Advances in Research on Polyunsaturated Fatty Acids and Their Derivatives in the Gut: Post-print

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Abstract

The gut microbiota constitutes a crucial component of human microecology and represents the largest and most complex microecosystem, playing essential roles in important physiological processes such as host nutrient absorption, intestinal and immune system development, and is intimately associated with human health and disease. The capacity of these symbiotic microorganisms to exclude intestinal pathogens primarily relies on bioactive substances they produce, including polyunsaturated fatty acids. Concurrently, these fatty acids can be further transformed into polyunsaturated fatty acid derivatives with unique structures and functions through the action of intestinal microorganisms. These derivatives are vital for maintaining a healthy and stable gut microbiota. Moreover, polyunsaturated fatty acids exert multiple critical functions in host defense and immunity, encompassing anticancer, anti-inflammatory, and antioxidant activities, as well as diminishing the competitive capacity of intestinal pathogenic bacteria. This review primarily summarizes the sources of polyunsaturated fatty acids in the gut and their important physiological functions, along with the mechanisms by which intestinal microorganisms transform and derive these fatty acids; and proposes that intestinal microorganisms serve as a potential seed bank and gene repository for specialized polyunsaturated fatty acid- and derivative-producing strains, thereby expanding the sources of functional lipid-producing microorganisms.

Full Text

Advances in Research on Polyunsaturated Fatty Acids and Their Derivatives in the Intestinal Tract

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Abstract

Gut microbiota constitutes a vital component of human microecology and represents the largest and most complex microbial ecosystem. These commensal microorganisms play crucial roles in host nutrient absorption, intestinal and immune system development, and other essential physiological processes, making them intimately linked to human health and disease. The ability of these microbes to exclude enteric pathogens partially depends on their production of bioactive compounds such as polyunsaturated fatty acids (PUFAs). Moreover, under the action of intestinal microorganisms, these fatty acids can be further transformed into PUFA derivatives with unique structures and functions. These microbial byproducts are critical for maintaining a healthy and stable gut flora. Additionally, PUFAs exert multiple key roles in host defense and immunity, including anti-cancer, anti-inflammatory, and antioxidant activities, as well as reducing the competitive capacity of intestinal pathogens. This review systematically summarizes the sources and important physiological functions of PUFAs in the intestinal tract, elucidates the mechanisms by which gut microbes transform and derivatize PUFAs, and proposes that intestinal microorganisms represent a potential seed and gene bank for producing strains of special PUFAs and their derivatives, thereby expanding the sources of functional oil-producing strains.

Keywords: polyunsaturated fatty acids; gut microbiota; biotransformation; hydroxy fatty acids

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The intestinal tract is the largest digestive organ and most complex microecosystem in the human body [**?**]. Gut microbiota, as a crucial component of human microecology, participates in essential physiological processes including host nutrient absorption and intestinal immune system development, with its compositional changes closely associated with the onset, progression, and treatment of various diseases [**?**, **?**]. The important physiological functions of these commensal microbes partially depend on their production of bioactive substances such as polyunsaturated fatty acids (PUFAs). The intestinal tract contains diverse PUFAs, primarily including -3, -6, -7, and -9 families [**?**, **?**]. These fatty acids exert various physiological functions, including preventing dementia and cardiovascular disease, anti-cancer activity, anti-inflammatory effects, and

antioxidant properties [?, ?]. The ratio of these fatty acids in the intestine directly or indirectly influences the gut microecosystem by altering the quantity and composition of intestinal microbiota and affecting the physiological characteristics and functions of the intestinal mucosal barrier. Moreover, research has demonstrated that gut dysbiosis is closely related to metabolic disorders and cancer in the host.

