Oncology
and Radiotherapy©
18(8) 2024: 001-007 • REVIEW ARTICLE

Metabolite identification of the gut microbes: Consequences for health and illness

Devanshu J. Patel[1], R. Jayanthi[2], Lokesh Verma[3], Nitin R. Nangare[4], Pravesh Kumar Sharma[5], Shikhar Gupta[6]

[1] Department of Pharmacology, Parul University, Vadodara, Gujarat, India
[2] Department of Computer Science and IT, School of Sciences, Jain (Deemed-to-be University), Bangalore, India
[3] Centre of Research Impact and Outcome, Chitkara University, Rajpura, Punjab, India
[4] Department of Surgery, Krishna Institute of Medical Sciences, Maharashtra, India
[5] Department of Pharmacy, Vivekananda Global University, Jaipur, India
[6] Chitkara Centre for Research and Development, Chitkara University, Himachal Pradesh, India

ABSTRACT

Human gut microorganisms consume proteins and amino acids to make many chemicals, including branched-chain fatty acids, phenolic, indole, and sulfur compounds, many of which are harmful to the host. In the distal colon, where symptoms of diseases including Ulcerative Colitis (UC) and Colo-Rectal Cancer (CRC) frequently manifest, bacteria mostly ferment amino acids and proteins. The Gut Microbiota's (GM) proteolytic metabolism hasn't been studied as much as its glucose metabolism. With low molecular weight, volatile molecules make up a large portion of metabolites. This research's goal is to give a general indication of the use of analysis techniques to identify and evaluate substances to comprehend the connections among several dietary protein substrates, their related metabolites, and their effects on gastrointestinal health.

Keywords: volatile analysis, indole, phenol, p-cresol, Colo-Rectal Cancer (CRC), protein fermentation

Address for correspondence:

Devanshu J. Patel

Department of Pharmacology, Parul University, Vadodara, Gujarat, India

E-mail: drdevanshu@paruluniversity.ac.in

Word count: 4945 **Tables:** 00 **Figures:** 06 **References:** 11

Received: 14 August, 2024, Manuscript No. OAR-24-145456
Editor Assigned: 17 August, 2024, Pre-QC No. OAR-24-145456(PQ)
Reviewed: 01 September, 2024, QC No. OAR-24-145456(Q)
Revised: 08 September, 2024, Manuscript No. OAR-24-145456(R)
Published: 16 September, 2024, Invoice No. J-145456

INTRODUCTION

Microbial communities coexist with the host in a mutualistic way. A stable, nutrient-rich micro-environmental benefits microbes, and in return, they carry out a crucial role for the hosts, such as the fermentation of food components to produce minerals, vitamins, and metabolites. The immune system's growth and education, as well as the preservation of tissue and immunological homeostasis, all depend on this interaction. Recent research confirms the significance of the immune system tuning toward healthy homeostasis through the constitutive detection of microorganisms and their metabolites. Furthermore, microorganisms give innate and adaptive immune system components local and systemic tonic signals that assist the development of defenses against a variety of diseases [1]. The GM is the dynamic and intricate community of bacteria and other microbes that lives within the digestive system. The composition and functioning of these gut commensals, which have coevolved closely with the host, are linked to a variety of physiologic and pathological features of the host. The GM often interacts with the host by producing dozens of short molecules and metabolites that build up in the digestive method or travel to other distant organs [2]. In host-microbiome interactions, GM metabolites have been identified as signaling molecules, while there is still work to be done in this area. The most essential components are Short-Chain Fatty Acids (SCFA) and Secondary Bile Acids (SBA) (Figure 1). For therapeutic interventions dependent on the GM, a greater comprehension of metabolic host-microbe interactions can be necessary [3].

