



Review Article

Roles of gut microbes in metabolic-associated fatty liver disease

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ABSTRACT

Metabolic-associated fatty liver disease (MAFLD) is the most common chronic liver disease. Gut dysbiosis is considered a significant contributing factor in disease development. Increased intestinal permeability can be induced by gut dysbiosis, followed by the entry of lipopolysaccharide into circulation to reach peripheral tissue and result in chronic inflammation. We reviewed how microbial metabolites push host physiology toward MAFLD, including short-chain fatty acids (SCFAs), bile acids, and tryptophan metabolites. The effects of SCFAs are generally reported as anti-inflammatory and can improve intestinal barrier function and restore gut microbiota. Gut microbes can influence intestinal barrier function through SCFAs produced by fermentative bacteria, especially butyrate and propionate producers. This is achieved through the activation of free fatty acid sensing receptors. Bile is directly involved in lipid absorption. Gut microbes can alter bile acid composition by bile salt hydrolase-producing bacteria and bacterial hydroxysteroid dehydrogenase-producing bacteria. These bile acids can affect host physiology by activating farnesoid X receptor Takeda G protein-coupled receptor 5. Gut microbes can also induce MAFLD-associated symptoms by producing tryptophan metabolites kynurenine, serotonin, and indole-3-propionate. A summary of bacterial genera involved in SCFAs production, bile acid transformation, and tryptophan metabolism is provided. Many bacteria have demonstrated efficacy in alleviating MAFLD in animal models and are potential therapeutic candidates for MAFLD.

KEYWORDS: *Bile acids, Gut microbiota, Metabolic-associated fatty liver disease, Short-chain fatty acid, Tryptophan*

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INTRODUCTION

Metabolic-associated fatty liver disease (MAFLD), or its synonym nonalcoholic fatty liver disease (NAFLD), is the most common chronic liver disease worldwide. MAFLD includes a continuum of liver pathologies ranging from nonalcoholic fatty liver to nonalcoholic steatohepatitis (NASH). A few MAFLD patients will progress to cirrhosis and hepatocellular carcinoma [1].

Gut microbes are essential in initiating and progressing fatty liver diseases. It is generally accepted that MAFLD pathogenesis involves gut microbiota dysbiosis, impaired intestinal barrier, entry of microbial cells and components into circulation, fat accumulation in the liver, aberrant bile acid production, oxidative stress in hepatocytes, and inflammation and fibrosis in the liver. Microbial components, especially lipopolysaccharides (LPS), are directly involved in disease development. Microbial metabolites, such as short-chain fatty acids (SCFAs), secondary bile acids, and other molecules, can also affect host physiology.

In this review, we aimed to provide a brief introduction to the roles of gut bacteria in MAFLD development [Figure 1]. Although many excellent reviews on MAFLD pathogenesis are available, we will emphasize how the gut microbes exert their influence and which bacterial taxa are involved in these processes.

GUT DYSBIOSIS AND METABOLIC-ASSOCIATED FATTY LIVER DISEASE

Metabolic-associated fatty liver disease microbiota


Gut dysbiosis has been frequently reported in MAFLD patients and animal models and is considered a significant contributing factor to this disease. Due to high inter-individual variation in gut microbiota composition, there is no clear-cut threshold to define dysbiosis. Using a

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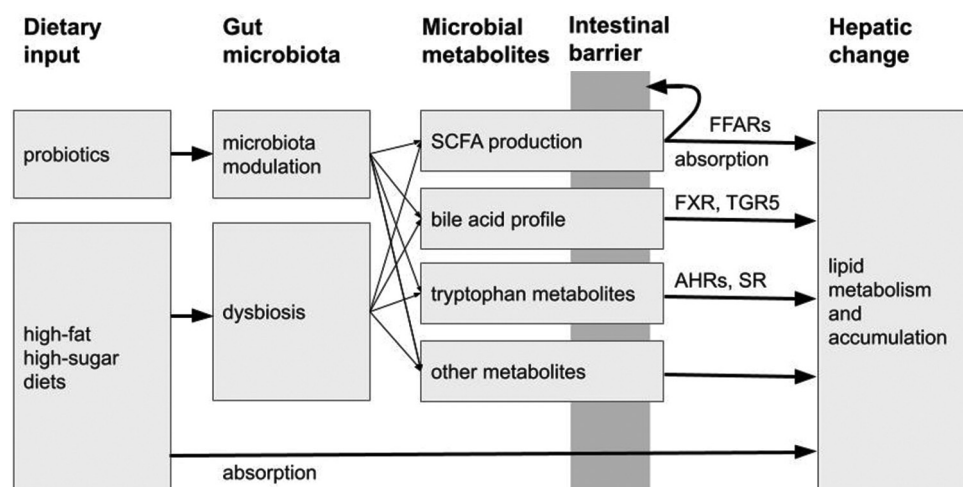


