The Human Microbiome and Metabolomics: Current Concepts and Applications

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ABSTRACT

The mammalian gastrointestinal tract has co-developed with a large number of microbes in a symbiotic relationship over millions of years. Recent studies indicate that indigenous bacteria are intimate with the intestine and play essential roles in health and disease. In the quest to maintain a stable niche, these prokaryotes influence multiple host metabolic pathways, resulting from an interactive host-microbiota metabolic signaling and impacting strongly on the metabolic phenotypes of the host. Since dysbiosis of the gut bacteria result in alteration in the levels of certain microbial and host co-metabolites, identifying these markers could enhance early detection of diseases. Also, identification of these metabolic finger prints could give us clues as to how to manipulate the microbiome to promote health or treat diseases. This review provides an overview of our current knowledge of the microbiome and metablomics, applications and the future perspectives.

Keywords

Co-metabolites, metabotype, biomarker, dysbiosis

INTRODUCTION

Contrary to the common perception that the human fetal gut is sterile and that the fetus develops in a sterile environment, recent studies have proved the presence of bacteria in the umbilical cord blood, amniotic fluid and the meconium of healthy neonates (Jimenez et al, 2005; Ardissone et al, 2014; Lee et al, 2015). Even though the roles of these early microbes are not yet known, they must be very essential since they are very intimate even during gestation. Recent metagenomic studies have shown that the human microbiome consists of three enterotypes that are independent of age, geographical location or physiological state (such as obesity). The enterotypes are *Bacteroides* (most abundant and most variable genus), *Prevotella* and *Ruminococcus* (Arumugam et al, 2011). The major microbial phyla in the gut are the Bacteroidetes and Firmicutes with minors of Acidobacteria, Actinobacteria, Fusobacteria, Lentisphaerae, Proteobacteria, candidate division TM7, Verrucomicrobia, and Deinococcus–Thermus (Xie and Jia, 2015). Factors such as gut morphology, pH variations in different gut compartments, oxygen and type of nutrient available may however account for the variation in host-specific gut microbiota as well as the variation in where different microbes are located in the gut (Licandro-Seraut et al., 2014). This implies that bacteria require specific properties to colonize different gut compartments. The gut harbors the largest proportion of microbes (≥70%) than any other part of the body (Xie and Jia, 2015) and the major microbiota may reveal the maternal hand-over “seed" microbiota which is subsequently altered by many environmental, genetic, and immunological factors to distinctively shape the microbial composition of an individual (DiBaise et al., 2012; Murphy et al., 2015; Ishimwe et al., 2015). Development of the immune system is strongly influenced by gut microbial metabolites (such as short chain fatty

acids and lipopolysaccharides) and the immune system also influences the composition of the gut microbiota through a host-microbe cross-talk involving complex pathways and different molecules (Candela et al., 2010). The gut microbiota has been compared to an organ due to its extensive metabolic ability and its numerous genes that influence the host metabolic pathways (Musso et al., 2011) and has therefore become an important target for pharmacological agents (Ursell et al., 2014). Attempts to therapeutically manipulate the microbiome range from completely resetting a subject's microbiota, as in the case of fecal microbiota transplant (Rohlke and Stollman, 2012) to the use of symbiotics (Bandyopadhyay and Mandal, 2014). Other interventions include the administration of substances containing defined sets of probiotics or prebiotics to produce specific microbial agents and all these interventions are aimed at modulating the microbiome in distinct ways (Olle, 2013).

The use of humanized mice has made it possible to study the effects of human microbiota in animal models, and this holds promise to a good understanding about how human microbiota influence their host metabolism under controlled conditions. The newly emerging field of "omics" research called metabolomics which aims at comprehensively characterizing small molecule metabolites in biological systems helps to decipher how gut microbes affect metabolism. The gut microbial communities can be studied using metagenomics; a technology which combines both genomic technologies and bioinformatics to access their genetic content (Thomas et al., 2012). Results from metabolomics provide information about the metabolic status and global biochemical events associated with a cellular or biological system (http://metabolomicssociety.org/metabolomics). Metabolomics and metabolic profiling analysis therefore makes it possible to identify biomarkers of diseases as well as the functions of

microbes of rare taxa (Ursell et al., 2014). This work provides an overview of our current knowledge of the microbiome and metablomics, applications and the future perspectives.

METAGENOMICS OF GUT MICROBIOTA

The gut harbors trillions of microbes among which only about 10-30 percent can be cultured by traditional culture-based approaches (Xie and Jia, 2015) and this makes it difficult to effectively identify the gut microbial composition. The most widely used traditional method to determine the numbers and types of bacteria is the viable cell count (Doyle and Buchanan, 2013). Such colony forming unit counting methods are only useful in detecting the numbers and types of viable colony forming bacterial. Apart from strictly anaerobic bacteria being exposed to air during fecal sampling, there are certain bacteria that are viable and culturable but do not form colonies on solid media. Such cells are therefore difficult to count and identify using traditional culture-based approaches. Viable but nonculturable (VBNC) bacteria include enteropathogenic *E. coli*, *Legionella pneumophila, Vibro cholerae, Helicobacter pylori, Vibro vulnificus* (types 1 and 2) and *Vibro alginolyticus* (Fakruddin et al., 2013). This therefore calls for the development of more efficient methods for enumeration and identification of gut microbes.