Figure 1 depicts several methods of manipulating the GM. The pathophysiology of Non-Alcoholic Fatty Liver Disease (NAFLD) is influenced with GM and it is the term for the 100 trillion or so microorganisms that live in the gastrointestinal system. It is becoming better understood that the presence of some GM species alters human physiology by converting the diet consumed into bioactive metabolites. After being absorbed in extra-intestinal organs, such as the liver, their initial target organ for metabolization, these compounds function as signaling messengers [4]. Obesity, diabetes, and other illnesses are influenced by GM, which is crucial to the host's metabolism. It has been difficult to pinpoint the methods behind microbiome-host interaction since the microbiome is incredibly diverse, contains hundreds of species, and is affected by a variety of variables [5]. Finding the metabolites created by GM is a crucial

region of investigate to might provide a fresh sympathetic of how GM influences person strength as well as disorders. Because other sulfur-containing substances also contribute considerably to luminal sulfur concentration, even though hydrogen sulfide (H_2S) appears to have greater evidence linking it to a decline in gut health, this suggests that future studies into how each substance affects the concentration of sulfur in the body as a whole requirement to pay attention to this area. The dietary loading is also influenced by sulfates, which are found naturally in several fruits and vegetables as well as those that have been included as processing additions. Also, it's possible that energy drink manufacturers' increased use of taurine could be a legitimate factor.

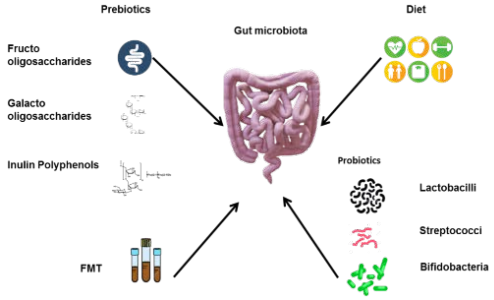

Fig. 1. Specifications are used to illustrate several ways to control the GM

Research covered the most recent research demonstrating how much GM influences disease development [6]. They can assess the potential for bacterial intervention to improve disease activity and positively regulate the makeup of the colonic microbiota based on the evidence they have so far. Study examined our current understanding of how GM and generated microbial chemicals might recount to the Healthy Host's (HH) metabolism or the pathogenesis of typical metabolic disorders [7]. They provide case studies of microbiota-targeted therapies aimed at enhancing metabolic health and offer suggestions for further basic and translational studies in this young and exciting area of study. Study presented a summary of the approaches available for measuring microbial metabolites, explain the main obstacles, and offer a possible paradigm for combining invention metabolites research with mechanistic study [8]. They highlight the research that it has significantly advanced the comprehension of host-microbiota interactions. Research determined the significant correlation between the prevalence of heart disease and changes within plasma Trimethylamine N-Oxide (TMAO) quantities during a 10-year period [9]. Research outlined the molecular markers that connect the prevalence of *Fusobacterium* with CRC characteristics [10]. *F cocultures* and nucleates with patient-derived colorectal cancer cells show a metabolic shift to increased formats synthesis and glutamine usage in cancer in combination with protumor genic effects. They further demonstrate how microbiome-derived formats promote cancer stemness by activating Aryl hydrocarbon Receptor (AhR) signaling, which in turn promotes CRC tumor invasion. Finally, mice treated with F, *nucleatum* exhibit raised tumor incidences or size in addition to a widening of cells that can improve pro-inflammatory profiles. Despite observational studies, they pinpoint formats as an on cometabolite from the gut that is important for the development of CRC. Study compared the metabolites of the mesenteric lymphatic and blood circulation methods and find considerably altered metabolites in the serum and mesentery [11]. They discover that the bulk of the lowered metabolites in the lymph system caused by diet with excessive fat are useful in treating metabolic diseases, demonstrating the lymph system's significant potential to control liver metabolism.