Figure 1: Microbial mechanisms on MAFLD. MAFLD: Metabolic-associated fatty liver disease, FFAR: Free fatty acid-sensing receptor, FXR: Farnesoid X receptor, TGR5: Takeda G protein-coupled receptor 5, AHR: Aryl hydrocarbon receptor, SR: Serotonin receptor

baseline microbial signature-based machine-learning model (random forest), Leung *et al.* have achieved high accuracies (auROCs of 0.72–0.80) in predicting MAFLD status and liver fat accumulation at the 4-year follow-up [2]. This suggests that the alteration of microbiota occurred before the appearance of clinical MAFLD symptoms.

Gut microbiota in MAFLD have been reviewed by many researchers [3,4]. An increase in Bacteroidota (previously known as Bacteroidetes) and Pseudomonadota (Proteobacteria), and a decrease in Bacillota [Firmicutes] are frequently reported in MAFLD patients and animals. However, variable or even contradictory results at the genus level were found among studies. This inconsistency is probably due to technical differences, for example, sampling methodology, sample size, and DNA processing procedures [5,6]. Host-related differences such as genetic background, diet, medication use, and comorbidities are the potential sources of variation [7-9].

Aron-Wisniewsky *et al.* provide an excellent summary of microbial signatures seen in the gut of human NAFLD patients, compared to two metabolic diseases, type II diabetes mellitus, and obesity [10]. They provided figures showing bacterial taxa (genera or species) with differential abundance between NAFLD/NASH patients and healthy controls. Here, we adopted their results and rearranged these taxa phylogenetically to show whether the trend is conserved phylogenetically [Table 1]. The table shows how each genus reacts to NAFLD/NASH conditions.

Table 1 shows that not all genera within the same phylum (or other higher-rank taxa) share the same trend. Taking phylum Bacteroidota as an example, there is an increase in *Porphyromonas*, a decrease in *Coprobacter* and *Alistipes*, and variable results in *Parabacteroides*, *Bacteroides*, and *Prevotella*. Unlike what has been reported in the literature, there is no consistent trend among genera within the same phylum or class. The Firmicutes-to-Bacteroidetes ratio is another indicator frequently used in the literature [15,16]. These phylum-level change does not reflect the more

physiology-related genus-level dynamic and should be avoided or replaced with a description of the turnover of genera.

Currently, only one clinical study on the gut microbiota of Taiwanese MAFLD patients is available in the literature [17]. In Taiwanese MAFLD patients, *Acidaminococcus*, *Alistipes*, *Escherichia-Shigella*, *Eubacterium*, *Faecalibacterium*, and *Subdoligranulum* have trends consistent with that reported in Aron-Wisniewsky *et al.* [10], but in *Akkermansia* and *Ruminococcus* opposite trend was seen. In this Taiwanese study, patients with broad age ranges (18–70 years old) were recruited. Genetic background is apparently a likely contributing factor for this difference. Due to the small number of samples included in this study (25 each for NAFL, NASH, and healthy control), and the fact that no other research on the Taiwanese population can be used for comparison, it is inconclusive whether Taiwanese MAFLD patients have different gut microbiota.

Intestinal barrier function and lipopolysaccharides

Dysbiosis leads to increased intestinal permeability and may move further into endotoxemia. Deterioration of intestinal barrier function is considered a significant contributor to the initiation and progression of MAFLD [18] and is commonly seen in MAFLD patients [19]. De Munck *et al.* performed a meta-analysis on 14 human clinical studies. They concluded that MAFLD patients had increased small intestinal permeability compared to healthy controls, but no clear difference was seen between simple steatosis and NASH patients [20].