Most PUFAs in the intestine originate from dietary sources. The U.S. National Institutes of Health recommends that healthy adults consume at least 220 mg/day of both DHA and EPA, with pregnant women requiring no less than 300 mg/day. In 2016, the International Scientific Association for Probiotics and Prebiotics (ISAPP) included polyunsaturated fatty acids in the prebiotic category for the first time. Beyond dietary intake, certain intestinal microorganisms possess the capacity to synthesize and transform PUFAs [?]. Intestinal microbes such as *Bifidobacterium*, *Lactobacillus*, and *Lactococcus* can synthesize -3 and -6 fatty acids and convert PUFAs into unique fatty acids with special molecular structures and functions, including hydroxy fatty acids and conjugated fatty acids [?]. Linoleic acid (LA) and conjugated linoleic acid (CLA) are recognized as the most beneficial PUFAs, attracting widespread attention for their health-promoting effects and potential as materials for functional food and chemical development. However, the biosynthetic and transformation mechanisms of these special fatty acids in the gut microbial community have rarely been reported. This review systematically elaborates on the types, production, and physiological functions of PUFAs in the intestinal tract, introduces recent research progress on the microbial transformation and derivatization mechanisms of PUFAs, and proposes that gut microorganisms serve as a potential seed and gene bank for producing strains of special PUFAs and their derivatives, effectively expanding the sources of functional oil-producing strains.

1.1 Common PUFAs in the Intestinal Tract

Intestinal PUFAs primarily originate from ingested food and microbial transformation. Based on the position of the first double bond from the methyl end (the numbering system), these PUFAs are classified into -3, -6, -7, and -9 families, with the first double bond located at the 3rd, 6th, 7th, and 9th carbon atoms, respectively [?, ?]. Common polyunsaturated fatty acids are summarized in Table 1 . The -3 and -6 families are essential fatty acids that cannot be synthesized *de novo* by humans or are produced in quantities far below physiological requirements [?]. The -3 family 主要包括以亚麻酸为母体经碳链延长酶和去饱和酶作用衍化生成的 -亚麻酸（-linolenic acid, ALA），二十碳五烯酸（Eicosapentaenoic，EPA），二十二碳六烯酸（Docosahexaenoic Acid，DHA）等；-6 族 PUFAs 主要包括以亚油酸为母体经碳链延长酶和去饱和酶作用衍化生成的 -亚麻酸（-linolenic acid, GLA），亚油酸（Linoleic acid），花生四烯酸（Arachidonic acid，ARA）等 [5,13]。在肠道中除了这些常见的 PUFAs 外，还存在一些具有特殊结构和功能的 PUFAs，如羟基脂肪酸、共轭脂肪酸等 [14]。

1.2 Sources of PUFAs in the Intestinal Tract

As a vital organ for digestion and nutrient absorption, the intestine contains abundant exogenous food materials and endogenous microbial communities. Since most PUFAs cannot be synthesized *de novo* in the human body or are synthesized inefficiently, they must be supplied through intestinal absorption. Intestinal PUFAs primarily derive from dietary intake and transformation by gut microbial communities (see Tables 1-1 and 1-2). For infants, breast milk serves as a rich source of PUFAs, including ALA, DHA, ARA, and GLA. Food ingredients also contain substantial PUFAs: flaxseed, rapeseed, soybean, and walnut are rich in ALA and GLA; peanut, liver, porcine adrenal gland, and egg yolk are abundant in ARA; while DHA, DPA, and EPA have traditionally been extracted from fish oil and algae [**?**].

Beyond dietary sources, numerous microorganisms in nature can synthesize PUFAs through two distinct pathways: the fatty acid synthase (FAS) pathway and the polyketide synthase (PKS) pathway. The PKS pathway primarily exists in certain marine bacteria and algae, where bacterial PUFA synthesis in low-temperature marine ecosystems is mediated by PKS enzymes. The FAS pathway is universally present in microorganisms, animals, and plants. Research shows that the marine microbe *Schizochytrium sp.* efficiently synthesizes DHA-rich oil via the PKS pathway, achieving lipid content exceeding 100 g/L with DHA content greater than 50% [**?**]. *Mortierella alpina* produces ARA-rich oil through the FAS pathway, with optimized fermentation conditions yielding lipid content of 20 g/L and ARA content over 60% [**?**, **?**]. DuPont has genetically engineered *Yarrowia lipolytica* into an EPA-producing strain. These microbial production efficiencies far exceed those of plant and animal sources, and these strains have been applied for commercial PUFA production as dietary supplements in the food industry. Additionally, certain microbes within the vast gut microbial community can synthesize fatty acids through both pathways.