LITERATURE REVIEW

Activity of saccharolytic

The digestion of carbohydrates is inhibited in the gut with bacteria by the genera *Lactobacillus, Bifidobacterium,* and Bacteroides similarly. These main product break downs include SCFA, which comprises butyrate, propionate, as well as acetate, lactate, and several other substances like methane, carbon dioxide, hydrogen, and ethanol. The Embden-Meyerhof-Parnas route that converts glucose molecules toward pyruvate be the primary mechanism by which SCFA as well as other Organic Acids (OA) are produced. Colonocytes use SCFA, primarily butyrate, to generate energy. Less than 5% of SCFA is expelled in feces, with the bulk being absorbed in the lumen. Many substances, including resistant starch, oligosaccharides, proteins, and amino acids are used to make SCFA. The majority of common SCFA is generally acetate, followed by propionate and butyrate. One of the main advantages of employing prebiotics is SCFA synthesis. Prebiotics are non-digestible carbohydrates that pass through the large intestine unharmed and intact before being specifically fermented by bacteria to have medicinal effects.

SCFA concentrations are higher in the ascending colon, where the majority of saccharolytic activity takes place, and gradually declines throughout the hindgut. Luminal SCFA levels can be raised by consuming more resistant starch and Non-Starch Polysaccharides (NSP). It has been found that SCFA inhibits colon cancer cell line proliferation and apoptosis.

Lipid metabolic activity in the gut

Dietary lipids are present as triglycerides in the small intestine. Bile emulsifies these lipids, causing pancreatic lipase to generate monoglycerides and free fatty acids that the stomach can absorb. After being taken up but instead packaged into chylo micrometers, liposomes are prepared on the way to distribute the necessary tissues as well as organs. After being emulsified by bile upon arrival, dietary lipids, which are initially present in the small intestine as triglycerides, can then be absorbed by the gut as free fatty acids and monoglycerides. They are then compressed into chylo micrometers and liposomes after being absorbed, and they are then ready for distribution to the necessary tissues and organs. Figure 2 shows how lipids are metabolized in the gut. In the gut, Saccharolytic action causes SCFA to develop and it has been demonstrated that these SCFA enter the portal circulation after being absorbed there. Colonocytes use the leftover fatty acids that are not absorbed or expelled as energy, and the liver uses them for glucogenesis.

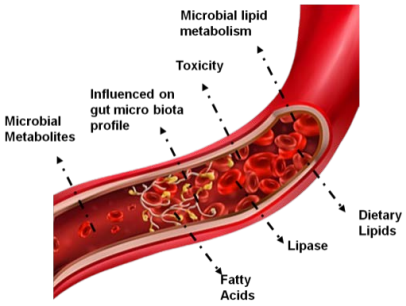

Fig. 2. Gut lipid metabolism

The fact that obese mice have much greater cecal SCFA levels suggests that the microbiota can have an impact on lipid metabolism. It has been proposed that the microbiota can influence lipid metabolism in serum and through the liver in a variety of ways. Particularly low-density lipoproteins and fatty acids are transported by chylomicrons of the liver to adipose tissues. Their release is facilitated by Lipoprotein Lipase (LPL). The microbiota might potentially impact the combination of lipid by enhancing various genes' reactions, Carbohydrate Response Element Binding Protein (CREBP), Fatty Acid Synthase (FAS) counting and Acetyl Coa carboxylase (ACC1), the last two that constitute lipogenesis's rate-limiting enzymes. This is demonstrated in GF mice exposed to the microbiome.

Activity of proteolytes

Proteins from supposedly highly digestible sources can pass upper Gastrointestinal (GIT) absorption through the colon after consumption. Each day, 6 g–18 g of the large intestine's nitrogenous material, which is mostly composed of proteins and amino acids, travels to the colon where it can be fermented by bacteria. Endogenous secretions like mucus in addition to pancreatic secretions contain an impact on what enters the colon, even though dietary protein consumption has a major impact. Figure 3 shows how proteins from food and other sources fare in the human big intestine. In particular, for those who don't consume animal-derived protein, legumes including "peanuts - *Arachis hypogaea (A. hypogaea),* chickpeas - *Cicer arietinum (C. arietinum)* and soybean - *Glycine max (G. max)"* are in the direction of dietary protein load. There are other types of protein besides animal protein that can enter the colon.