Intestinal barrier function has four components: physical barrier formed by the intestinal epithelium, mucus layer secreted by goblet cells, chemical barriers including gastric acid and digestive enzymes, and immunological barrier composed of antimicrobials and immune cells [21]. High-fat diet (HFD) is known to alter gut microbiota. Safari *et al.* have shown that recovering from HFD-induced dysbiosis to normal gut microbiota required < 7 days [22]. Therefore the dysbiosis is expected to occur within days after receiving HFD. Dysbiosis leads to altered host-microbiota interaction and

Table 1: Bacterial genera that have increased or decreased abundance in metabolic-associated fatty liver disease patients as compared to healthy controls, with their abilities to produce short chain fatty acids

Genus	Change in MAFLD*	SCFA producer [†]			Taxonomy (class-order-family)
		Butyrate	Propionate	Acetate	
Phylum Bacteroidota					
<i>Bacteroides</i>	+/-	-	+	+	Bacteroidia-Bacteroidales-Bacteroidaceae
<i>Coprobacter</i>	-	-	+	-	Bacteroidia-Bacteroidales-Barnesiellaceae
<i>Porphyromonas</i>	+	-	+	-	Bacteroidia-Bacteroidales-Porphyromonadaceae
<i>Prevotella</i>	+/-	-	+	+	Bacteroidia-Bacteroidales-Prevotellaceae
<i>Alistipes</i>	-	-	+	+	Bacteroidia-Bacteroidales-Rikenellaceae
<i>Parabacteroides</i>	+/-	-	+	+	Bacteroidia-Bacteroidales-Tannerellaceae
Phylum Pseudomonadota					
<i>Bradyrhizobium</i>	+	-	-	-	Alphaproteobacteria-Hyphomicrobiales-Nitrobacteraceae
<i>Sutterella</i>	+/-	+	-	-	Betaproteobacteria-Burkholderiales-Sutterellaceae
<i>Escherichia</i>	+	-	-	+	Gammaproteobacteria-Enterobacterales-Enterobacteriaceae
<i>Shigella</i>	+	-	-	+	Gammaproteobacteria-Enterobacterales-Enterobacteriaceae
<i>Haemophilus</i>	-	-	-	+	Gammaproteobacteria-Pasteurellales-Pasteurellaceae
Phylum Verrucomicrobiota					
<i>Akkermansia</i>	+	-	+	-	Verrucomicrobiae-Verrucomicrobiales-Akkermansiaceae
Phylum Actinomycetota					
<i>Bifidobacterium</i>	+/-	-	-	+	Actinomycetes-Bifidobacteriales-Bifidobacteriaceae
<i>Propionibacterium</i>	+/-	-	+	-	Actinomycetes-Propionibacteriales-Propionibacteriaceae
<i>Eggerthella</i>	+	-	-	-	Coriobacteriia-Eggerthellales-Eggerthellaceae
Phylum Bacillota					
<i>Lactobacillus</i>	-	-	-	-	Bacilli-Lactobacillales-Lactobacillaceae
<i>Eubacterium</i>	-	+	+	+	Clostridia-Eubacteriales-Eubacteriaceae
<i>Anaerosporebacter</i>	-	-	+	+	Clostridia-Eubacteriales-Lachnospiraceae
<i>Blautia</i>	+/-	-	+	+	Clostridia-Eubacteriales-Lachnospiraceae
<i>Coproccoccus</i>	-	+	+	+	Clostridia-Eubacteriales-Lachnospiraceae
<i>Dorea</i>	+	-	+	-	Clostridia-Eubacteriales-Lachnospiraceae
<i>Moryella</i>	-	-	+	+	Clostridia-Eubacteriales-Lachnospiraceae
<i>Pseudobutyrvibrio</i>	-	-	+	+	Clostridia-Eubacteriales-Lachnospiraceae
<i>Roseburia</i>	+/-	+	+	-	Clostridia-Eubacteriales-Lachnospiraceae
<i>Anaerofilum</i>	+/-	-	-	-	Clostridia-Eubacteriales-Oscillospiraceae
<i>Faecalibacterium</i>	-	+	-	-	Clostridia-Eubacteriales-Oscillospiraceae
<i>Flavonifractor</i>	+	-	-	-	Clostridia-Eubacteriales-Oscillospiraceae
<i>Oscillibacter</i>	-	-	-	-	Clostridia-Eubacteriales-Oscillospiraceae
<i>Oscillospira</i>	-	-	-	-	Clostridia-Eubacteriales-Oscillospiraceae
<i>Ruminococcus</i>	+	-	+	+	Clostridia-Eubacteriales-Oscillospiraceae
<i>Subdoligranulum</i>	-	+	-	-	Clostridia-Eubacteriales-Oscillospiraceae
<i>Acidaminococcus</i>	+	-	-	-	Negativicutes-Acidaminococcales-Acidaminococcaceae
<i>Allisonella</i>	+	-	-	-	Negativicutes-Veillonellales-Veillonellaceae
<i>Anaerococcus</i>	+	+	-	-	Tissierellia-Tissierellales-Peptoniphilaceae
<i>Peptoniphilus</i>	+	-	-	+	Tissierellia-Tissierellales-Peptoniphilaceae

*Result from [10], showing changes in MAFLD compared to healthy control, [†]Data are compiled from multiple sources, including [11-14]. +: Increase, -: Decrease, +/-: Variable results among studies, MAFLD: Metabolic-associated fatty liver disease, SCFA: Short-chain fatty acids

thus may disrupt intestinal barriers through the mechanisms described below.