Over the years, a number of methods based on DNA extraction have been developed to more efficiently identify bacteria and classify them into their taxa. These methods overcome problems such as those posed by VBNC microorganisms and strict anaerobes to give more convincing and accurate results (Ercolini, 2004). Metagenomics, a culture-independent and sequencing-based technique makes it possible to study the functional characteristics of genomes of various microbial populations (Tong et al., 2014). Before the completion of the human genomic sequence in 2003, the first generation sequencing (the Sanger sequencing) was the nucleic acid

sequencing method of choice. The Sanger technique sequences DNA by selectively incorporating chain-terminating dideoxynucleotides by DNA polymerase during *in vitro* DNA replication (Liu et al., 2012). Challenges associated with the Sanger sequencing method includes poor quality in the first 15-40 bases of the sequence due to primer binding and deteriorating quality of sequencing traces after 700-900 bases. However, the recent introduction of one-step Sanger sequencing which combines amplification and sequencing methods allow rapid sequencing of target genes without cloning or prior amplification (Sengupta and Cookson, 2010). In the last decade however, the quest for cheaper and faster genome sequencing methods have increased greatly leading to the development of the next generation sequencing (NGS).

The next generation sequencing (NGS) technology

NGS platforms carry out highly parallel sequencing during which millions of DNA fragments from a single sample are sequenced simultaneously (Grada and Weinbrecht, 2013). This technology facilitates high-throughput sequencing and allows an entire genome to be sequenced in less than one day. NGS platforms include the Genome Sequencer FLX+ System (454 Life Sciences), Genome Analyzer System (Illumina), Ion Semiconductor Sequencing and PacBio Single Molecule Real Time Sequencing (Novais and Thorstenson, 2011). While most NGS platforms follow certain general steps (Figure 1), each uses a different strategy. Generally, DNA sequencing libraries are first generated by clonal amplification by PCR *in vitro*. Secondly, the DNA is sequenced by synthesis (SBS) in such a way that the DNA sequence is determined by the addition of dNTP to the complementary strand rather than through chain-termination. Lastly, the amplified DNA templates are sequenced simultaneously in a massively parallel manner (Anderson and Schrijver, 2010).

The 454 life sciences GS FLX system sequencing procedure.

In this platform, amplification of the target region involves the use of primers with 454 Life Sciences Titanium 5′ A or B adapters for recovering the forward and reverse sequence reads, and multiplex identifier sample tags to allow for up to 44 samples to be sequenced at a time. After the primary PCR amplification, the amplicons are purified, quantified, diluted, and pooled to yield the amplicon library. The next step is the emulsion PCR (emPCR) step at which the amplicon library is mixed with the appropriate A or B complimentary beads in a ratio designed to generate beads with a single DNA fragment bound (Liu et al., 2012). These bead-bound sequences are emulsified with amplification reagents in an oil–water mixture to yield aqueous "microreactors," within which, each DNA is amplified to give numerous clones of DNA molecules per bead. Beads that lack amplified DNA are washed away. The DNA containing beads, DNA polymerase and pyrosequencing enzymes are then loaded onto the PicoTiter Plate for sequencing on the GS FLX, which generates runs with over 600,000 high-quality reads (Trachtenberg and Holcomb, 2013). Though this technology has a fast run time and provides long reads (up to about 1kb) making it easier to match to a reference genome (http://www.454.com), its relatively low throughput (about 1million reads) and high cost of reagents limit its use. Also, the resolution of homopolymer DNA segments (A, G, C and T) remains a challenge. This is because pyrosequencing relies on the amount of light emitted to determine the number of repetitive bases, and hence erroneous base calls can be a problem with homopolymers (http://www.454.com/publications-and-resources).

Illumina genome analyzer

Just like the other platforms, the DNA library is first ligated to oligonucleotide adaptors which incorporate a sequence complementary to oligonucleotides covalently bonded to the surface of the flow cell. After annealing to the bound oligonucleotides, the template DNA molecules are clonally amplified in a modified isothermal PCR reaction in which the DNA molecules bend and form a "bridge" with an adjacent anchor oligonucleotide. The process generates millions of individual clusters containing over one thousand copies of clonally amplified DNA molecules on the surface of the flow cell (Liu et al., 2012). The clusters are then denatured to provide a single stranded template, and a sequencing primer oligonucleotide is hybridized to the strand. During each sequencing cycle, the clonally amplified clusters are exposed to DNA polymerase and a mixture of four nucleotides, each labeled with a distinct fluorescent label. The nucleotides are modified at the 3' end with a cleavable terminator moiety so that only a single nucleotide can be incorporated in each sequencing cycle. At the end of each cycle, the fluorescent signal of each cluster is measured, and both the fluorescent label and 3' terminator moiety are cleaved and removed to regenerate the growing strand for another cycle of nucleotide addition (Anderson and Schrijver, 2010). Though this technology offers the highest throughput of all the platforms and the lowest per second cost, the random scattering of clusters across the flow cells library concentration require strict control. This makes the technology technically challenging (van Dijk et al., 2014). Also, the Illumina software is limited by its inability to handle artifact sequences or trim adapters and hence, some portions of insert-adapter-chimeras or pure adapter dimers may cause false alignment. Unsuccessful alignment can also result in the exclusion of short-insert-size molecules from analysis (Kircher et al., 2011).