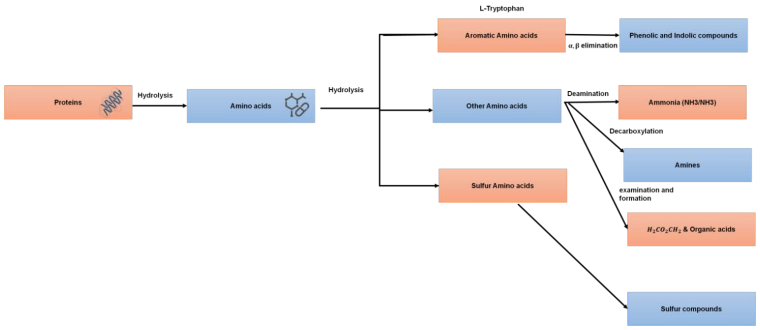

Fig. 3. Protein fate in the human big intestine, both dietary and non-dietary

Compounds of sulfur

Desulfomonas spp. and *Desulfovibrio spp.* are two examples of sulfate-reducing bacteria that can corrode Organic Substrates (OS) into Carbon Dioxide (CO_2) used to sulfur compounds resembling H_2S near reducing sulfate. The sulfur-containing amino acids fermented by bacteria similar to colon H_2S levels enhance by cysteine. Furthermore, pyruvate, H_2S, and NH_3 are produced from cysteine by the enzyme cysteine desulfhydrase. *E. coli* and *Clostridium spp.* are examples of bacteria that have this ability. Figure 4 shows the process by which N-nitroso dimethylamine is created by nitro sating dimethylamine with dinitrogen trioxide.

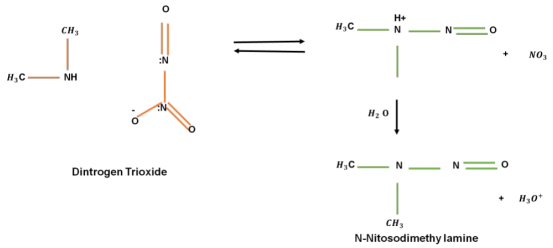

Fig. 4. Reaction mechanism for N-Nitroso dimethylamine synthesis through dinitrogen trioxide nitrosation of dimethylamine

The European Food Standards Agency (EFSA) used to raise dietary protein intake together with amino acid intake, and it also increases the colonic load of the produced metabolites of the amino acids, which can also have harmful effects; Energy drink intake of taurine exceeds that of a typical omnivore diet. This could put a heavy demand on the lumen. Chinese Hamster Ovary (CHO) cells for human HT-29 colonic epithelial cells have both been used to study the genotoxicity of sulfur dioxide. When CHO was present in concentrations lower than those found in feces, as little 250 mol/L significantly damaged the Deoxyribonucleic Acid

(DNA). Even though HT-29 cells seemed to be more resistant to apoptosis, significant genetic damage was discovered. This demonstrates how elevated luminal sulfide levels can affect how well cells act as barriers, resulting in improved chemical penetration. Once within the cell, it can continue doing damage by changing genes.

N-nitroso substances

N-Nitroso Compounds (NOC) is produced internally when nitrite sources nitro sate organic substances like amines. Endogenous N-nitrosation can create unstable and stable nitrosamines from primary and secondary amines, respectively. Base pair transitions have been associated with base pair alkylation of DNA in GI tissues in some cases of colon cancer, and some secondary NOC has been linked to cancer. Hem, a substantial kind of organic iron, has been proposed because of the potential contributor toward high NOC levels, they are frequently measured using terms apparent Total N-Nitroso Compounds (ATNC). Nitric oxide acts as a free radical with a short lifespan caused by its strong reactivity.

The building components for various nitric oxide synthase enzymes are the amino acid arginine, oxygen, as well as Nicotinamide Adenine Dinucleotide Phosphate (NADPH). Tri Methyl Amine (TMA) is a cofactor in the creation of TMAO, which has been connected with increased atherosclerosis 68 within mice along with non-alcoholic fatty liver illness. Phosphodicholine (PC), a dietary component that triggers the liver to create TMAO, releases choline during lipolysis and makes it available for bacterial conversion to TMA.