Among these mechanisms, intestinal epithelial integrity is probably the most studied. This epithelial integrity is maintained by tight junctions between two adjacent epithelial cells. Reduction in tight junction proteins zonula occludens-1 and claudin are always seen in MAFLD animals [19]. Local inflammation can disrupt the intestinal barrier. Mice with dextran sulfate sodium-induced intestinal inflammation have increased plasmalemma vesicle-associated protein-1 (an endothelial permeability marker) and decreased zonula occludens-1 and claudin expression [23].

A disrupted intestinal barrier enables gut microbes and microbial metabolites to pass through, and they may reach the liver and facilitate MAFLD development. Endotoxemia is associated with systemic inflammation and metabolic syndrome [24]. NASH patients have elevated levels of LPS in their blood [25]. Entry of LPS into circulation is typical in MAFLD, and high blood LPS level has even been suggested to be used as a MAFLD biomarker [26]. In the liver, LPS can activate Kupffer cells by binding to TLR4 and activating NF- κ B, therefore inducing inflammation and developing liver diseases [27].

HFD, HFD-responding gut microbes (HFD microbes), and impaired intestinal barriers are all considered essential factors

in MAFLD. HFD has been shown to induce dysfunction in the intestinal barrier [28] and is commonly used to induce MAFLD in the murine model. HFD-fed germ-free mice gained less weight than HFD-fed conventional mice [29], indicating that HFD itself is insufficient to induce MAFLD. HFD-fed germ-free mice receiving gut microbes from HFD-responding mice developed MAFLD, showing that combining HFD and HFD microbes leads to MAFLD development [30]. Regular chow-fed mice receiving regular chow and gut microbes from hepatic steatosis developed hepatic steatosis, indicating HFD microbes is sufficient in developing hepatic steatosis [31].

Fei *et al.* isolated LPS-producing bacterial strains (LPS producer) from obese patients and used them to study MAFLD [32]. In their study, HFD-fed mice receiving LPS producer developed the disease, but mice receiving other gut microbes did not, indicating that LPS producer is required for disease development. HFD-fed mice receiving wildtype LPS producers developed the disease, but mice receiving LPS deficient mutants did not, indicating that LPS is required for this disease. HFD-fed wildtype mice receiving LPS producer developed the disease, but HFD-fed TLR4 deficient mice receiving the same inoculation did not, indicating TLR4 is required for disease development. These data suggest that LPS from HFD microbes is more likely the primary determinant in MAFLD development, and HFD and impaired intestinal barrier play a more supportive role. The importance of LPS in MAFLD also explains bacterial changes commonly reported in the literature, with an increase in Bacteroidota and Pseudomonadota, which have LPS in their cells, and a decrease in Bacillota, which has no LPS.

MICROBIAL METABOLITES ON METABOLIC-ASSOCIATED FATTY LIVER DISEASE

Host-microbe interaction depends mainly on chemical signals. In the following discussion, we will describe how microbial metabolites affect hosts and contribute to the development of MAFLD. We will focus on three groups of metabolites involved in MAFLD pathogenesis [33]: SCFAs, secondary bile acids, and tryptophan metabolites.

Short-chain fatty acids and metabolic-associated fatty liver disease

SCFAs are fermentation products derived from gut microbes. Acetate, propionate, and butyrate are the dominant SCFAs in the human gut, with their molar ratio in the colon and feces at approximately 3:1:1 [34]. SCFAs are known to affect host physiology [35]. These SCFAs, especially butyrate, can be used by human colonocytes as a major energy source. They are sensed by various nutrient-sensing G-protein coupled receptors, collectively known as free fatty acid (FFA) receptors, including FFA2, FFA3, GPR109a, and OLF78 [36]. Signaling through these receptors can affect immune function [37], the nervous system [33], adipose tissue, and the endocrine system [38]. In the intestine, SCFAs usually help to improve intestinal barrier function and suppress inflammation.