In 1996, Japanese scientists first reported the isolation of *Shewanella putrefaciens* SCRC2378 from marine animal intestines, a strain that produces EPA via PKS, with EPA comprising 24–40% of total fatty acids. Studies have also demonstrated that intestinal microbes such as *Escherichia coli*, *Lactococcus lactis*, *Clostridium acetobutylicum*, and *Enterococcus faecalis* can perform fatty acid synthesis through the FAS pathway.

PUFAs also include rare functional fatty acids with unique molecular structures, such as hydroxy fatty acids and conjugated fatty acids, which are widely present in the intestine. Research indicates these unique PUFAs beyond conventional structures are obtained through transformation and derivatization by gut microbial communities [**?**]. Studies on intestinal anaerobic microbes have revealed unique PUFA transformation mechanisms. These microorganisms can isomerize PUFAs such as LA, ARA, and EPA into corresponding conjugated fatty acids. Intestinal anaerobes possess distinctive biohydrogenation pathways that further reduce PUFAs to partially saturated fatty acids—for example, EPA and

ARA are reduced to 5(Z),8(Z),13(E),17(Z)-conjugated eicosapentaenoic acid and 5(Z),8(Z),13(E)-conjugated eicosatrienoic acid, respectively. This reduction process generates special hydroxy fatty acids such as 10-hydroxy, 13-hydroxy, and 10,13-dihydroxy fatty acids [**?**].

Butyrivibrio fibrisolvens was the first recognized producer of conjugated linolenic acid [**?**, **?**], and other gut microbes with similar capacity for synthesizing various secondary metabolites including PUFAs include *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactococcus lactis*, and *Streptococcus thermophilus* [**?**]. However, the content and composition of these PUFAs are determined by the spatial distribution of microbes in the gut and the types of food consumed (as shown in Table 2). The functions of these unique PUFAs have attracted attention for their health benefits and potential as novel materials for functional foods and chemicals [**?**], yet reports on the metabolic derivatization pathways of special fatty acids in gut microbes remain scarce.

1.3 Physiological Functions of PUFAs in the Intestinal Tract

As essential fatty acids, PUFAs exert numerous physiological functions against various diseases. They are crucial components of cell membranes, maintaining normal cellular physiological properties, participating in regulation of the cell cycle, membrane-bound protein behavior, and plasma membrane permeability [**?**, **?**]. Additionally, PUFAs are highly concentrated in important tissues and organs such as the brain and visual system [**?**, **?**]. Studies have demonstrated that -3 fatty acids (particularly -linolenic acid) and -6 fatty acids can reduce the risk of cardiovascular disease, genetic disorders, dementia, breast cancer, and prostate cancer, while inhibiting the survival and growth of pathogenic microorganisms in the intestine. Linoleic acid (LA) and conjugated linoleic acid (CLA) are recognized as the most beneficial PUFAs, offering broad health benefits including anti-cancer, anti-inflammatory, antioxidant, and anti-pathogen activities [**?**]. Other widely applied PUFAs in healthcare include ARA, EPA, and DHA. ARA serves as an important precursor for eicosanoids (such as prostaglandins, prostacyclins, and leukotrienes) and plays vital roles in promoting brain development, esterifying cholesterol, preventing and treating cardiovascular disease, and exerting anti-inflammatory and immune-enhancing effects [**?**]. EPA reduces platelet aggregation and blood lipids while improving blood rheology, thereby preventing and reducing the risk of coronary heart disease and atherosclerosis [**?**]. DHA prevents cardiovascular disease, protects vision and brain health, enhances memory and judgment, prevents brain aging, and exhibits anti-cancer and anti-inflammatory effects [**?**].

Furthermore, PUFAs play important roles in maintaining intestinal health by combating foodborne pathogenic infections and improving gut health, directly or indirectly influencing the gut microecosystem and protecting the intestine from pathogen invasion and infection through modulation of intestinal immu-

nity, thereby maintaining fundamental physiological properties [**?**]. The human gut microbiota constitutes an essential component of the innate defense mechanism on the intestinal mucosal surface, playing a crucial role in protecting the host from exogenous pathogen invasion [**?**]. Both commensal and pathogenic bacteria require stable growth environments to colonize host epithelial cells and proliferate throughout the intestine. Commensal microbes inhibit pathogen adhesion, proliferation, survival, and colonization through nutrient competition and production of antimicrobial compounds. *Bifidobacterium* and *Lactobacillus* counter pathogen invasion, including enterohemorrhagic *E. coli* O157:H7 (EHEC), by synthesizing and secreting PUFAs and acidifying the surrounding environment [**?**].