Ion semiconductor sequencing (Ion Torrent sequencing)

In ion semiconductor sequencing, a single strand of DNA serves as a template for the sequencing strand (Rothberg et al., 2011). Polymerase and dNTP are added sequentially as in pyrosequencing. When a nucleotide is added to the growing DNA strand, a hydrogen ion is released which changes the pH of the solution. The unattached dNTP molecules are washed out before the next cycle when a different dNTP species is introduced (Eganet al., 2012). The hydrogen ion is detected by a field effect transistor based sensor. The technology is known for its rapid sequencing speed and low operation cost (Metzker, 2005). The system also records natural polymerase-mediated nucleotide incorporation events which enables sequencing to occur in real time. However, just like 454 life sciences GS FLX system, it is difficult to use this system to enumerate long nucleotide repeats because it is difficult to distinguish between signals generated from high repeat numbers of a similar nucleotide but different lengths (Rusk, 2011). It also has a short read length and therefore cannot be used for de novo genome assembly.

PacBio single molecule real time (SMRT) *sequencing*

SMRT sequencing depends on the zero mode wave guide (ZMW) (Cumming et al., 2013). The ZMW is a small structure where a single polymerase molecule is attached to the bottom of a well. The well is illuminated so that a florescent molecule can be detected. Each nucleotide is tagged with a fluorescent molecule. As a fluorescently tagged nucleotide is incorporated, it is identified by a detector and when the next nucleotide is added, the florescent molecule is cleaved (Eid et al., 2009). SMRT not only produces relatively longer and highly accurate DNA sequences from individual unamplified molecules, but can also show where methylated bases occur. It can therefore provide functional information about the DNA methyltransferases that are encoded by

the genome (Roberts et al., 2013). The technology however has high error rates (10-18%) relative to the other sequencing technologies, low total number of reads per run and high capital cost.

Nanopore DNA sequencing technology

This technology is different from the previous ones since it does not require amplification or optical labelling of the DNA. The instruments consist of the device and a consumable flow cell. The flow cell has a sensor chip containing multiple micro-scaffolds, each one supporting an individual biological or solid-state nanopore combined with an enzyme designed to chop a DNA. Other platforms may however pull DNA strands through the pore without enzymatic digestion. A voltage is applied across the membrane in which the pore is set, and the resulting ionic current through the pore is measured. Each DNA base that passes through the pore or near its aperture creates a characteristic disruption in current and the change in current is measured by an application-specific integrated circuit. The resulting electronic signals are interpreted to give information about the DNA sequence (https://www.nanoporetech.com/about-us/summary). However, a challenge for this sequencing technique is that the DNA may separate from the exonuclease attached to the nanopore meaning that, the reading length may be limited.

Other techniques such as quantitative PCR coupled to denaturing gradient gel electrophoresis (DGGE) and fluorescent in situ hybridization (FISH) have also yielded positive results in characterizing the bacteria population and diversity in the gut (Piterina and Pembroke, 2013; González et al., 2014).

METABOLIC POTENTIAL OF THE INTESTINAL MICROBIOTA

The gut microbiota have numerous genes that are responsible for substrate breakdown, protein synthesis, biomass production, production of signaling molecules and anti-microbial compounds (Conlon and Bird, 2015). They biotransform complex indigestible molecules such as dietary fibers and mucin into short chain fatty acids which are of physiological importance to the host (Table 1). Acetate from microbial breakdown of polysaccharides bind to receptors GPR43 and GPR109A in non-hematopoietic cells and protect gut epithelia against colitis (Macia et al., 2014). Bifidobacteria and lactic acid bacteria synthesize folate, riboflavin, cobalamin and other vitamins which serve as coenzymes in host metabolism (LeBlanc et al., 2013). *B. pseudocatenulatum, B.infantis* and other *Bifidobacteria* species produce phytases in the large intestine to digest phytic acids in grains to make calcium, magnesium and phosphates available to the host (Haros et al., 2007). Psycobiotics like *Lactobacillus helveticus, Bifdobacterium longum*, *Escherichia,* and *Enterococcus* produce gamma-aminobutyric acid (GABA), serotonin, dopamine, catecholamines and acetylcholine affect brain functions and behavior (Wall et al., 2014).

Furthermore, gut microbiota play crucial roles in the hosts' xenobiotic metabolism (Figure 2). In the liver, absorbed drugs are detoxified usually by the addition of glucuronic acid, glycine, sulfate, glutathione and taurine to make them water soluble for easy excretion in urine or bile. The metabolites (drug conjugates) secreted with bile into the intestinal lumen are further hydrolyzed by microbial β-glucuronidases, sulfatases and glucosidases before excretion (Pandeya et al., 2012). This is can be observed when a prodrug is administered to germ free mice and their conventional counterparts. Fecal matter of germ free mice administered with prodrug sulfasalazine contained the unmetabolized prodrug while the fecal matter of conventional mice in

the same experiment contained the active drug (5-aminosalicylic acid). This was because the gut microbial azoreductases cleaved the N-N double bond of the prodrug to release active 5-aminosalicylic acid (Carmody and Turnbaugh, 2014).