Bacterial species vary in the fermentative pathways they adopt and the final product produced; therefore, change

in microbiota composition will alter SCFAs produced by the community. Bacteria may adopt different fermentation pathways and produce different SCFAs, depending on environmental conditions such as substrate availability, pH, and co-inhabiting microbes. Some bacterial species mainly produce one product, while others may vary their choice. SCFA producers have been reviewed elsewhere [11-14]. We summarize this information and present it in Table 1.

Butyrate and intestinal barrier function

Among SCFAs, butyrate has more roles in physiological modulation than propionate and acetate [39]. Butyrate can help to restore gut microbiota. The administration of sodium butyrate restored bleomycin-induced changes in fecal microbiota in mice [40]. Butyrate also helps to maintain intestinal barrier function. It has been shown to facilitate the assembly of tight junctions [41] and increase trans-epithelial electrical resistance in Caco-2 cells [42]. Butyrate can improve mucus barrier function. Butyrate and propionate increased MUC2 expression in human goblet cell-like LS174T cells [43] and mucin production in the mouse model [44]. Butyrate was shown to decrease pro-inflammatory and increase anti-inflammatory cytokines in the collagen-induced arthritis mouse model [45]. Butyrate supplementation reduced intestinal inflammation in *Citrobacter rodentium*-infected mice [46] and the pancreatitis rat model [47]. Butyrate concentration generally decreases in MAFLD conditions. Among the bacterial genera affected by MAFLD, butyrate-producing genera *Eubacterium*, *Coprococcus*, *Roseburia*, and *Faecalibacterium* decrease in relative abundance [Table 1].

Propionate and intestinal barrier function

Propionate modulates gut microbiota, intestinal barrier function, and immune response like butyrate. Oral or rectal administration of propionate in rats altered the intestinal microbiota, increased SCFA production, improved intestinal barrier function, and reduced inflammation [48]. Sodium propionate administration restored gut microbiota dysbiosis and SCFA production in HFD-fed mice [15]. However, propionate supplementation did not alleviate this disease in a high-fructose-induced steatosis and gut dysbiosis mouse model [49]. Propionate can strengthen tight junction barrier integrity in Caco-2 cells [42,50]. Propionate administration attenuated intestinal epithelial barrier dysfunction, restored mucus production, altered gut microbiota dysbiosis, and reduced intestinal inflammation in mice with alcoholic-related liver disease [51]. Among the bacterial genera affected by MAFLD, most Lachnospiraceae propionate producers decrease, and *Akkermansia* increases, while Bacteroidota producers and *Propionibacterium* have inconsistent results [Table 1].

Bile acids and metabolic-associated fatty liver disease

Bile acids are a group of structurally similar molecules. MAFLD patients generally have altered bile acid profile, which has been proposed to be used as biomarkers for the disease [52]. Bile acids can modulate gut microbiota, and in reverse, bile acid composition is affected by gut microbiota [53]. They are produced as primary bile acids and transformed into secondary bile acids by gut microbes.

About 95% of these bile acids are reabsorbed into the portal circulation and can affect host physiology.

The primary bile acids, synthesized and conjugated to glycine or taurine in the hepatocyte, include cholic acid (CA), chenodeoxycholic acid (CDCA), and ursodeoxycholic acid (UDCA). Gut bacteria act on bile acids through a two-staged transformation [54]. They use bacterial bile salt hydrolases to de-conjugate glycine or taurine and release free CA and CDCA. Bacterial bile salt hydrolases are found in all major lineages of gut bacteria [55], as *Bacteroides* and *Lactobacillus* are considered the leading bile salt hydrolase producers in the human gut [56,57].

Bacterial hydroxysteroid dehydrogenase will further convert CA and CDCA to deoxycholic acid (DCA) and lithocholic acid, respectively. Bacterial species equipped with hydroxysteroid dehydrogenase are much more limited. It was estimated that only < 1% of total gut bacteria have the 7 α -dehydroxylating *bai* gene cluster, based on analysis of metagenomic and metatranscriptomic data [58]. Since not all the gut microbes have these enzymes, the composition of gut microbial community will directly affect bile acid composition. Bacterial hydroxysteroid dehydrogenase has been reported in *Eubacterium*, *Clostridium*, *Collinsella* [59], *Eggerthella* [60] and *Ruminococcus* [61].

