Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

China Food Publishing Co.

Recent advancements in butyric acid research: health effects, sources, and high-efficiency bioconversion production strategies

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ARTICLE INFO

Article history:
Received 28 March 2024
Received in revised form 3 July 2024
Accepted 30 August 2024

Keywords:
Butyric acid
Health effects
Sources
Bioconversion production strategies

ABSTRACT

Butyric acid, a representative short-chain fatty acid, has gained considerable attention due to its noteworthy health benefits and widespread application. Nonetheless, the majority of commercially available butyric acid is currently synthesized through environmentally harmful chemical synthesis methods, which pose a significant obstacle to the development of the butyric acid industry. Therefore, there is a need to develop cost-effective, efficient, and environmentally friendly methods for butyric acid production. This review commences with a systematic evaluation of the health effects and mechanisms of action of butyric acid. Subsequently, it outlines the advantages and disadvantages of different sources of butyric acid, including chemical synthesis and biosynthesis, along with their development trends. Finally, the review delves into high-efficiency strategies to enhance butyric acid production through targeted biosynthesis. This review not only serves as a valuable reference for researchers and practitioners interested in the diverse aspects of butyric acid but also offers insights into future trends in the development of butyric acid.

1. Introduction

Butyric acid, a typical short-chain fatty acid (SCFA), has been reported to have important applications in diverse fields including chemicals[1], food additives[2], pharmaceuticals[3], and animal feed[4]. Intestinal butyric acid is mainly derived from the metabolism of undigested carbohydrates by the intestinal microbiota, including plant polysaccharides, oligosaccharides, and resistant starch[5]. Although butyric acid is a SCFA with relatively low levels in the gut (approximately 15% of the total fermentable acids), it is still an important substance with many physiological functions[6]. Butyric acid is important in promoting human health as it exhibits various beneficial effects such as anti-inflammatory and anti-cancer properties[7]. Studies have shown that butyric acid can act on cells primarily through G protein-coupled receptors (GPRs) and further reduce the levels of cellular cyclic adenosine monophosphate (cAMP) and pro-inflammatory cytokines[8-9]. In addition, butyric acid has the potential to modulate the immune system and alleviate cancer by inhibiting histone deacetylases (HDACs)[10]. Thus, butyric acid is an important physiologically active substance in maintaining human health, and therefore targeting to increase intestinal butyric acid level may be one of the hotspots for future research.

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Peer review under responsibility of Beijing Academy of Food Sciences.

SciOpen Publishing services by Tsinghua University Press

http://doi.org/10.26599/FSHW.2024.9250384

Currently, the main source of butyric acid is chemical synthesis, but this method may cause environmental pollution and increase the burden of isolation and purification of reaction products. Therefore, the environmentally friendly and inexpensive butyric acid production methods, such as microbial conversion, will be widely concerned. For example, the utilization of low-cost renewable feedstocks for the production of butyric acid holds immense potential and is anticipated to be a sustainable method for the future large-scale industrial production of butyric acid[11]. Considering the current research progress, the low-cost and efficient production of butyric acid by biotransformation method mainly includes the following aspects: 1) selecting and breeding superior strains with high butyric acid production capability[12]; 2) designing bioreactors that can effectively utilize the synergistic effects between different strains[13]; 3) improving butyric acid metabolism of strains by genetic engineering[14], and 4) enhancing the metabolism capability of intestinal butyric acid-producing strains[15]. These multi-pronged approaches may not only significantly increase the yield of butyric acid, but also significantly reduce the cost of butyric acid production, which paves the way for broader applications of butyric acid.

To promote the application of butyric acid and the development of butyric acid-related industries, this review provides a comprehensive overview of butyric acid, including its health effects, sources, and the advantages and disadvantages of different production methods. Moreover, it provides insights into new advances and strategies to enhance the biological production of butyric acid. Additionally, this review sheds light on the future development trends of butyric acid, which can be pivotal in shaping its broader application and industrial production. Overall, this review is an important reference for researchers and practitioners interested in areas such as increasing butyric acid production or targeting increased human intestinal butyric acid level.

2. Health effects of butyric acid

Butyric acid is one of the most comprehensively studied SCFAs in terms of its health effects. From the research reports in recent years, it can be seen that the health effects of butyric acid mainly include providing energy to the intestinal epithelial cells[16], enhancing colon cell function[17], maintaining intestinal barriers[18], enhancing intestinal immunity[19], regulating inflammatory responses[20], and modulating intestinal microbiota[21] (Fig. 1). These health effects of butyric acid make it play an important role in ameliorating diarrhea, inflammatory bowel disease, metabolic disorders, and colorectal cancer[22]. Butyric acid can also be absorbed into the bloodstream *via* intestinal cells and act *via* the blood circulation in the central nervous system and peripheral tissues such as the liver, fat, and kidney tissues, which can further affect the metabolic activity throughout the body[16,23]. For example, dietary supplementation or colonic infusion of butyric acid can increase thermogenesis, decrease appetite, regulate glycolipid metabolism, and reduce adipose tissue weight[24]. Moreover, numerous studies confirm that butyric acid also has great potential in the treatment of obesity, fatty liver, cardiovascular disease, and diabetes[3]. In addition to improving gastrointestinal and metabolism-related disorders, butyric acid can also improve brain function and ameliorate neurodegenerative diseases and behavioral disorders by acting on the gut-brain axis[25].