Characterizing metabolic interactions between host and microbes

With the recent advances in 16S rRNA gene based metagenomic sequence methods coupled with metabolic profiling, it has become possible to study and understand the host-microbial metabolic axis. Since specific gut microbes may have specific effects on the metabolome, alterations or changes in the microbiome can result in important differences in the extracellular metabolome and this may present important findings associated with pathology and toxicology. Analytical methods such as liquid chromatography (LC), gas chromatography (GC), mass spectrometry (MS), MS/MS, ultraviolet/visible spectroscopy (UV/Vis) and nuclear magnetic resonance (NMR) spectroscopy allow sensitive identification of microbial and host cell metabolites in samples such as feces, urine, intestinal contents and tissues. Comparing pretreatment and post treatment metabolomic profiles using multivariate statistics can provide insights into intra- and extracellular regulatory processes involved in the host's metabolic homeostasis (Shim et al., 2015). Metabolomics therefore allow researchers to simultaneously analyze the quantity and quality of metabolites in a host after some treatment and this may help to explore individual needs, foods, and nutrient functionalities.

LC-MS

MS accurately measures the mass-to-charge ratio of ions. In most MS applications, the metabolites in the sample are first separated by techniques such as LC, GC or capillary

electrophoresis before they are detected. The most widely used techniques are GC-MS and LC-MS for detecting a wide variety of compounds (Moco et al., 2007). LC-MS and GC-MS identify metabolites by combining the physical separation ability of LC and GC (e.g. retention time) with the mass analysis ability of MS (accurate mass to charge ratio, fragmentation pattern, isotopic pattern, etc) and comparing them to available MS information or standard compounds. Though LC-MS is a powerful tool for metabolite identification, LC–MS spectra are highly variable and fragmentations are not reproducible even with the same instrument (Gika et al. 2014). GC-MS however gives a relatively characteristic and reproducible figure-print of analyzed compounds (Gika et al. 2014). GC-MS is also limited by its poor performance in analyzing large and nonvolatile samples greater than 700 amu (Theodoridis et al. 2011).

NMR

NMR takes advantage of the spin properties of the nucleus of atoms. The technique enhances structural elucidation of metabolites. NMR fingerprinting is commonly used in acquiring NMR spectra of biofluids, tissue and fecal samples to identify biomarkers. The nuclear transition energy of NMR is low and this makes it less sensitive relative to other techniques such as infrared (IR) or UV/Vis spectroscopy (Ardenkjaer-Larsen et al., 2015). A combination of several analytical methods such as MS-NMR, LC-NMR, and LC-MS-NMR for identifying metabolites is therefore a powerful strategy for biomarker discovery since the full description of a metabolite is best obtained by combining the information acquired from different sources (Moco et al., 2007). Such strategies also require little amounts of the samples for the identification process.

Fourier Transform Infrared Spectroscopy (FTIR)

FT-IR spectroscopy is a technique for nondestructive analysis of biological specimens such as tissue sections. The technique measures vibrational frequency of molecular bonds in response to IR stimulation (Baker et al. 2014). FT-IR spectroscopy has been effective in metabolic fingerprinting of samples for a wide range of metabolite classes including carbohydrates, lipids and amino acids (Ellis and Goodacre, 2006). The minimal sample preparation and the speed, and the non-invasive and non-destructive nature of FTIR allow its application for rapid *in situ* chemical compositional analysis (Chen et al. 2015). However, the limitations of this technique include high signal-to-noise ratio and overlapping spectra (Liu and Xiao, 2014).

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI)

MALDI-MSI is a histology-based mass spectrometry technique that allows for the spatially resolved detection, comprehensive and specific characterization and measurement of compounds in tissue sections or single heterogeneous organ samples (Seeley and Caprioli, 2008). This technology is a powerful tool for investigating biological samples because the identities of compounds or metabolites under investigation do not need to be known in advance (Chaurand et al. 2005). Depending on the matrix used, a wide range of molecular classes, such as proteins, peptides, glycans, lipids and exogenous molecules can be detected (Ly et al. 2016). MSI allows the simultaneous visualization of molecules including a parent molecule and its metabolites by 2D ion intensity maps (Fujimura and Miura, 2014). Although MALDI-MSI is promising for imaging small molecules, many technical problems may exist in its ionization and ion separation procedures. Thus, the numerous competing molecular species can result in ion suppression

effects which affect the detection capability, precision and accuracy of the results obtained (Fujimura and Miura, 2014).

Many researchers have used different metabolomic approaches to study the host-gut microbiota interactions in various animal models (Martin et al., 2008; Swann et al., 2011; Lee et al., 2015; Kim et al., 2015). Wikoff et al, (2009) applied a broad MS-based metabolomic technique to study the effect of gut microbes on blood biochemistry. When plasma extracts from germ-free mice were compared with samples from conventional animals by using several MS-based methods, the plasma concentrations of tryptophan was 40% higher while *N*-acetyltryptophan was 60% higher than the levels recorded in their respective conventional counterparts. Many molecules resulting from phase II drug-like chemical processing of microbial metabolites were also significantly elevated in the plasma of conventional animals indicating the involvement of gut microbiota in the host metabolism. Zheng et al. (2011) also applied untargeted GC-MS and LC-MS based metabolomics to profile the urinary and fecal metabolites of Wistar rats after antibiotic pre-exposure and recovery. They observed a significant alteration in human metabolic profiles as well as a disruption of energy metabolism after antibiotic treatment. Large amounts of tryptophan and tryptamine were found in both feces and urine samples while large amounts of 6-hydroxymelatonin sulfate were found in urine samples and this indicates a depletion of the bacteria that metabolize these molecules. To determine how chronic ethanol consumption alters bile acids in the liver, gastrointestinal tract and serum, Xie et al., (2013) fed rats with a Lieber-DeCarli liquid diet with 38% of calories as ethanol. Large amounts of conjugated bile acids were found in the liver, duodenum and ileum while unconjugated bile acids comprised the largest proportion of measured bile acids in serum, the cecum, and the rectum. Ethanol