Many gut bacteria are susceptible to bile acids. Different bile acid molecules vary in their spectrum and strength of inhibitory activities, consequently controlling the gut's ecology and microbiota composition. For example, minimum inhibitory concentrations (MICs) of unconjugated bile acid against *Staphylococcus aureus* is 20 mM for CA and 1 mM for DCA, and MICs for tested conjugated bile acids are over 200 mM [62]. Tian *et al.* demonstrated that bile acids have a differential inhibitory effect on gut microbes, and this differential inhibition varies among different bile acids [63].

Besides their roles in fat digestion and modulation of gut microbial composition, bile acids can also affect host energy management. Bile acids can be detected by the nuclear farnesoid X receptor (FXR) and the cell surface Takeda G protein-coupled receptor 5 (TGR5), and are associated with the regulation of glucose, lipid, and energy metabolism [64]. FXR can affect hepatic fatty acid metabolism [65]. FXR deficiency in mice enhanced glucose clearance, but increased liver steatosis due to repression of β -oxidation genes [66] and developed liver damage resembling NASH [67]. TGR5 has an immunomodulatory effect and can inhibit LPS-induced cytokine production [68]. Gillard and Leclercq reviewed the effect of bile acids on MAFLD and concluded that the administration of bile acids (CA, DCA, UDCA) could alleviate MAFLD symptoms [69]. *Bacteroides fragilis* modulates bile acid synthesis using bile salt hydrolase, and through FXR signaling, this change led to excessive bile acid production [70]. This indicates the possibility of using bile-metabolizing bacteria to modulate bile acid composition, which has been proposed as a potential therapeutic approach for MAFLD [69].

Tryptophan metabolites

Tryptophan is an essential amino acid for humans commonly found in everyday diets. Recently the roles of tryptophan metabolites in disease development have been reported in various diseases, including MAFLD [71], dermatological diseases [72], neurodegenerative diseases [73] and kidney diseases [74]. Tryptophan can be metabolized through the following pathways in the human gut: the kynurenine pathway (90%–95% of tryptophan metabolism), the serotonin/melatonin pathway (1%–2%), and indole pathway (5%) [71].

Gut microbes are known to produce kynurenine and kynurenine derivatives [75]. Fecal samples of NASH patients have lower L-tryptophan and higher kynurenine [76]. Germ-free mice receiving gut microbes from MAFLD have increased kynurenine [76]. Supplementation of kynurenine in HFD-fed mice led to body mass gain, liver steatosis, and hyperglycemia [77]. This effect is conducted by sensing kynurenine through aryl hydrocarbon receptors. These data support the view that microbially-derived kynurenine may contribute to MAFLD development.

Serotonin can regulate hepatic energy metabolism and affect MAFLD development [78]. Blocking serotonin receptors can reduce hepatic steatosis and fibrosis in MAFLD mice [79]. Germ-free mice have reduced blood serotonin, indicating that gut microbes can modulate host serotonin production [80]. Some bacteria have been shown the capability to produce serotonin [81]. However, whether this microbially-produced serotonin can affect host physiology is unclear.

Gut microbes can metabolize tryptophan through the indole pathway to indole and its derivatives indole-3-acetate, indole-3-propionate (IPA), and skatole [82]. Interestingly, IPA stimulated the expression of tight junction proteins and exerted anti-inflammatory and anti-oxidant effects and has been proposed to be used as a therapeutic option for metabolic diseases, including MAFLD [83]. Bacterial taxa previously shown to be positively correlated to IPA production include species from *Lactobacillus* [84], *Akkermansia* and *Clostridium* [85], *Allobaculum*, *Bifidobacterium*, Lachnospiraceae, and *Allobaculum* [86]. However, only *C. sporogenes* has been experimentally validated for IPA production [87].

Other microbial metabolites

Metagenomic analysis has shown that patients with steatosis have dysregulated aromatic and branched-chain amino acid metabolism [31], although more evidence is needed to clarify their importance in MAFLD pathogenesis. Tyrosine metabolite 3-(4-hydroxyphenyl) lactate has been found to be associated with liver steatosis and fibrosis [88]. Palmitic acid-treated hepatocyte cell lines PH5CH8 and HepG2 showed typical features of steatosis, and differential changes in tyrosine and phenylalanine pathways, fatty acid metabolism, and bile acids [89]. MAFLD microbiota facilitated phenylalanine production in the MAFLD mouse model [90]. Chronic treatment with phenylacetic acid, a microbial product of aromatic amino acid metabolism triggered steatosis [31]. Elevated plasma concentrations of branched-chain amino acids have been reported in MAFLD patients [91]. However,

it also positively correlated with insulin resistance and BCAA supplements were previously linked to a beneficial outcome in various liver diseases [92].