Simultaneously, gut microbes can obtain PUFA derivatives through their unique fatty acid transformation pathways, which regulate immune responses and control apoptosis. Lactic acid bacteria in particular can produce various beneficial lipid molecules. Fatty acids are crucial for immune function as they play important roles in energy balance. Potential immunomodulatory mechanisms involve gene regulation, alterations in membrane fluidity, and formation of lipid peroxides. Based on these mechanisms, PUFAs also exert important functions in limiting intestinal pathogens, especially foodborne pathogens. Sun et al. confirmed through animal experiments that PUFAs play vital roles in host immunity, demonstrating that CLA effectively inhibits tumor formation, proliferation, and metastasis [**?**]. PUFAs promote the inhibition and clearance of intestinal pathogenic microorganisms causing foodborne diseases, including *Salmonella*, EHEC, *Campylobacter*, *Listeria monocytogenes*, *Shigella*, *Vibrio*, and *Yersinia* [**?**]. Babu et al. showed that ARA, ALA, and DHA can promote chicken macrophage clearance of *Shigella* Enteritidis [**?**]. The defensive efficacy of PUFAs against intestinal pathogens, particularly in inhibiting *Salmonella* proliferation and colonization, has been demonstrated in multiple organisms including rodents, birds, fish, and pigs [**?**].

2.1 Biohydrogenation of PUFAs by Anaerobic Microorganisms

Fatty acid saturation metabolism, known as biohydrogenation, is considered a detoxification process in anaerobic bacteria. This biotransformation converts toxic free PUFAs into less toxic saturated fatty acids. Driven by the pursuit of healthy lifestyles and the development of health-promoting foods with low saturated fatty acid content, high -3 PUFA levels, and enriched conjugated linoleic acid, the biohydrogenation process by ruminal anaerobic microbes has become a research hotspot in recent years. Early studies demonstrated that the intestinal anaerobe *Butyrivibrio fibrisolvens* could convert linoleic acid into 9(Z),11(E)-octadecenoic acid and further into trans-octadecenoic acid [**?**]. Recent research has elucidated the detailed biohydrogenation process in *Lactobacillus plantarum* AKU 1009a [**?**, **?**]. The fatty acids recognized and utilized as substrates by *L. plantarum* AKU 1009a are C18 fatty acids with 9(Z),12(Z)-diene structures. These 9(Z),12(Z)-diene fatty acids can be further transformed into 9(Z),11(E)-

and 9(E),11(E)-diene series fatty acids, which are subsequently saturated to 10(E)-monoene series fatty acids. These biohydrogenation pathways involve numerous potential reactions for fatty acid transformation as described below.

2.2 Basic Metabolism of PUFA Biohydrogenation in Gut Anaerobic Microorganisms

The biohydrogenation pathway in *Lactobacillus plantarum* involves multiple enzymatic reactions [**?**, **?**, **?**]. The first step is catalyzed by conjugated linoleic acid hydratase, which hydrates the Δ9 double bond of linoleic acid to produce 10-hydroxy-12(Z)-octadecenoic acid. The second reaction is catalyzed by conjugated linoleic acid dehydrogenase, which dehydrates 10-hydroxy-12(Z)-octadecenoic acid to generate 10-oxo-12(Z)-octadecenoic acid. The third step involves conjugated linoleic acid isomerase, which isomerizes the Δ12 double bond of 10-oxo-12(Z)-octadecenoic acid to form 10-oxo-11(E)-octadecenoic acid with a conjugated ketone structure. The fourth reaction is catalyzed by conjugated linoleic acid enone reductase, which hydrogenates the Δ11 double bond of 10-oxo-11(E)-octadecenoic acid to form a single bond, producing 10-oxo-octadecanoic acid. The fifth step involves conjugated linoleic acid dehydrogenase, which reduces the Δ10 oxo group of 10-oxo-octadecanoic acid to a hydroxyl group, generating 10-hydroxy-octadecanoic acid. The final reaction is catalyzed by conjugated linoleic acid hydratase, which reduces the Δ10 hydroxyl group of 10-hydroxy-octadecanoic acid to produce 9(Z)-octadecenoic acid (oleic acid) and 10(E)-octadecenoic acid (Figure 1 [Figure 1: see original paper] A, C).