administration caused a decrease in taurine-conjugated bile acids in the liver and gastrointestinal tract while unconjugated and glycine-conjugated species increased. The authors proposed that ethanol induced changes in gut microbiota might have caused the alterations in the bile acid profiles. Walkeret al., (2014) also studied the nature and specificity of metabolic profiles related to gut microbiota and obesity using metabolomics and gut microbiome analysis. Obese mouse strains C57BL/6J (C57J) and lean strains C57BL/6N (C57N) were fed with high-fat diet for 3 weeks, but only the C57J mice became obese. 16S-ribosomal RNA comparative sequence analysis revealed higher abundances of *Firmicutes* and *Deferribacteres* in C57J mice and higher abundances of *Bacteroidetes,* and *Proteobacteria* in C57N mice. Metabolomic profiling showed high levels of deoxycholic acid, taurocholic acid sulfate, arachidonic acid and taurocholic acid in the obese group while eicosadienoic acid, I-urobilinogen and urocortisol were present in the lean group. By this study, the researchers accessed some functional aspects in the host–gut microbiome interactions and metabolism as well as new factors that could contribute to high fat diet-induced obesity. In another interesting study to determine if chronic coffee consumption could mitigate negative gut microbiota and metabolomic profile changes induced by a high-fat diet, Cowan et al., (2014) observed a reduction in the *Firmicutes*-to-*Bacteroidetes* ratio usually associated with high-fat feeding. In the serum, high levels of aromatic amino acids, short-chain fatty acids and low levels of branched-chain amino acids were found. Such broad metabolomics profiling studies together with the selective colonization of germ-free animals with specific bacterial population hold promise to decipher the complex interplay between host-gut microbial co-metabolic processes.

Gut microbial-host co-metabolism

Bacteria and their end products of metabolism act as signaling molecules and have direct impacts on the gut, liver, brain, adipose and muscle tissues, which affect host physiology. For instance, the conditions of patients suffering from inflammatory bowel disease (IBD) improve after metronidazole treatment suggesting the role of gut microbes in the disease. Reduced gut microbial diversity due to dysbiosis may mean that specific components that protect the host from pathogenic invaders are absent. Members of the phylum *Bacteroidetes* produce acetate and propionate while the *Firmicutes* phylum produce butyrate from complex carbohydrates and these short chain fatty acids function to suppress inflammation and cancer (Louis et al., 2014). A reduction in short chain fatty acids therefore implies a decrease in members of these phyla and this has been observed in conditions such as metabolic syndrome and cancer (den Besten et al., 2013). During dysbiosis, significantly high or low levels of certain bacteria metabolites are produced in body fluids (blood and urine), exhaled air or on certain body tissues (tongue coating) as well as in feces and these may give clues to disease conditions. De Angelis et al., (2013) found that changes in the levels of *Clostridium* species in the gut cause corresponding changes in the amount of butanoic acid methyl esters, acetic acid methyl esters, pentanoic acid methyl esters and indoles in feces of children with autism. They also observed a positive correlation between *Bacteroides* genus and total free amino acids, NH_3 and propionic acid in the feces of the children. The feces of IBD patients also have fewer short-chain fatty acids, while their urine contain altered amounts of hippurate and *p*-cresyl sulfate relative to those of healthy controls (Le Gall et al., 2011). Much effort are therefore being made to identify non-invasive metabolic fingerprints associated with dysbiosis and their associated diseases (Table 2) so as to develop simple but highly sensitive means of disease detection for early treatment.

Apart from the aforementioned metabolomics techniques, selective ion flow tube mass spectroscopy (SIFT-MS) has been used to identify biomarkers of various disease conditions that result from gut microbial dysbiosis. Patel et al., (2014) used SIFT-MS to identify very high levels of 1-octene, 1-decene and (E)-2-nonene in the breadth of IBD children but these were absent in healthy children. Alkhouri et al., (2015) also used the same technique (SIFT-MS) to identify significantly high levels of isoprene, ammonia and hydrogen sulfide in the breadth of obese children which distinguished them from their lean counterparts. Dragonieri et al., (2007) however failed to use an electronic nose to identify which specific volatile organic compound in exhaled breadth that could distinguish asthmatic patients from controls. Meanwhile, other researchers have used the electronic nose to detect volatile organic compounds in various samples (Montuschi et al., 2013) and all these techniques could be used for rapid detection of biomarkers of various diseases arising from dysbiosis.