It has been found that butyric acid regulates human health mainly through 2 signaling mechanisms (Fig. 1): activation of GPRs[26] and inhibition of HDACs[27]. On the one hand, butyric acid can activate GPRs, including GPR41, GPR43, and GPR109A[28]. These receptors are widely expressed in various cell types throughout the intestine tissue, including enteroendocrine cells, epithelial and smooth muscle cells, enteric neurons, and immune cells[8]. GPR41 can couple with Gαi protein after activation by butyric acid, which in turn inhibits

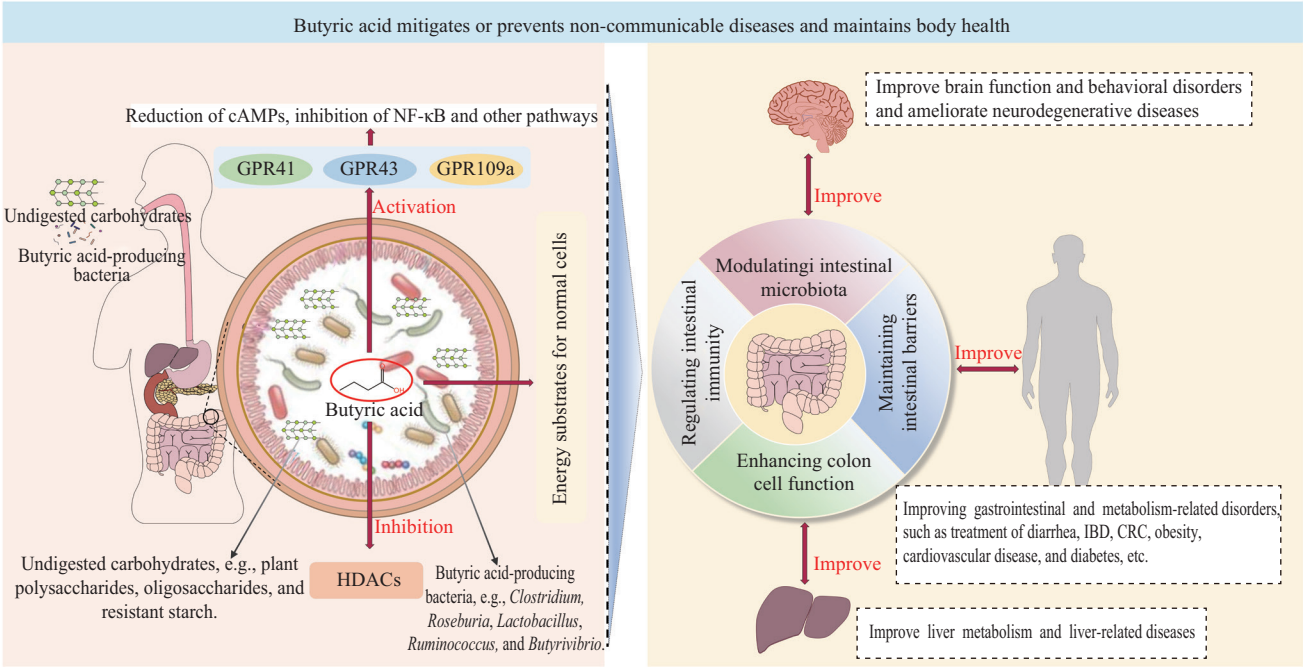

Fig. 1 Health effects of butyric acid. IBD, inflammatory bowel disease; CRC, colorectal cancer.