A branch pathway exists throughout the PUFA biohydrogenation reactions, primarily catalyzed by conjugated linoleic acid hydratase, dehydrogenase, and isomerase, through which conjugated fatty acids are produced by the combined action of these three key enzymes (Figure 1 A, B). This branch pathway is initiated by conjugated linoleic acid dehydrogenase, which reduces the Δ10 oxo group of 10-oxo-11(E)-octadecenoic acid to generate 10-hydroxy-11(E)-octadecenoic acid. The final reaction is catalyzed by conjugated linoleic acid hydratase, which reduces the Δ10 hydroxyl group of 10-hydroxy-11(E)-octadecenoic acid to produce 9(Z),11(E)-conjugated linoleic acid and 9(E),11(Z)-conjugated linoleic acid. C18 fatty acids with Δ9,Δ12-diene structures, such as -linolenic acid, -linolenic acid, and stearidonic acid, undergo similar transformation mechanisms in *Lactobacillus plantarum* AKU 1009a, indicating that similar intermediates containing hydroxyl, keto, conjugated, and partially saturated structures are also produced through the combined catalysis of these enzymes [**?**].

2.3 Production of Conjugated Fatty Acids by Gut Anaerobic Microorganisms

Conjugated fatty acids are a class of PUFAs where two double bonds are separated by only one single bond or no intervening carbon atoms (e.g., conjugated linoleic acid). These compounds exhibit strong antioxidant functions and have attracted widespread attention as beneficial functional oils. Kishino

et al. proposed *Lactobacillus plantarum* AKU 1009a as a potential production strain for converting linoleic acid into conjugated linoleic acid [**?**, **?**]. Through 108 hours of fermentation, *L. plantarum* can convert 12% (w/v) linoleic acid into 40 mg/mL conjugated linoleic acid, achieving a molar conversion rate of 12%. The produced oil contains 50% conjugated linoleic acid in free form, with 9(Z),11(E)-conjugated linoleic acid and 9(E),11(Z)-conjugated linoleic acid accounting for 38% and 62%, respectively (Figure 2 [Figure 2: see original paper] A). Lactic acid bacteria exhibit similar oil transformation capabilities, converting -linolenic acid and -linolenic acid into corresponding conjugated fatty acids [**?**, **?**]. The main conjugated fatty acids produced from -linolenic acid are 9(Z),11(E),15(Z)-conjugated linolenic acid and 9(E),11(E),15(Z)-conjugated linolenic acid. Those produced from -linolenic acid are primarily 6(E),9(E),11(Z)-conjugated linolenic acid and 6(Z),9(E),11(E)-conjugated linolenic acid (Figure 2 [Figure 2: see original paper] B). Studies have also shown that overexpressing hydratase, dehydrogenase, and isomerase in *Escherichia coli* can further improve conjugated fatty acid production efficiency [**?**].

Following the discovery of hydroxy fatty acids as intermediates in conjugated linolenic acid synthesis, research on hydroxy fatty acid production using lactic acid bacteria has been initiated. Lactic acid bacteria can convert ricinoleic acid (12-hydroxy-9(Z)-octadecenoic acid) into conjugated linoleic acid (a mixture of 9(Z),11(E)-conjugated linoleic acid and 9(E),11(Z)-conjugated linoleic acid) [**?**]. *Lactobacillus plantarum* also serves as a candidate biocatalyst for converting ricinoleic acid into conjugated linoleic acid [**?**]. In this reaction, 3.4 mg/mL free ricinoleic acid can be converted into 2.4 mg/mL conjugated linoleic acid within 90 hours, achieving a conversion rate of 71%. The obtained conjugated linoleic acid exists primarily in free form, with 9(Z),11(E)-conjugated linoleic acid and 9(E),11(Z)-conjugated linoleic acid comprising 21% and 79%, respectively. Castor oil, which is rich in ricinoleic acid, has been extensively studied as an efficient substrate for conjugated linoleic acid production by lactic acid bacteria in the presence of lipase [**?**].