CHALLENGES

Since the metabolome is influenced by the gut microbiome, genetic (gene polymorphism and gender) and environmental factors, thus significant differences in metabolite concentrations may be observed even when experimental subjects are given the same treatment. Such factors are therefore worth considering when studying the effects of specific interventions on a system so as to stratify populations. For instance, Robosky et al., (2005) observed that Sprague-Dawley rats housed in different rooms had different urinary metabolic profiles from their cohoused counterparts and this shows how critical environmental consistency is in metabolomics experiments. Although cohousing experimental subjects ensures the same environmental conditions, however, there is evidence that cohousing may alter the metabolic profiles of rats,

probably due to the exchange of gut microbes (Ridaura et al., 2013). Therefore, health status, age and body mass index of experimental subjects must be strictly scrutinized before cohousing. Another challenge has been the situations where some cage-mates tend to eat more than others. This situation would obviously lead to differences in the concentrations of metabolites. Therefore, increasing the population of experimental subjects or the number of volunteers could eliminate such nonspecific variations. Humans and rats are genetically different and hence, making human conclusions from rat experiments could be deceptive. Though orthologues of some human genes are present in mice and may have similar functions, differences in activity may exist (Pang et al., 2011). Hence, developing transgenic humanized gnotiotic mice models could be helpful in obtaining very important information regarding the human microbiome and metabolomics. Disorders such as systemic lupus erythematosus and arthritis have been found to be influenced by gut microbes since many animal and human studies have shown that systemic lupus erythematosus patients have very low *Firmicutes*/*Bacteroidetes* ratio (Hevia et al., 2014). However, human studies on gut microbial-host cometabolites that can serve as evidence and better still markers of the disease are scarce. More human studies are therefore needed to better understand the genesis and treatment of the disease through manipulation of the gut microbiota.

CONCLUSION AND FUTURE PROSPECTS

The benefits derived from the symbiotic relationship between hosts and their microbiome has been known for centuries. However, the complex nature of the host-microbiome co-metabolism makes it quite challenging to clearly understand the mechanisms behind this fascinating field. An understanding of this mechanism through the application of metagenomics and metabolomics would help in devising new strategies for preventing and treating diseases as well as improving

health. In the near future, nutritional research goals may be geared towards predicting future disease susceptibility within the context of an individual's overall heath since metabolomics would reveal biomarkers of diseases at their early stages for appropriate dietary interventions to be made. Moreover, a deeper knowledge of the biochemical signals that enhance stable host–microbe as well as microbe–microbe communication may open doors to new classes of drugs useful for maintaining our host-microbiome associations. The approach would also open up new possibilities for personalizing healthcare and to determine the mechanisms by which microbes influence production of metabolites from drugs and diet. This is possible because metabolites report an individual's response to stimuli and reflect the endpoints of genomic, transcriptomic and proteomic changes as well as gut microbial effects and hence grouping populations of similar metabotypes will be useful in designing drugs for specific individuals.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1 Some gut microbiota, their metabolites, and functions.

Metabolites	Bacteria	Biological functions	References
Vitamins: vitamin K, B group vitamins, pyridoxine, biotin, folate, thiamine, riboflavin.	*Bifidobacterium*, *Lactobacilli*	Serve as cofactors for enzymatic reactions, strengthen immune function, regulate cell proliferation.	Mora et al.,(2008); Nicholson et al., (2012); LeBlanc et al.,(2013)
Conjugated fatty acids, acylglycerols, sphingomyelin, cholesterol, phosphatidylcholines, phosphoethanolamines, triglycerides	*Bifidobacterium*, *Clostridium*, *Roseburia*, *Lactobacillus*, *CitrobacterKlebsiell*, *Enterobacter*.	Enhance intestinal permeability; conjugated fatty acids reduce fatty cell size, decrease body weight and fat mass. Cholesterol is the basis for sterol and bile acid production.	Martins et al., (2015); Shadman et al.,(2013).
Bile acids: cholate, deoxycholate, chenodeoxycholate, taurocholate, glycocholate, ursodeoxycholate, etc	*Bifidobacterium*, *Lactobacillus*, *Enterobacter*, *Clostridium*, *Bacteroides*,	Facilitate lipid absorption, maintain intestinal barrier function, some Bifidobacteria accumulate bile acid into their cell membrane.	Daliri and Lee (2015); **de Diego-Cabero et al., (2015); Kurdi et al., (2003)**

Phenolic, benzoyl, and phenyl derivatives	*Clostridium difficile*, *F. prausnitzii*, *Bifidobacterium*, *Subdoligranulum*, *Lactobacillus*	Urinary hippuricacid may be a biomarker of hypertension and obesity in humans. Urinary3-Nitrotyrosine and 3-Nitro-4-hydroxyphenylacetic acid may indicate asthma, chronic herpatities or diabetes.	Nicholson et al.,(2012); Chao et al.,(2015)
Short-chain fatty acids such as acetate, propionate, hexanoate, butyrate, isobutyrate, 2-methylpropionate, valerate, isovalerate.	*Bifidobacterium sp*, *Roseburia*, *Clostridium*, *Faecalibacterium*, and *Coprococcus*	Decreased colonic pH, decreases circulating cholesterol; inhibit the growth of pathogens; stimulate water and sodium absorption; provide energy to the colonic epithelial cells, prevent high-fat diet-induced obesity by stimulating fat oxidation.	Djamilatou and Daotai (2013); Ringel-Kulka et al.,(2013); Kelly et al.(2015); den den Besten et al.,(2015)
Choline metabolites: methylamine,	*Bifidobacterium*, *Faecalibacteriumpra*	Contribute to cell membrane function, methyl transfer	Craciun and Balskus