Ammonia

Amino acid deamination occurs in the colon, where bacteria produce majority of the ammonia in the body. Most amino acid fermentation results in the distal colon, with greater ammonia concentrations. The body regulates its ammonia amounts precisely as the majority is engrossed and rehabilitated toward urea in the liver, leaving only a little quantity in the blood of healthy persons.

Hterocyclic amines

The Maillard process, which occurs between 150°C and 200°C when free amino acids, reducing sugars, creatine, as well as creatinine, interact, produces Hetero Cyclic Amino (HCA) acids. HCA is a substance that typically accumulates on food surfaces and is triggered by intense heat and little moisture. The most common HCA were 2-amino-3, 8-dimethylimidazo quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazopyridinebased on the research on cooking methods conducted in several different countries throughout the world. Figure 5 shows the mechanisms through which tryptophan and tyrosine metabolites degrade.

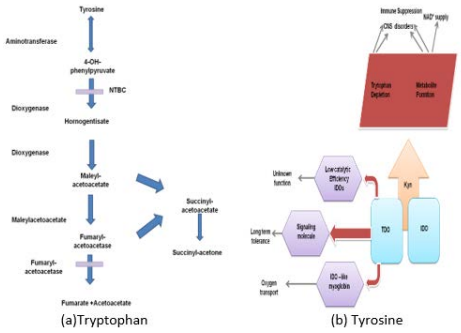

Fig. 5. Tryptophan and tyrosine metabolite degradation mechanisms

Organic Acid (OA)

SCFA produced by the fermentation of carbohydrates and SCFA created by the breakdown of proteins and amino acids breakdowns in the large intestine are the two components. A single source of Branched-Chain Fatty Acids (BCFA) is proteins and amino acids. BCFA produces aldehydes and Longer-Chain Fatty Acid (LCFA) includes isobutyrate. More essential amino acids, including as alanine and glutamine that together give energy for the digestive system, can use BCFA as nitrogen donors. It was made up of leucine, isoleucine, and valine, three BCFAs. Since colonocytes mostly use butyrate as an energy source, SCFAs primarily have favorable effects. With its reputation for being bad for cluster IV, XVI, colon fitness and clostridia clusters XIV a, b are break down carbohydrates to produce significant quantities of butyrate. The formation of SCFA involves many different genera. Proteobacteria, Bifidobacterium species, and the phylum Bacteroides-prevotella all create additional SCFAs such as acetate and propionate.

Phenolic and indolic compounds

Indolic (indole and skatole) and phenolic (phenol and p-Cresol) compounds are created among the terrible aromatic amino acid circumstances. Bacteroides species and Clostridium species are two types of bacteria that contribute in the metabolism of aromatic amino acids. Urine contains phenol (p-cresol) that has been cleaned up in the liver, as it can also be detected in considerable amounts in the lumen of sudden deaths and the feces of both healthy persons and those with Inflammatory Bowel Diseases (IBD). Phenol has been associated as a cocarcinogen similarly it can contribute to the N-nitrosation of various amines for producing nitro-amines called carcinogens. In an acidic environment, nitrite nitro sated phenol more quickly than amines could, produce diazo quinone, a mutagen which acts as bactericidal in larger concentrations, and p-nitrophenol.

Large bowel disorders and dietary metabolites

For the host to remain healthy, connections between the microbiota in addition to host immunity are crucial. Although the fundamental processes are still unknown, IBD such as CRC and Irritable Bowel Syndrome (IBS) with UC have all been connected to microbiota components.

Inflammatory Bowel Disease (IBD)

Colon mucosal inflammation, or UC, can cause sores or ulcers. It can cause ulcers or bleed across the entire colon. As germ-free mice

don't exhibit mucosal inflammation, the microbiota's role in these animals has been examined. Crohn's Disease (CD) can affect GIT regions, including the anus and oral region, compared to UC and it affects only the rectum and colon. Generally, CD first occurs in the ileocecal area.