adenylate cyclase activity and reduces intracellular cAMP levels[29]. GPR43 is associated with both Gαi and Gαq proteins, which can not only reduce cAMP levels but also increase intracellular Ca^{2+} concentrations. The activation of GPR43 can initiate downstream signaling cascades, including activation of the *β*-arrestin-2 pathway, inhibition of the nuclear factor kappa-B (NF-κB), and reduction of the pro-inflammatory cytokines production[30]. GPR109A can be expressed in macrophages and neutrophils, and it plays a role in inducing the differentiation of regulatory T cells (Tregs) and interleukin (IL)-10-producing T cells[31]. A previous study demonstrated that the activation of GPR109A is associated with the aggregation of the inhibitory G proteins Gi/Go and *β*-arrestin into the cell membrane[32]. On the other hand, butyric acid also exerts potent immunomodulatory effects by inhibiting HDACs to affect neutrophils, monocytes, and macrophages. For example, butyric acid can reduce lipopolysaccharide-induced pro-inflammatory factors such as nitric oxide, IL-6, and IL-12 by inhibiting HDACs to activate histone acetyl transferase (HAT) in macrophages and colonocytes[33]. However, butyric acid exhibits the paradox of inhibiting the proliferation of cancer cells but promoting the growth of colonic epithelial cells, which can be explained by the Warburg effect[10]. Briefly, the normal colon cells prefer the tricarboxylic acid cycle and can metabolize butyric acid as energy[22]. However, the cancer cells prefer aerobic glycolysis and do not utilize butyric acid, so the butyric acid accumulated in cancer cells can act on HDACs to inhibit the proliferation of cancer cells[34]. In addition, butyric acid can also modulate health by simultaneously acting on both HDACs and GPRs pathways. A prior study reported that butyrate can synergistically promote IL-22 production in $CD4^+$ T cells and innate lymphoid cells (ILCs) to modulate intestinal immunity through HDACs inhibition and GPR41 activation[19].

Taken together, butyric acid plays a broad role in maintaining human health. It has great potential in alleviating or treating gastrointestinal diseases (e.g., colitis and cancer), metabolism-related diseases (e.g., obesity and diabetes), and neurological-related diseases (e.g., inflammation and neurodegenerative diseases). This is mainly achieved through the activation of GPRs and inhibition of HDACs pathways. This suggests that intestinal butyric acid level is closely related to human health, which also suggests that intestinal butyric acid level is expected to be a potential biomarker for assessing human health. In addition, it also suggests that dietary supplementation or targeting to increase intestinal butyric acid level is an important way to maintain health.

3. Main sources of butyric acid

Currently, the commercial butyric acid is mainly derived from chemical synthesis (oxidative of fossil raw materials) and microbial fermentation (Fig. 2), of which chemical synthesis is particularly prevalent. However, the production of butyric acid by chemical synthesis method not only consumes fossil raw materials but also pollutes the environment with its reaction by-products (e.g., toxic spent metallic catalysts sulfide, cyanide, and chloride)[2]. Therefore, the utilization of microbial conversion methods to convert cheap biomass raw materials into butyric acid has a broader application prospect in the future[11]. In addition, compared to butyric acid produced by chemical synthesis, butyric acid derived from microbial fermentation has a higher biosafety and it is more suitable for some specific applications in the food (e.g., used as pure acid to intensify butter-like notes in food flavors) and pharmaceutical industries (e.g., used as the pharmaceutical component to treat colorectal cancer, stomach, and intestine diseases)[2]. Currently, increasing the level of intestinal butyric acid is one of the hot topics of research. Except for a small portion of foods (such as SCFAs-rich dairy products and fats), most of the butyric acid in the human gut comes from the fermentation of dietary fiber by the intestinal microbiota[8].

3.1 Chemical synthesis

Industrially, butyric acid is mainly produced by butyraldehyde oxidation, in which butyraldehyde is predominantly derived from the oxidative synthesis of propylene from crude oil[35]. For instance, butyraldehyde can be oxidized to butyric acid by reacting at 90 °C for 7 h with 30% H_2O_2 as the oxidant and the sulfonic acid resin Amberlyst 15 as the catalyst[36]. The underlying principle of this reaction is shown in Fig. 2.

Although the production of butyric acid using the chemical synthesis method described above has advantages such as a single substrate, this method also encounters some challenges. On the one hand, the substrate used in chemical synthesis is derived from petrochemicals, which may face the threat of the depletion of fossil raw materials and the increase of crude oil prices in the future. On the other hand, the chemical synthesis method not only requires the use of catalysts but also cannot completely eliminate the production of reaction by-products, which will inevitably cause a certain amount of environmental pollution. Therefore, some environmentally friendly and low-cost butyric acid production methods will be one of the hot topics for future research, such as the use of microbial fermentation to convert cheap biomass raw materials into butyric acid.

3.2 Biological synthesis

In recent years, a variety of bacteria have been reported to produce butyric acid, mainly including *Clostridium*, *Butyribacterium*, *Butyrivibrio*, *Eubacterium*, *Sarcina*, *Megasphaera*, *Clostridium* and *Fusobacterium*[1]. Among them, *Clostridium* species are commonly employed microorganisms in butyric acid fermentation due to its high production and high yield. Notably, *Clostridium butyricum* and *Clostridium tyrobutyricum* are the main high-yielding butyric acid bacteria[37]. Although *C. butyricum* can utilize a wider range of substrates, including starch, lignocellulose, hexose, pentose, and glycerol, some of these *C. butyricum* may carry pathogenic genes, which have been reported to be associated with necrotizing small intestinal colitis in newborns[38]. In contrast, *C. tyrobutyricum* has a more limited spectrum of substrates, specifically glucose, xylose, fructose, and lactate. However, *C. tyrobutyricum* has the advantages of high yield, high productivity, high selectivity, and better tolerance to butyric acid when compared to *C. butyricum*[2,35]. Therefore, *C. tyrobutyricum* is more promising for butyric acid biosynthesis during the industrial production of butyric acid in the future.

