate [131]. Rapid imbalance in the metabolites associated with TCA cycle (succinate, 2-oxoglutrate and citrate) and catecholamine metabolic pathway (phenylalanine) in rat urine in response to exposure to acute heat stress was observed by Gandhi, et al. [132]. They emphasised a possible role of reduced creatinine and hippurate levels as an initial marker for renal function impairment and altered gut microflora respectively in heat stress. NMR-based metabolomic profiling identified 1- and 3-methylhistidine as potential serum and urine biomarkers of cerivastatin-induced skeletal muscle toxicity and hypertrophy in Sprague–Dawley rats [133].

Medical Metabolomics

Disease specific studies have demonstrated application of metabolomics in diverse clinical areas that influence diagnostics, therapeutics and development of drugs. As primary focus of metabolomics is the exploration of endogenous small metabolite molecules, metabolic profiling of biofluids, VOCs etc. can illustrate associated pathophysiogical changes. This will strengthen early detection, preventive and improved management strategies for several chronic diseases [8, 9, 44, 75, 76, 134, 135].

The world cancer burden continues to increase largely because of aging and growth of world population in conjunction with growing implementation of cancer causing behaviours [136]. Metabolome based studies on carcinogenesis and cancer biology enabled deep examination of specific aspects and allows discovery based analysis. The Warburg effect shed light into cancer cell's selective adoption of anaerobic glycolysis for energy over oxidative phosphorylation. Many common cancer specific mutations were later identified including many metabolic enzymes and hypoxia inducing factors which further supported the positive association of carcinogenesis with Warburg effect [137]. Denkert and co-workers [138] in their study identified α-glycerophosphate and uracil as significant differential metabolites between ovarian carcinoma and borderline tumors. Sarcosine in urine has been identified as a biomarker in the progression of prostate cancer in metabolic studies reported by Sreekumar et al. [139]. Extracellular profiling of metabolites with proliferating cancer and non-cancerous cell lines indicated that the consumption of glycine specifically correlated with cancer cell growth [137]. Quantification of multiple metabolites as against a single entity can offer improved

tumor classification, staging and metastasis identification. This approach even contributes to characterize treatment efficacy / toxicity and to understand overall survival rates in cancer. Hence research on the use of metabolic profiles instead of traditional tumor markers is being carried out [137]. NMR spectra from patients with benign and malignant condition of biliary tract presented distinct patterns on analysis validating the ability of metabolomics approach to possibly complement the available diagnostic approaches [140]. NMR based metabolomic study of gastric cell lines identified 21 metabolites that could differentiate gastric cancer spheroids from normal gastric spheroids. These metabolites were found to correspond mostly to changes in energy metabolism and composition of lipid or lipid derivatives [141]. Correlation of faecal volatile organic metabolites with diarrhoea predominant inflammatory bowel syndrome have also been observed [142].

Central nervous system disorders present difficulty in establishing quantitative diagnostic criteria, hence there still exists a need for establishing marker/s for diagnosis and confirmation of treatment effects. Holmes et al.,[143] reported an imbalance in CSF glucose homeostasis in drug-naive schizophrenics. This suggests that targeted neural metabolomics can help identify patients from healthy subjects and track drug response [143]. Altered pyruvate metabolism in Parkinson's disease patients, and fatty acid and aliphatic amino acid (AA) metabolic perturbations in Huntington disease patients reveal that brain degeneration affects the blood metabolite profile, despite the blood-cerebrospinal barrier [114]. ^{1}H and ^{31}P NMR based plasma and hepatic lipid metabolome was studied in Male Fischer rats with ethanol induced fatty liver. Significant changes in plasma lipid profile was observed that could help early stage diagnosis of this condition [144]. Differential metabolic profile between transplantation survivors and non-survivors projects its potential utility in the assessment of liver transplant status [145]. Differential perturbations were observed in serum metabolites following primary and secondary infections with different dengue virus serotypes. These potential small metabolite biomarkers can delineate pathways / pathogenic and immunologic mechanisms associated with dengue [146]. A comparative analysis of plasma from rheumatoid arthritis patients following anti-tumor necrosis factor therapy showed clear distinction between responders and non-responders [147]. Within preclinical toxicology, areas such as screening and predicting the outcome of therapeutic interventions, biomarkers of safety and mechanism of action continue to have significant impact of metabolomics [14, 148, 149]. Table3 comprehends the perturbations in metabolic pathways associated with diverse human pathologies.