Pro-inflammatory cytokines include Tumor Necrosis Factors (TNF) and Interleukin-1 (IL-1) are present, mutually that are connected to apoptosis, increases the likelihood that the sickness is an autoimmune ailment even if multiple studies have not found a clear connection. Inflammation in the impacted tissues has also been connected to associated gut bacteria.

Colorectal cancer

Malignant neoplasms, or cancers, are growths of tumors, frequently in the epithelium, brought on by unchecked replication of damaged cells that have the capacity to metastasis and migrate to other tissues or harm nearby cells. When examining the connections between nutrition and cancer, environmental factors are crucial. Recently, dietary metabolites of specific food components have been connected to cancer incidence. It was discovered that there is a significant relationship between fat and protein, especially animal and processed meat proteins. The favorable relationship between cancer risk and fat removal altered when animal proteins were eliminated from the diet, proving that consuming complete proteins plays a substantial role in the association between CRC incidence and fat removal. An increasing diet of animal-derived proteins, predominantly red as well as processed pork has been linked to CRC in three analyses of epidemiological and prospective studies. The hemoglobin concentrations of red and white meat are significantly different, especially in terms of iron content, it has been thoroughly investigated as a CRC risk factor based on its catalytic role in NOC production.

Metabolite assessments

Many techniques can be used to study human metabolomes, which are made up of intrinsic and extrinsic components. The study of the metabolome's chemical components, often referred to as "metabonomic," which are found in biological sample as a consequence of metabolic activity, is known as "metabolomics." Based on the modulation of intrinsic and extrinsic factors such as aging, health status, and body composition as well as extrinsic factors such as diet and drug intake, several analytical methods are used in metabolomics to identify, recognize and measure a range of chemical alterations in biological materials.

Volatile metabolite analysis

Chemicals that can transition from a phase of gaseous to one of liquid or solid are referred to as volatile compounds. Hydrocarbons, alcohols, ketones, aldehydes, ester compounds, and organic acids are some of these carbon-based compounds. Volatile Organic Compounds (VOCs) are present in a wide range of environments, including people breathe, urine, skin, perspiration, blood, and feces. Endogenous metabolic processes or ambient chemical absorption result in the production of an enormous number of distinct molecules. Over 1000 volatiles found in healthy person's breath, some of which are common (such as acetone, ethane, and methanol) and can indicate that the individual has a healthy metabolism. It has been possible to identify volatile patterns in volunteer participants' feces that had UC and Campylobacter Jejuni (CJ)-related gut illnesses thanks to the efficient application of these approaches. For the intent of Nuclear Magnet Resonance (NMR), Liquid Chromatography and Mass Spectrometry (LC-MS) were used to identify portable and non-volatile components.

Non-volatile metabolites assessments

NMR spectroscopy, one of the most frequently used methods in metabolomics, is thought to offer a reasonably comprehensive perspective of metabolites obtained under particular circumstances. The key factors influencing its widespread use are its capacity to quantify compounds at micro-molar concentrations and non-destructive sample analysis. Since NMR can provide detailed information on the metabolites present as well as structural data on molecules, which is crucial when unidentified substances are found, it is utilized to evaluate a variety of physiological fluids. As a result, NMR is frequently employed in investigations involving dietary nutrients and the host-micro-biome in both animal and human research. This appears to be overcome by hydrophilic interaction chromatography and LC-MS, enabling more accurate identification of substances like amino acids and nucleotides. Chromatographic separation of several metabolites from human biological samples can occasionally be issue to their polar nature. It is crucial to calculate relative concentrations before identifying a likely chemical structure in metabolite investigations since LC-MS can collect relative peak abundances of relatively unidentified chemicals and their m/z. Combining approaches is always preferable because it enables a much more comprehensive understanding of the metabolic profile; however, it is simple to fall into the danger of gathering excessive amounts of data, which is dangerous because it increases the variability in the host's response and calls for more data to reduce it. However, depending on the metabolic reaction one is interested in, choosing the right instruments requires careful consideration. The knowledge that is not gathered via other techniques will always be interesting.