3.2.1 Butyric acid production by Clostridium fermentation

Clostridium is a genus within the family of *Bacillus*, which includes most butyric acid-producing strains such as *C. butyricum*, *C. tyrobutyricum*, and *C. acetobutylicum*. These strains belong to

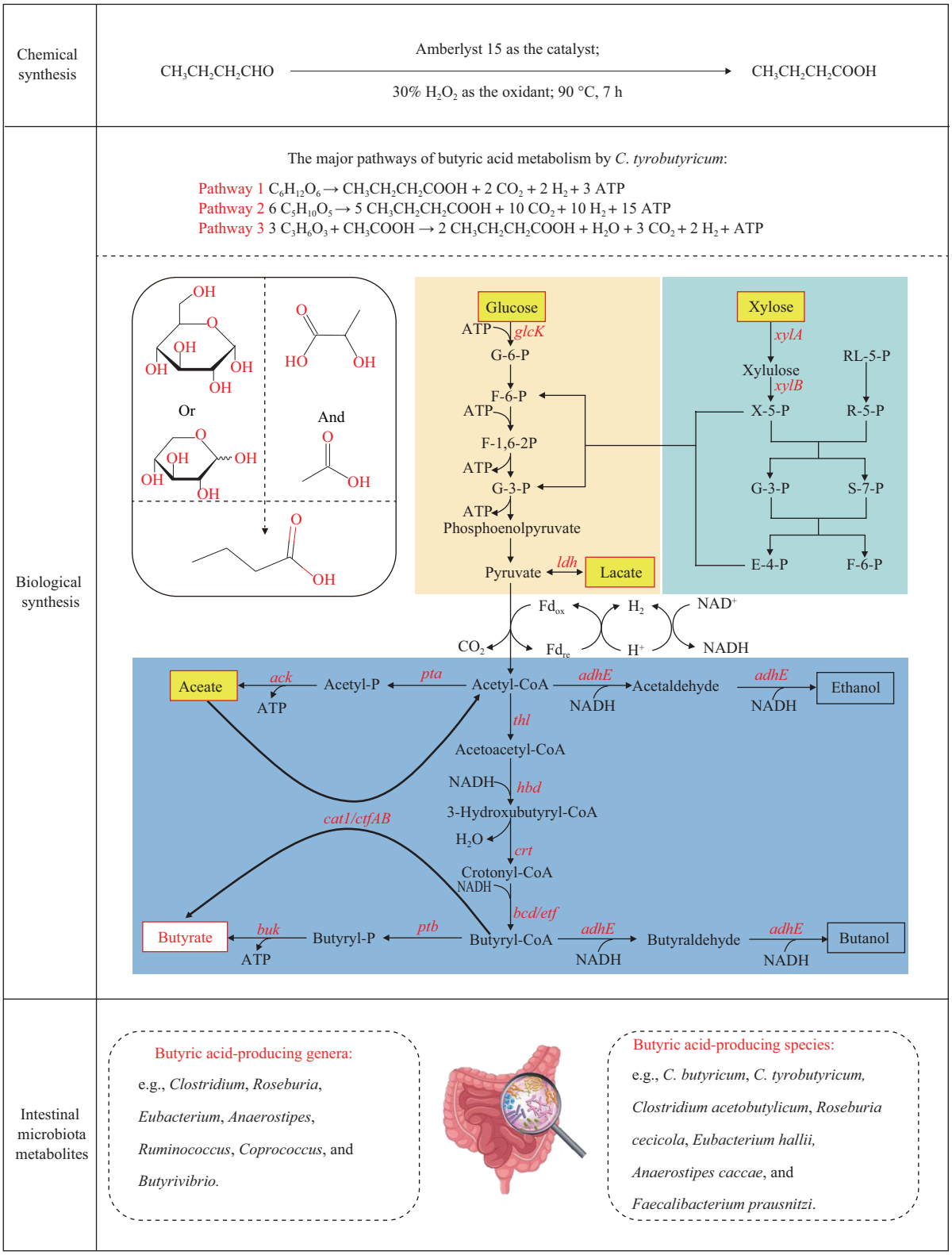

Fig. 2 Main sources of butyric acid, including chemical synthesis, biological synthesis, and intestinal microbiota metabolites. *xylA*, xylose isomerase; *xylB*, xylose kinase; *pfkA*, 6-phosphofructokinase; *pykA*, pyruvate kinase; *ldh*, lactic dehydrogenase; *pfor*, pyruvate:ferredoxin oxidoreductase; *ack*, acetate kinase; *pta*, phosphotransacetylase; *thl*, thiolase; *hbd*, *β*-hydroxybutyryl-CoA dehydrogenase; *crt*, crotonase; *bcd/etf*, butyryl-CoA dehydrogenase and electron transfer flavoprotein; *cat1*, butyryl-CoA/acetate-CoA transferase; *ptb*, phosphotransferase; *buk*, butyrate kinase; *adhE1*, aldehyde/alcohol dehydrogenase.