2.4 Production of Hydroxy Fatty Acids by Gut Anaerobic Microorganisms

Hydroxy fatty acids represent important intermediates in microbial fatty acid transformation, with their esterified structures having significant pharmaceutical applications. Specifically, 3-hydroxy fatty acid esters are widely used in preventing and treating mitochondrial damage-related diseases, brain atrophy-associated conditions, and products for supplementing brain energy supply. Most intestinal microorganisms possess metabolic pathways for hydroxy fatty acid synthesis. In *E. coli*, overexpression of conjugated linoleic acid hydratase (catalyzing the first step of fatty acid saturation) enables hydroxy fatty acid production. Through fermentation optimization, this engineered strain can convert oleic acid into 10-hydroxy-octadecanoic acid, achieving a titer of 30 g/L

with 90% conversion and strict S-configuration [**?**]. Similar synthetic pathways for hydroxy fatty acids exist in lactic acid bacteria, which can utilize 9(Z),12(Z)-nonconjugated octadecenoic acids to synthesize various hydroxy fatty acids, including dihydroxy fatty acids such as 10-hydroxy, 13-hydroxy, and 10,13-dihydroxy fatty acids.

2.5 Key Intermediates in the Reduction of Long-Chain PUFAs by Gut Anaerobic Microorganisms

As an important class of intestinal microbes, *Clostridium bifermentans* can reduce eicosapentaenoic acid (EPA) and arachidonic acid (ARA) to 5(Z),8(Z),13(E),17(Z)-conjugated eicosapentaenoic acid and 5(Z),8(Z),13(E)-conjugated eicosatrienoic acid, respectively (Figure 2 [Figure 2: see original paper] C) [**?**]. Similar reactions occur in -6 and -9 C18 and C20 fatty acids with (Z),(Z)-nonconjugated structures (such as -linolenic acid, -linolenic acid, and dihomo- -linolenic acid), which are transformed into -7 (E)-monoenone fatty acids. Structural characterization has identified a series of intermediates produced during EPA reduction by *C. bifermentans*, primarily conjugated isomers of EPA such as 5(Z),8(Z),11(Z),13(E),17(Z)-conjugated eicosapentaenoic acid and 5(Z),8(Z),11(E),13(E),17(Z)-conjugated eicosapentaenoic acid (Figure 2 [Figure 2: see original paper] C). Similar metabolic processes occur during ARA transformation by *C. bifermentans*, with key intermediates in the reduction of ARA to 5(Z),8(Z),13(E)-conjugated eicosatrienoic acid including 5(Z),8(Z),11(Z),13(E)-conjugated eicosatetraenoic acid and 5(Z),8(Z),11(E),13(E)-conjugated eicosatetraenoic acid (Figure 2 [Figure 2: see original paper] C).

Polyunsaturated fatty acids and their derivatives play crucial roles in maintaining intestinal health, particularly in combating foodborne pathogenic infections and improving gut health, while directly or indirectly influencing the balance of the gut microecosystem. These compounds protect the intestine from pathogen invasion and infection by regulating intestinal immunity, thereby maintaining fundamental physiological properties of the gut. This provides new insights into the mechanisms of intestinal disease development and offers novel approaches for disease prevention and treatment. Intestinal PUFAs and their derivatives are not solely obtained from dietary sources; most intestinal microorganisms possess metabolic pathways for PUFA synthesis, and certain microbes can transform PUFAs into rare fatty acids with special structures and physiological functions, such as hydroxy fatty acids, through a series of derivatization mechanisms. This review has elucidated these fatty acid transformation and derivatization mechanisms in detail. Gut microorganisms possessing these mechanisms will serve as seed banks for rare fatty acid production strains and provide a rich genetic element library for metabolic pathway engineering, thereby expanding the sources of functional PUFA-producing strains.

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