Association of trimethylamine-N-oxide (TMAO) to MAFLD has been noticed recently. Trimethylamine is produced by bacterial metabolism of choline or carnitine and can be further oxidized to TMAO in the liver. Individuals with histologically proven MAFLD have higher plasma TMAO [93]. TMAO has been linked to many MAFLD-related diseases, such as obesity, diabetes, dyslipidemia, and hypertension [94]. However, a direct mechanistic link to MAFLD has yet to be established.

Bacterially-derived ethanol is another potential cause of MAFLD. An alcohol-producing *Klebsiella pneumoniae* HiAlc has been reported and is found to be associated with up to 60% of MAFLD patients in a Chinese cohort [95]. This strain can cause MAFLD in mice, as confirmed by FMT experiments. The mutant of this strain, which has reduced alcohol production, has less prominent hepatic damage [96]. Ethanol-producing yeasts in the *Pichia*, *Candida*, and *Galactomyces* have been isolated from NASH patients and are suspected to be the causative agents [97]. Meijnikman *et al.* have shown that hepatic alcohol dehydrogenase could greatly reduce circulating ethanol and obscures the endogenous ethanol production [98].

Microbial modulation of lipid metabolism

MAFLD is characterized by aberrant metabolism and the accumulation of lipids. Gut microbes can act as a regulator of lipid metabolism in the intestine by regulating lipid digestion/absorption and energy balance [99] and therefore has great potential in participating in MAFLD development through manipulating lipid metabolism.

Gut microbes affect hepatic lipid metabolism through several mechanisms. They can exert their control by modulating bile acid composition. These bile acids can, in turn, modulate host hepatic or systemic lipid and glucose metabolism [100,101]. They produce SCFAs and provide them to host as an alternative energy source. They can also affect cholesterol and steroid metabolism. Amino acid choline and carnitine can be metabolized to trimethylamine, which is further oxidized to trimethylamine N-oxide in the liver, affecting cholesterol and sterol metabolism and increasing the risk of cardiovascular diseases [102].

Gut microbes can also regulate lipid metabolism by suppressing fasting-induced adipocyte factor (Fiaf) in the intestinal epithelium [99]. This will reduce circulating lipoprotein lipase levels and enhance liver-derived triacylglycerols storage in adipocytes [103]. Germ-free mice have a 60% increase in body fat content after receiving gut microbiota from conventionally-raised mice, due to suppression of Fiaf by gut microbes [104]. Gut microbes *Lactobacillus rhamnosus* [105] and *Akkermansia muciniphila* [106] have been shown to suppress Fiaf expression. Liver-specific overexpression of lipoprotein lipase has been shown to attenuate lipid droplet accumulation in the liver and improve glucose metabolism in HFD-fed mice [107].

Although gut microbes can apparently affect lipid metabolism, direct evidence showing gut microbes facilitate hepatic lipid accumulation through Fiaf is still waiting to be established.

Microbial modulators of metabolic-associated fatty liver disease

Many bacterial species have shown properties that might be used to prevent or treat MAFLD [Table 2]. These potential microbial modulators include genera commonly used as probiotics, such as *Lactobacillus* and *Bifidobacterium*, as well as other gut inhabitants.

Lactobacillus isolates reported to alleviate MAFLD include *L. reuteri* [120], *L. plantarum* [116-118], *L. pentosus* [119], *L. rhamnosus* [121], *L. paracasei* [114,115], *L. gasseri* [113], and *Latilactobacillus sakei* [122]. Generally, these isolates demonstrate the capability to reduce hepatic fat accumulation, suppress immune activation and improve intestinal barrier integrity. *Bifidobacterium* species also show great potential. Supplementation of *B. adolescentis* reduced HFD-induced visceral fat accumulation, insulin sensitivity, and steatosis in Wistar rats [111]. *B. animalis* modulated gut microbiota, restored intestinal barrier function, and reduced LPS entry into the circulation and inflammation to alleviate HFD-induced NAFLD in mice [112].

Certain “new generation probiotics” have been shown to have MAFLD-alleviating effects. *A. muciniphila* can improve metabolic disorders, improving hepatic inflammation mainly by suppressing pro-inflammatory immune responses [127]. The supplementation of *Faecalibacterium prausnitzii* in the NASH mouse improved glucose homeostasis, prevented hepatic lipid accumulation and liver damage, and restored damaged gut barrier functions [123,124]. *Roseburia intestinalis* is anti-inflammatory and has been shown to increase anti-inflammatory cytokine production [125] and maintain tight junction integrity during colitis [128]. A *Roseburia* species has been shown to alleviate the alcohol-related fatty liver disease by reducing hepatic steatosis and inflammation, recovering gut barrier integrity, and restoring gut microbiota [110]. Supplementation of *Clostridium butyricum* improved HFD-induced intestinal inflammation in rats, probably through its capability of butyrate production [47,126].