Gram-positive bacteria and are strictly anaerobic heterotrophic fermentation strains that can metabolize organic carbon sources such as glucose and pentose into butyric acid[39]. The metabolic pathway of *Clostridium* converting substrate carbon sources (glucose or pentose) to butyric acid is shown in Fig. 2. Briefly, i) *Clostridium* can first convert substrate carbon sources to pyruvate, which is then converted to acetyl coenzyme A (acetyl-CoA, a precursor for butyric acid formation) or lactate. For example, *Clostridium* can convert glucose to pyruvate *via* the glycolytic pathway (Embden-Meyerhof-Parnas pathway, EMP). When the substrate carbon source is xylose, these bacteria are also able to catabolize xylose to xylulose *via* the pentose phosphate pathway (PPP), and then transformed into pyruvate by a series of enzymatic reactions[40]. Subsequently, pyruvate can be further converted to acetyl-CoA, which is a critical hub in the butyric acid metabolic pathway[40]. ii) During metabolism, acetyl-CoA can be enzymatically converted to acetate through the action of phosphotransacetylase (PTA) and acetic acid kinase (ACK). Additionally, acetyl-CoA can be also further transformed into butyryl coenzyme A (butyryl-CoA) by a series of enzymes, including acetyl-CoA transferase (THL), *β*-hydroxybutyryl-CoA dehydrogenase (HBD), crotonase (CRT), butyryl-CoA dehydrogenase and electron-transferring flavoprotein (BCD & ETF)[22]. iii) Butyryl-CoA is an important precursor for the synthesis of butyric acid. Currently, there are 2 known pathways by which butyryl-CoA can form butyric acid. One pathway is the conversion of butyryl-CoA to butyric acid through the action of phosphotransferase (PTB) and butyrate kinase (BUK)[11]. The PTB-BUK pathway is predominantly employed by butyric acid-producing *Clostridium* species, including *C. butyricum* and *C. acetobutylicum*[2]. The second pathway utilizes butyryl-CoA: acetate coenzyme A (acetate-CoA) transferase (CAT) to transfer CoA from butyryl-CoA to acetate, resulting in the production of acetyl-CoA and butyric acid. It was found that *C. tyrobutyricum* was able to utilize the second pathway to produce butyric acid[41].

Although *Clostridium* has good potential for butyric acid production, there are many factors affecting the butyric acid fermentation of *Clostridium*, mainly including fermentation substrates, pH, fermentation products, and fermentation methods. For example, the growth and metabolism of *Clostridium* are significantly impacted by the fermentation substrate, and glucose is the favored carbon source for most species. However, the economic costs of different substrates vary significantly, so reducing the expenses associated with butyric acid fermentation in *Clostridium* is a key area of research. One approach to reduce costs is to replace expensive nutrients in media with affordable and renewable biomass feedstocks. A previous study reported that the butyric acid metabolism in *C. tyrobutyricum* DSM 2367 could be achieved by solid-state fermentation of certain agricultural wastes[42]. However, it should be noted that *C. tyrobutyricum* cannot directly utilize most agricultural wastes or lignocellulosic materials for butyric acid fermentation. Additionally, the presence of glucose can affect the metabolism of xylose by *C. tyrobutyricum*[43]. Moreover, the fermentation pH can significantly affect *Clostridium* metabolism and its metabolite distribution. For example, *C. tyrobutyricum* exhibits a high yield of butyric acid (approximately 57.9 g/L) at pH 6.3, but this strain will produce mainly acetic and lactic acids along with small amounts of butyric acid (approximately 48.2 g/L) once pH less than 5.7[44]. For *Clostridium*, the fermentation products (like butyric acid) belong to its growth and fermentation inhibitor, and its accumulation can cause a decrease in intracellular pH and a reduction in available ATP for cell growth and metabolism. Consequently, this in turn leads to a significant decline in the growth and metabolism rates of this strain[2]. Different fermentation methods also have notable effects on the biotransformation of butyric acid. For example, natural fermentation tends to have a low yield and low concentration of butyric acid. While using a typical bioreactor for butyric acid production can lead to a substantial decrease in butyric acid yield during the mid- to late-stage of fermentation due to the excessive accumulation of nonviable cells as well as suboptimal utilization of fermentation equipment[41]. Therefore, some efficient fermentation strategies are needed to overcome these practical problems in the production process, such as utilizing bioaugmented reactors.