Analysis of tyrosine metabolites

Tyrosine was easily iodinated by thyroid peroxidase under the same circumstances that were utilized to iodinate protein. In figure 6, the outcomes of a recent examination contrasting the early rates of iodination of tyrosine are displayed.

The rate of tyrosine is 0.88, and 73% of Iodide is utilized in this research. The investigation findings compare the iodination of tyrosine, and mono iodotyrosinen using a less purified thyroid peroxidase. Hydrogen peroxide (H_2O_2) was produced during the 15-min incubation period by glucose-glucose oxidase. In these circumstances, the ability of mono iodotyrosine to act as an iodine acceptor for thyroid peroxidase-catalyzed iodination was comparable to that of tyrosine.

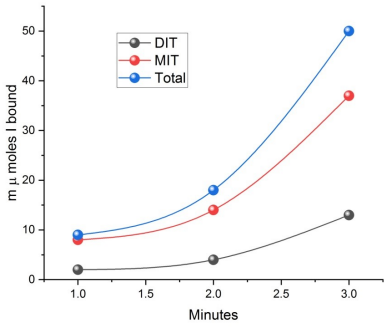

Fig. 6. Early rates of iodination of tyrosine

CONCLUSIONS

The study gives a general overview of the impacts of food components on the catabolic microbiota types and the consequences of consumption on host health. The growing body of clinical evidence suggesting health attenuation depending on specific food decisions and failure in health status relying on population-level dietary choices is fueling interest in diet-host interactions. Because various metabolomics methodologies rely on the impacts on metabolite activities depending on communications with host tissues and other prevailing metabolites, their application in biological modeling and metabolomics could be complementary.

REFERENCES

1. Dang AT, Marsland BJ. Microbes, metabolites, and the gut–lung axis. Mucosal Immunol. 2019;12:843-850.
2. Liu Y, Hou Y, Wang G, Zheng X, Hao H. Gut microbial metabolites of aromatic amino acids as signals in host–microbe interplay. Trends Endocrinol. Metab. 2020;31:818-834.
3. Zheng X, Cai X, Hao H. Emerging targetome and signalome landscape of gut microbial metabolites. Cell metabolism. 2022;34:35-58.
4. Cao Y, Aquino-Martinez R, Hutchison E, Allayee H, Lusis AJ, et al. Role of gut microbe-derived metabolites in cardiometabolic diseases: Systems based approach. Molecular metabolism. 2022;64:101557.
5. Driuchina A, Hintikka J, Lehtonen M, Keski-Rahkonen P, O'Connell T, et al. Identification of gut microbial lysine and histidine degradation and CYP-dependent metabolites as biomarkers of fatty liver disease. MBio. 2023;14:2663-2622.
6. Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. Microb Ecol Health Dis. 2015;26:26191.
7. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. Nat Rev Microbiol. 2021;19:55-71.
8. Krautkramer KA, Fan J, Backhed F. Gut microbial metabolites as multikingdom intermediates. Nat Rev Microbiol. 2021;19:77-94.
9. Heianza Y, Ma W, DiDonato JA, Sun Q, Rimm EB, et al. Long-term changes in gut microbial metabolite trimethylamine N-oxide and coronary heart disease risk. J Am Coll Cardiol. 2020;75:763-772.
10. Ternes D, Tsenkova M, Pozdeev VI, Meyers M, Koncina E, et al. The gut microbial metabolite formate exacerbates colorectal cancer progression. Nat Metab. 2022;4:458-475.
11. Yu Y, Liu B, Liu X, Zhang X, Zhang W, et al. Mesenteric lymph system constitutes the second route in gut–liver axis and transports metabolism-modulating gut microbial metabolites. J Genet Genom. 2022;49:612-623.