Table 3: Overview of Studies for Disease-Related Marker Metabolites in Human Biofluids [114, 150-154]

Disorders	Perturbed metabolic pathways / significantly altered metabolite
Diabetes and related disorders	3-Indoxylsulfate, glycerophospholipids, bile acids
Cancer	Alpha-glycerolphosphate, uracil, glycine, ^{13}C-enrichment in lactate, alanine, succinate, thiodiglycolic acid, lactic acid, 7-hydroxyoctanoic acid, xylitol, urea, hydroxy proline dipeptide, succinate, N-acetyl-aspartate, 2-hydroxyhippurate, Pyrroline, leucine þ isoleucine, taurine, Taurine, putrescine, leucine þ isoleucine, Leucine þ isoleucine, phenylalanine, alpha-amino butyric acid, phospholipids, triacylglycerides, cholesterol, retinol, L-proline, glycochenodeoxycholic acid, gycocholic acid, taurocholic acid, chenodeoxycholic acid, bile acids, histidine, inosine, acylcarnitines, quinolinate, 4-hydroxybenzoate, gentisate, choline, 2-hydroxybutarate, aspartic acid, kynurenine, cystamine
Respiratory diseases	Lactate, taurine, threonine, arginine, aspartic acid, glutamate, acetate
Metabolic syndromes and related condition	Lysophosphatidylcholine, phosphatidylcholine
Neurological disorders	Threonate, myoinositol, suberate, Glycerol, urea, valine, Free fatty acids, triglycerides, Lactate, citrate, glucose, phosphatidylethanolamine
Inborn errors of metabolism	Propionyl carnitine, γ –butyrobetaine, isovaleryl carnitine, argininosuccinate, methionine, phenylalanine, 4-hydroxyphenylacetate, tyrosine, 2-hydroxyl-3-methylvalerate, 2-hydroxyvalerate, isoleucine, leucine, valine
Crohn´S Disease and Ulcerative	Tryptophan, phenylalanine, arachidonic acid, oleic acid, stearic acid, palmitic acid, 6Z-, 9Z- and 12Z-octadecatrienoic acids, linoleic acid, 4-hydroxyphenylacetylglycine, (Z)-4-

Colitis	hydroxyphenylacetaldehyde-oxime
Celiac Disease	glucose and 3-hydroxybutyric acid, indoxyl sulphate, meta-[hydroxyphenyl]propionic acid, phenylacetylglycine,
Cardiovascular diseases and related conditions	Gamma-amino-butyric acid, uric acid, citrate, trimethylamine N-oxide, choline, betaine
Interstitial Cystitis	Phenylacetylglutamine
Uremia	Alanine, lucine, linoleic acid, myristic acid
Sarcoidosis	3-hydroxybutyrate$^{\uparrow}$, acetoacetate$^{\uparrow}$, carnitine$^{\uparrow}$, cysteine$^{\uparrow}$, homocysteine$^{\uparrow}$, pyruvate$^{\uparrow}$, trimethylamine *n*-oxide$^{\uparrow}$, glutamine$^{\downarrow}$, isoleucine$^{\downarrow}$, succinate$^{\downarrow}$

Compiled from [114, 150-154]

Pathogenesis of a disease can be delineated by the metabolomics in tissues taken from patient and/or animal models. For an improved understanding of the associations between changes in body and pathogenesis, a combined effort of all omics sciences is ideal. Medical metabolomics aims at interpretation of pathogenic mechanisms of a condition to identify drug targets along with highly sensitive-specific biomarkers. These may contribute markedly in the prognosis / diagnosis of such conditions where setting a diagnosis criterion is difficult, thus facilitating early diagnosis of cancer, psychiatric diseases etc. Diagnostic indices constructed based on multiple metabolite concentrations through metabolomic approach are found to be more promising than conventional biochemical markers in terms of specificity and/or sensitivity [150]. Authentication of these markers on a bigger patient population will confirm the efficacy of metabolomics in clinical science.

The growth of environmental metabolomics has paved a way for exposome (build-up of environmental exposures across a lifetime) research. Studies have been carried out on various organisms under diverse stressors at both field and laboratory levels. These include studies on humans for xenobiotic obesity, cadmium exposure etc.; rodents and microorganisms for environmental contaminants; earthworms for soil health; *D melanogaster* for cold stress; terrestrial plants for drought, CO_2, ozone and UV-B radiation exposure; mussels for eco-toxicity; behavioural studies in aquatic animals and a few studies on aquatic plants and arthropods. Significant metabolites have been identified from these studies which could be biomarker candidates for assessment of environmental stressors. Studies have demonstrated that variations in serum metabolites related to smoking are revocable post giving up smoking [151-153]. This may serve as prospective biomarkers to evaluate smoking cessation status and to characterize smoking-related diseases.