3.2.2 Bioaugmented reactor for butyric acid production

The efficient utilization of bioreactors to produce butyric acid has been a hot topic in the butyric acid fermentation industry. For instance, a fibrous bed bioreactor (FBB) was used to study the fermentation ability of *C. tyrobutyricum* on agricultural wastes (such as corn fiber, inulin, rapeseed stover, sugarcane bagasse hydrolysate, and waste paper) and the tolerance of the strain to butyric acid[45-47]. Furthermore, modifications based on FBB technology have been explored to develop innovative reactor designs, including internal disc substrate reactors, stirred kettle reactors, and microbubble-coupled fiber bed bioreactors[48-50]. All these reactor systems have demonstrated significant improvements in the efficiency and yield of butyric acid fermentation compared to conventional fermentation methods. For example, Shahab et al.[13] developed a biofilm reactor that mimics a stable microbial community and creates an ecological environment from aerobic to anoxic, which is suitable for integrated bioprocessing of lignocellulose to SCFAs. Initially, the reactor was enclosed by an oxygen-permeable membrane to exclude air while allowing oxygen penetration. Subsequently, the aerobic fungus *Trichoderma reesei* produced cellulolytic enzymes and consumed oxygen, resulting in the release of soluble sugars (xylose and glucose). Following this, lactic acid and acetic acid were metabolized by partially anaerobic lactic acid bacteria. Ultimately, anaerobic *C. tyrobutyricum* facilitated the biotransformation of lactic acid and acetic acid into butyric acid. The results demonstrated that, when incubated with a mixture of cellulose and xylose as substrates, this biofilm reactor produced 0.35 g butyric acid per 1 g consumed carbohydrate. Therefore, this bioaugmented reactor not only enhances the yield of butyric acid, surpassing the results achieved with soluble substrates or the addition of commercial cellulolytic enzymes[13], but also markedly reduces the cost of butyric acid production.

Overall, in contrast to the chemical synthesis of butyric acid, which depends on the consumption of fossil resources and raises environmental pollution concerns, bioconversion provides a more sustainable alternative by transforming inexpensive biomass feedstocks into butyric acid. Consequently, the use of the bioconversion method to produce butyric acid will become one

of the key directions for future research. Currently, among the naturally isolated butyric acid-producing bacteria, butyric acid fermentation by *C. tyrobutyricum* is widely regarded as the most promising strain for commercial application due to the presence of high purity, high selectivity, lower nutritional requirements, and better butyric acid tolerance[11]. Nonetheless, the production of butyric acid using *C. tyrobutyricum* through natural fermentation or conventional bioreactor fermentation has certain drawbacks, such as cell aging and product inhibition. Therefore, the rational use of bioaugmented bioreactor for high-efficiency fermentation of butyric acid is the future development trend in the production of butyric acid by bioconversion methods.

3.3 Intestinal microbiota metabolites

Except for a minor proportion of butyric acid derived from foods in dietary supplements, the majority of intestinal butyric acid is generated through the metabolism of gut microbiota. The human gut represents the most intricate ecosystem, harboring diverse microbial populations dominated by Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia. Among them, Firmicutes and Bacteroidetes account for approximately 90% of the intestinal microbiota[51]. Butyric acid, a vital metabolite, is mainly derived from the microbial metabolism of undigested carbohydrates[52], and its main metabolic pathways are shown in Fig. 2.

Currently, a number of gut microbiota have been reported to produce butyric acid, including both some genera (e.g., *Clostridium*, *Roseburia*, *Eubacterium*, *Anaerostipes*, *Ruminococcus*, *Coprococcus*, and *Butyrivibrio*) and some specific species (e.g., *C. butyricum*, *C. tyrobutyricum*, *C. acetobutylicum*, *R. cecicola*, *E. hallii*, *A. caccae*, and *F. prausnitzi*)[15]. Among these butyric acid-producing bacteria, *Roseburia*, *F. prausnitzii*, *E. hallii*, and *C. tyrobutyricum* are more inclined to utilize acetic acid for butyric acid synthesis, whereas the core group of *Clostridium* spp. prefers to synthesize butyric acid *via* the PTB-BUK pathway. It remains unclear whether there exist strains that utilize both pathways simultaneously[53-54]. The composition of the gut microbiota is largely influenced by various factors, such as the host's diet, age, disease state, physical activity, and antibiotic usage[55], which in turn affect the butyric acid level in the host's gut. Therefore, maintaining the balance of gut microbiota and increasing the abundance and metabolic capacity of intestinal butyric acid-producing bacteria can effectively target the elevation of host intestinal butyric acid level.

4. The high-efficient strategies to improve butyric acid yield by biosynthesis

Currently, there are 3 main highly efficient strategies to enhance butyric acid production through biosynthesis (Fig. 3): 1) mining butyric acid-producing bacteria that can efficiently utilize inexpensive biomass resources and have strong butyric acid metabolism ability *via* high-throughput screening techniques; 2) improving butyric acid metabolism capacity of bacterial strains through genetic engineering approaches; and 3) targeting to increase the abundance and metabolic capacity of butyric acid-producing bacteria by giving full play to the mutualistic effects of prebiotics and probiotics interactions with butyric acid-producing strains.