Bacteroides species have been proposed as probiotics in dealing with many diseases. No report on the application of *Bacteroides* species in dealing with MAFLD, despite the reduction in *Bacteroides* has been associated with MAFLD. However, the closely related *Parabacteroides distasonis* has recently been reported in mice to ameliorate hepatic fibrosis, potentially through modulating bile acid metabolism and pyroptosis in hepatocytes [129]. Consumption of butyrate or butyrate-producing *C. butyricum* reduced intestinal injury and decreased the plasma levels of inflammatory cytokines, diamine oxidase, and LPS in rats [47].

A meta-analysis on the efficacy of probiotic treatments in NAFLD shows improvement in liver function, decreases in blood lipid, glucose, and insulin levels, and reduction in hepatic steatosis, based on 21 clinical trials [130]. Thus, probiotic treatment may be a potentially useful tool in treating

Table 2: Bacterial species showing properties with metabolic-associated fatty liver disease-improving potentials

Species	Host	Reported mechanisms				Reference
		Reverse dysbiosis	Fix intestinal barrier	Improve liver functions	Suppress inflammation	
Phylum Verrucomicrobiota						
<i>Akkermansia</i> (Verrucomicrobiae-Verrucomicrobiales-Akkermansiaceae)						
<i>A. muciniphila</i>	Human			+	+	[108]
<i>A. muciniphila</i>	Mouse			+	+	[109]
<i>A. muciniphila</i>	Mouse		+	+		[110]
Phylum Actinomycetota						
<i>Bifidobacterium</i> (Actinomycetes-Bifidobacteriales-Bifidobacteriaceae)						
<i>B. adolescentis</i>	Rat			+		[111]
<i>B. animalis</i>	Mouse	+	+	+	+	[112]
Phylum Bacillota						
<i>Lactobacillus</i> (Bacilli-Lactobacillales-Lactobacillaceae)						
<i>L. gasserii</i>	Rat	+		+	+	[113]
<i>L. paracasei</i>	Mouse	+		+	+	[114]
<i>L. paracasei</i>	Mouse	+		+		[115]
<i>L. plantarum</i>	Rat	+	+	+		[116]
<i>L. plantarum</i>	Mouse	+	+	+		[117]
<i>L. plantarum</i>	Mouse	+		+	+	[118]
<i>L. pentosus</i>	Mouse		+	+	+	[119]
<i>L. reuteri</i>	Mouse			+	+	[120]
<i>L. rhamnosus</i>	Rat			+		[121]
<i>L. sakei</i>	Mouse	+		+		[122]
<i>Faecalibacterium</i> (Clostridia-Eubacteriales-Oscillospiraceae)						
<i>F. prausnitzii</i>	Mouse	+		+	+	[123]
<i>F. prausnitzii</i>	Mouse	+	+	+	+	[124]
<i>Roseburia</i> (Clostridia-Eubacteriales-Lachnospiraceae)						
<i>R. hominis</i>	Mouse		+	+		[110]
<i>R. intestinalis</i>	Mouse		+		+	[125]
<i>R. intestinalis</i>	Mouse		+	+	+	[110]
<i>Clostridium</i> (clostridia-Eubacteriales-Clostridiaceae)						
<i>C. butyricum</i>	Rat	+	+		+	[47]
<i>C. butyricum</i>	Rat		+		+	[126]

MAFLD. However, so far evidence showing clinical efficacy in improving MAFLD is scarce, and more clinical trials on different populations are therefore urgently needed.

CONCLUSION

The importance of gut microbes in MAFLD has been recognized. However, we usually consider the gut microbes in bulk and need to identify the specific role each bacterial species plays in disease development. The next generation (NGS) sequencing and bioinformation revolution enables us to monitor the changes in each bacterial species, giving us an excellent opportunity to dissect their respective contribution to host-microbe interaction. Dietary supplementation of beneficial microbes or microbial metabolites to achieve desired bile acid or SCFA composition can be considered a promising MAFLD-alleviating approach. The high diversity among gut microbes provides us with a plethora of candidate beneficial microbes, to be used as replacement parts to manipulate and fix host physiology and improve health.

Data availability statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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Conflicts of interest

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