dimethylamine, trimethylamine, trimethylamine-*N*-oxide, dimethylglycine, betaine	*usnitzii*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria*,	events, and neurotransmission. Choline is a precursor for the biosynthesis of phospholipids.	(2012); Russell et al.,(2013)
Indole derivatives such as 5-hydroxyindole, *N*-acetyltryptophan, indoxyl sulfate, indole-3-propionate, melatonin, melatonin 6-sulfate, serotonin.	*Clostridium sporogenes*, *E. coli*	Protect against stress-induced GI epithelial damage; indolesulphate is associated with endothelial dysfunction and increased cardiovascular disease.	Wu et al.,(2013); Shimada et al.,(2013)
Polyamines: putrescine, cadaverine, spermidine, spermine	*Campylobacter jejuni*, *Clostridium saccharolyticum*	Polyamines are essential for promoting cell growth, apoptosis, and increased Ca^{2+} accumulation in mitochondria	Graham et al.,(2015); Minois et al.,(2011)
γ-Aminobutyric acid (GABA)	*Lactobacillus brevis*, *L.paracasei*	Inhibits central nervous system functions, promotes hypotension, promotes diuresis, decreases weight loss	Pandeya et al.,(2012)

Polysaccharide A, Polysaccharide B, Exopolysaccharides	*Bacteriodes fragilis, Lactobacillus reuteri, Lactobacillus acidophilus*	Deceases cytokines levels, decreases neutrophil infiltration, modulate the host immune response by activating the mononuclear phagocyte system	Wang et al.,(2012); Chung et al.,(2012); Li et al.,(2014)

Table 2 Studies on human gut microbial-host co-metabolites and their associated diseases.

Disease condition	Cometabolite	Associated microbes	Reference
Obesity	High levels of 2-hydroxyisobutyrate, low concentration of hippuric acid, trigonelline, and xanthine in urine.	*Bacteriodetes* decrease, *Firmicutes* increase, *Roseburia* decrease, *Bifidobacteria* decrease	Calvani et al, (2010); Lecomte et al., (2015).
Crohn's disease	Significantly low levels of hippuric acid in urine compared to healthy controls.	*Significantly high levels of Enterobacteriaceae*, *Pasteurellaceae*, *Veillonellaceae*, and *Fusobacteriaceae and significantly low levels of Erysipelotrichales*, *Bacteroidales*, and *Clostridiales in the rectum.*	Williams et al., (2010); Gevers et al., (2014).
Cardiovascular disease	High levels of Choline,betaine, and TMAO in plasma. Indoxyl sulfate, TMAO	*Increased Desulfovibrio desulfuricans, increased Prevotella genus* in feces.	Viaene et al, (2014). Huang et al, (2012); Tang et al., (2014); Koeth

	and *p*-cresyl sulfate in urine.		et al., (2013).
IBD (Crohn's *Disease* and Ulcerative Colitis)	High levels of 1-octene, 1-decene, (E)-2-nonene and pentane in exhaled breadth.	*Lactobacillus spp* decrease, *Veillonella spp.* increase, *Alphaproteobacteria* and *Proteobacteria* increase.	Patel et al, (2014); Dryahina et al, (2013); Durbán et al., (2013)
Type -2-diabetes	Very high levels of dimethylamine, trimethylamine, and trimethylamine-N-oxide in urine.	*Firmicutes* decrease, *Clostridia* decrease, *Betaproteobacteria* increase, *Bacteroidetes/Firmicutes* ratio increase	Leite et al., (2009); Messana et al, (1998); Tilg and Moschen (2014)
Ciliac disease	Significantly higher levels of acetic, *i*-butyric, and *i*-valeric acid, propionic, and *n*-butyric acids in feces.	High *Bacteroides–E. coli* ratio than *Lactobacillus–Bifidobacterium* species ratio	Tjellström et al., (2005); de Sousa et al., (2014)

Chronic gastritis	Ethylene, γ-aminobutyric acid, and 5-pyroglutamic acid in tongue coating	High levels of *Helicobacter pylori*	Liu et al, (2015); Lee et al., (2015).
Bacterial vaginosis	High levels of putrescine, cadaverine, 2-methyl-2-hydroxybutanoic acid and diethylene glycol in urine.	High levels of *Prevotella, Dialister*, *Veillonella* spp., *Peptostreptococcus*, *Parvimonas*, and *Megasphaera* spp. as well as *Mobiluncus* spp.	Yeoman et al., (2013); Nelson et al., (2015).
Autism	High levels of 3-(3-hydroxyphenyl)-3-hydroxypropionic acid, indoxyl sulfate, N-α-acetyl-L-	Increase in *Bacteroidetes* and *Proteobacteria*; Decrease in *Actinobacteria* and *Firmicutes* levels	Dieme et al., (2015). Shaw (2010); Krajmalnik-Brown et al., (2015).

	arginine, methyl guanidine, and phenylacetylglutamin in urine.		
Renal disease	High levels of indoxyl sulfate and p-cresol in urine	Low levels of *Lactobacillaceae and Prevotellaceae* families. lower numbers of *Bifidobacteria* and higher *Clostridium perfringens*	Vaziri et al., (2013).