4.1 Screening of high-yielding butyric acid-producing bacteria for efficient biomass conversion

Currently, *Clostridium* is considered to be a typical class of the most commercially valuable genera for butyric acid production, but they usually require strict anaerobic heterotrophic fermentation. Reinforced *Clostridium* medium (RCM, containing peptone 10 g/L, beef extract 10 g/L, yeast extract 3 g/L, glucose 5 g/L, NaCl 5 g/L, soluble starch 1 g/L, sodium acetate 3 g/L, and *L*-cysteine hydrochloride 0.5 g/L) is a commonly used medium for cultivating and isolating *Clostridium*. For instance, Fu et al.[56] isolated a strain of *C. butyricum* SCUT 620 that can directly convert raw tapioca starch into butyric acid under anaerobic conditions using RCM medium, and its butyric acid yield was as high as 21.72 g/L. Alternatively, pasteurized samples can be added to a modified RCM medium containing 1.8% sodium lactate (pH 6.1) and then covered with a 1.5 cm layer of paraffin wax.

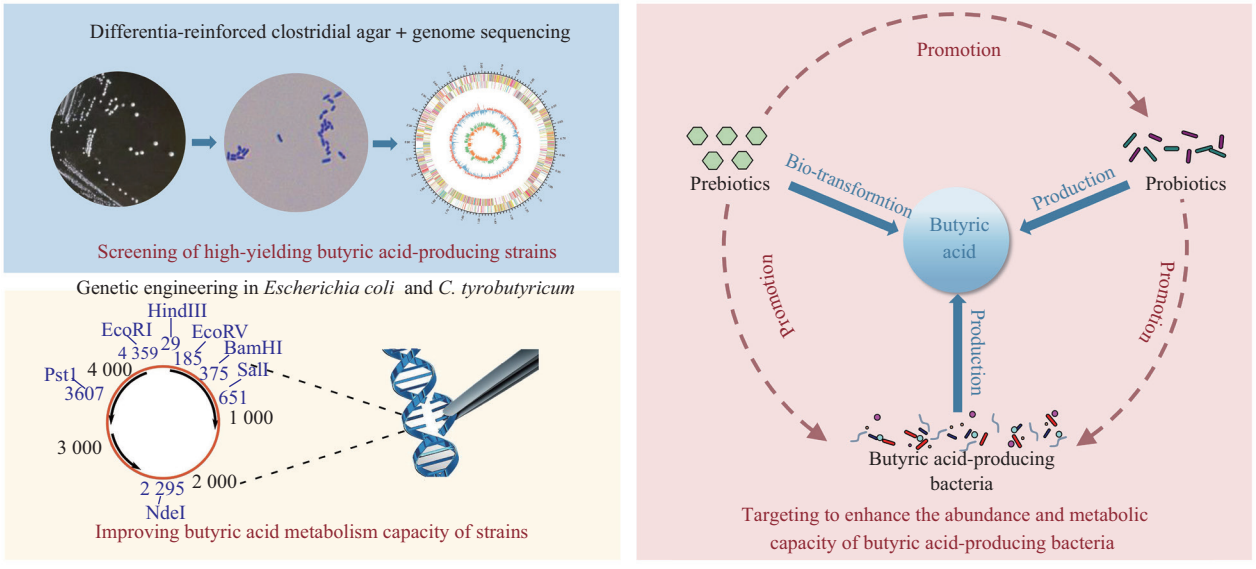

Fig. 3 High-efficient strategies to improve butyric acid yield by biosynthesis.

Subsequently, anaerobic incubation was carried out at 37 °C and the growth of *Clostridia* was confirmed by the elevation of the paraffin layer[57]. In addition, the addition of neutral red, serine, and methoxybenzamidopyrimidine into the RCM medium also can accelerate the detection of *Clostridium* in the samples[57]. This is because the presence of butyric acid-producing bacteria can change the color of the medium from red to yellow due to the reducing effect of neutral red. In general, this method simplifies the observation of strain growth in the medium by the production of gas[12]. In comparison, the medium with the addition of neutral red as an indicator will have better selectivity. For example, when detecting *Clostridium sporogenes* in pasteurized milk samples, RCM medium covered with paraffin wax at 37 °C gave 88% selectivity for the detection of *Clostridium* after 2 days, while the selectivity of the indicator method was as high as 96%[57]. In some studies, differentia-reinforced *Clostridial* agar (DRCA, containing beef extract powder 8 g/L, casein peptone 5 g/L, peptone 5 g/L, yeast extract powder 1 g/L, glucose 1 g/L, soluble starch 1 g/L, sodium acetate 5 g/L, *L*-cysteine hydrochloride 0.5 g/L, ammonium ferric citrate 0.5 g/L, sodium sulfite 0.75 g/L, and blade asphaltene 0.002 g/L) is used as common media for the preliminary identification of *Clostridium*. In comparison, DRCA is more suitable for the identification of *Clostridium* than RCM because DRCA contains hydrogen sulfide indicator (ammonium ferric citrate and sodium sulfite).