TRENDS IN METABOLOMICS RESEARCH IN INDIA

Though accumulated data demonstrates that researchers throughout the world have scientifically contributed towards the advancement of metabolomics, high income countries are found to be the key contributors. India in its course of advancement from a developing to a newly industrialised country is exploring new avenues as well as being exposed to diverse challenges. Population size with cultural complexity and high incidence of systemic/lifestyle diseases in India bring about a need for continuous research towards disease management strategies. Significant work has been carried out in the diagnosis, treatment and prognosis of infectious diseases. Elevated incidence and mortality from diseases such as CVDs, DM, cancer etc. in India, prompt an urgent need for novel specific and sensitive markers for timely diagnosis and improved management. Condition specific metabolite based diagnostic / prognostic markers may complement and speed up the existing treatment procedures and aid population screening operations for non-communicable diseases (NCDs) in high risk areas. Changes in lifestyle and environmental pollution are among its present day priority concerns. This has led to a dynamic research and development sector working towards state of the art strategies for pressing issues like health care, pollution abatement, improving nutritional value of food etc. In agriculture metabolomics finds application in selection of high yielding and disease resistant cultivars. Agriculture being the most important sector of Indian economy, phytometabolomics may help in finding solutions for food shortage and improving the nutrient value of food crops. In addition, India also encounters diverse unfavourable environmental changes resulting from unregulated human interventions. The potentials metabolomics offer may be extended to assess such exposure associated changes for the overall well-being of living organisms. The last five years have seen an escalating leap in metabolomic studies including on human health and diseases, plant, animal/worm /insect models, micro-organisms, food-omics etc. In this part of the

review we have attempted to summarise the Indian contribution along with future directive to advance in this field. A PubMed search for the past five years using keywords 'Metabolomics' and 'India' fetched a total of 272 articles including both review and research papers (Figure 2). Thirty articles were excluded as they fell outside the scope of this review. Table 4a-e compiles the specific areas of a few Indian metabolomics research groups.

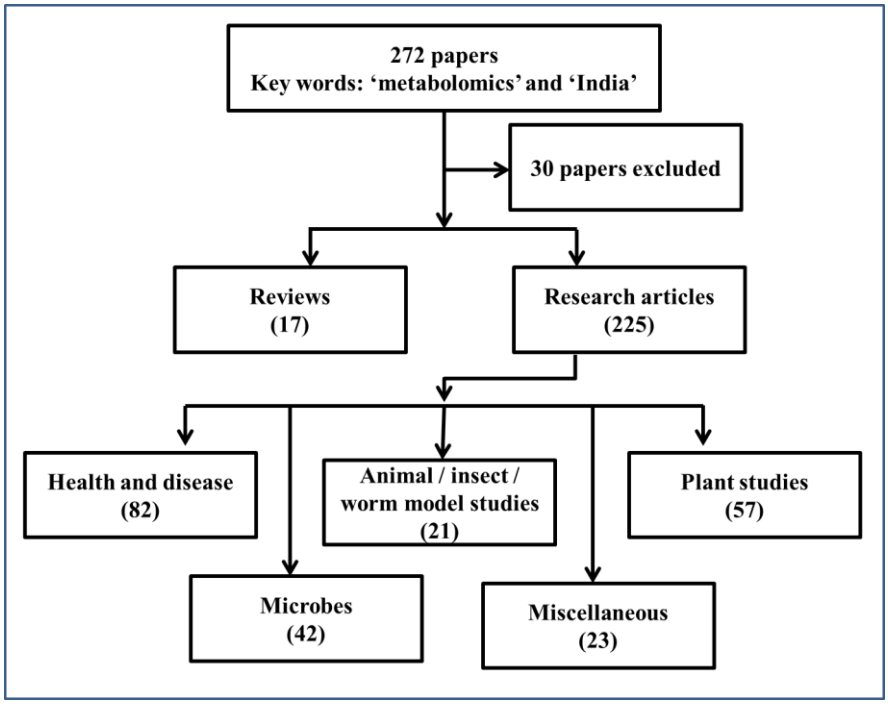

Figure 2: PubMed search outcome

FUTURE DIRECTIONS

Metabolomics is an evolving field of science which has made a marked presence in varied fields of research worldwide. Extensive work is being carried out in diverse fields using the metabolomics approach. However this is majorly concentrated in the developed pockets of the world. Application of metabolomics in research in the developing countries has also been initiated. However there still remains a huge potential to be explored. The current data acquisition and analysis tools are relatively expensive. Development of cost effective and easy-to-use supporting techniques may support the extended implementation of metabolomics. More extensive application of metabolomics beyond the research sector is beckoning. Metabolomics platform also do offer an attractive career option and employment opportunity to a large array of technically trained / skilled individuals from various backgrounds. Researchers ambitious towards establishing new challenging avenues and contributing to the scientific community and social causes alike will find this platform inspirational.

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