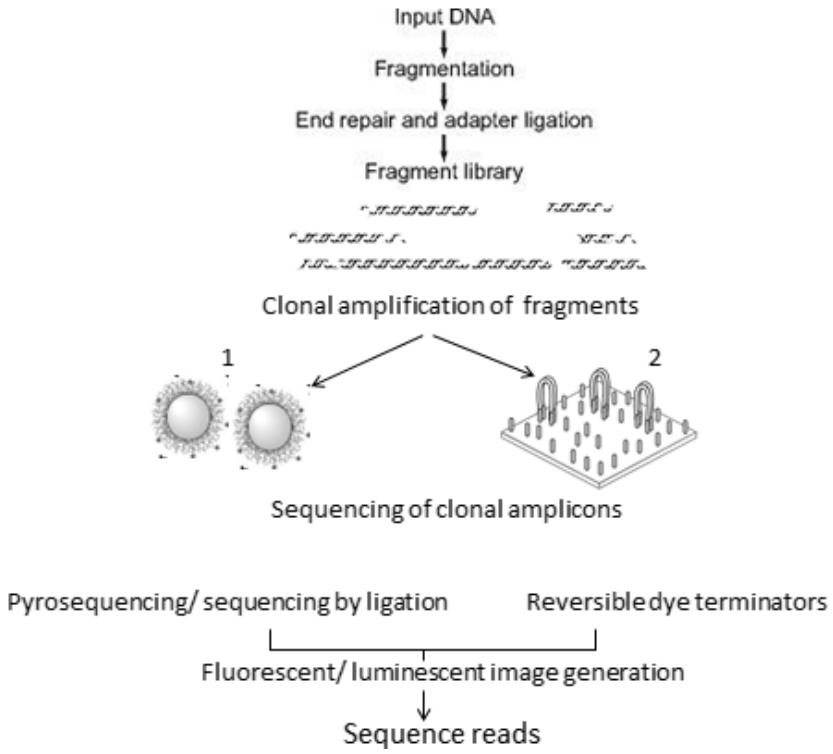

Figure 1 Next generation sequencing process steps for platforms requiring clonally amplified templates (Roche 454, Illumina and Life Technologies). Input DNA is converted to a sequencing library by fragmentation, end repair, and ligation to platform specific oligonucleotide adapters. Individual library fragments are clonally amplified by either (1) water in oil bead–based emulsion PCR or (2) solid surface bridge amplification. Flow cell sequencing of clonal templates generates luminescent or fluorescent images that are algorithmically processed into sequence reads.

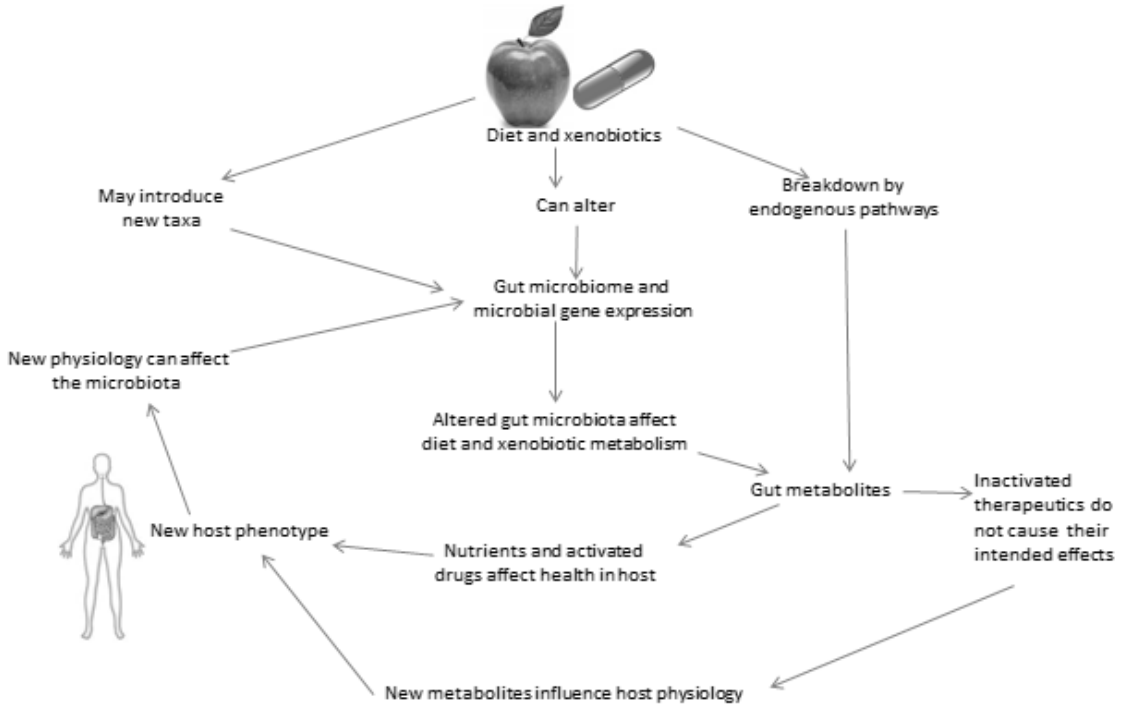

Figure 2 Interactions among host, microbiota, and metabolites. The gut microbiota metabolizes diet and xenobiotics, into metabolites that can be absorbed into the host's bloodstream to affect the host. For instance, gut microbiota may inactivate therapeutic drugs rendering them less effective. Alternatively, drugs may be biotransformed into active or even toxic derivatives. Therefore, alterations in these input substrates influence the available microbial substrates and alter the metabolomic profile of the gut to yield variable effects on the host. The new host phenotype can in turn have a feedback effect on the microbial community.