C. tyrobutyricum ATCC 25755 is a representative strain that efficiently converts lignocellulosic hydrolysate to butyric acid at a final fermentation concentration of up to 86.9 g/L, which is the highest reported yield in the conventional fermentation processes[58]. Genome sequencing of *C. tyrobutyricum* ATCC 25755 revealed that this strain lacks the PTB-BUK gene sequence, which was contradictory to the results of some previous studies[59-60], indicating that *cat1* is the sole gene responsible for butyric acid synthesis in *C. tyrobutyricum*[39]. *C. tyrobutyricum* ATCC 25755 has been annotated in the NCBI database (https://www.ncbi.nlm.nih.gov) with gene sequences CP014170 (chromosome) and CP014171 (plasmid). The key genes associated with the butyric acid synthesis in *C. tyrobutyricum* ATCC 25755, along with their corresponding primers are shown in Table 1.

4.2 Application of genetically engineered bacteria in improving butyric acid metabolism

Genetic engineering involves biotechnologies that manipulate genes at the molecular level. The biggest advantage of genetic engineering is that it can break through the constraints between different species. For example, genetic engineering techniques can be applied to improve the tolerance of *C. tyrobutyricum* to butyric acid while reducing the production of acetic acid as the by-product (Table 2). In addition, researchers also often use the introduction of exogenous genes associated with butyric acid metabolism in *E. coli* for butyric acid production, which can significantly cut down the costs of butyric acid production (Table 2).

4.2.1 Application of genetic engineering in C. tyrobutyricum

During butyric acid fermentation, the utilization of inexpensive renewable lignocellulosic materials as fermentation substrates is a primary strategy to reduce the cost of butyric acid production. Nevertheless, the utilization of *C. tyrobutyricum* in conventional fermentation processes to achieve low-cost and efficient butyric acid production encounters various challenges. These challenges encompass bacterial growth inhibition, the interconnection of acetic acid with butyric acid synthesis, and carbon catabolite repression (CCR) associated with lignocellulosic hydrolysates[11,43,61]. These issues can be effectively solved by modifying the relevant genes in the strains through genetic techniques such as gene knockdown, expression inhibition, or overexpression.

Furan derivatives and phenolic compounds are the primary inhibitors of *C. tyrobutyricum* in lignocellulosic hydrolysates. However, recent studies have confirmed that the overexpression of natural *GroESL* in *C. tyrobutyricum* significantly increases its tolerance to lignocellulosic hydrolysis product-derived inhibitors, especially phenolic compounds[62]. Based on this finding, simultaneous expression of natural GroESL and short-chain dehydrogenase/reductase (*SDR*) from *Clostridium beijerinckii* NCIMB 8052 in *C. tyrobutyricum* improves furfural tolerance in *C. tyrobutyricum*. This modification led to a notable increase in butyric acid productivity of ATCC 25755/*sdr* + *GroESL* from 0.19 to 0.29 g/(L·h)[63].

Table 1
Relevant enzymes, genes, and primers related to butyric acid metabolic in *C. tyrobutyricum.*

Locus tag in NCBI database	Enzymes	Target gene	Primers (5′-3′)	Reference
CTK_C21080	XylA	*xylA*-F *xylA*-R	AAAAGTAGGAGCGCGAGGAGGAATTAAAATGAATAATACACCAA AAACAGCTATGACCGCCGCGGATTACTCAAAAGGATTTTCTGTTTT	[88]
CTK_C21070	XylB	*xylB*-F *xylB*-R	ATTTAAATTTGGATCC AGATTGAGGAGGAATATAAAATGAATAA AAACAGCTATGACCGAGCTCGCGCTCCTACTTTTAACTATTTATATATCT	[88]
CTK_C18230	Pta	*pta*-F *pta*-R	GA(A/G)(C/T)T(A/T/G)AG(A/G)AA(A/G)CA(T/C)AA(A/G)GG(A/T)ATGAC (A/T)GCCTG(A/T)(G/A)C(A/T)GC(A/T/C)GT(A/T)AT(A/T)GC	[89]
CTK_C18220	Ack	*ack*-F *ack*-R	GATAC(A/T)GC(A/T)TT(C/T)CA(C/T)CA(A/G)AC (G/C)(A/T)(A/G)TT(C/T)TC(A/T)CC(A/T)AT(A/T)CC(A/T)CC	[90]
CTK_C06520	Cat or Ctf or BCoAT	*cat1*-F *cat1*-R	GCTCTAGAATAAATATTTAGGAGGAATAGTCATGAGTTTTGAGGAATTGTATAA TCCCCGCGGTCAATATTTACATCCAAATCTTTTT	[61]

Note: xylose isomerase (xylA); xylose kinase (xylB); butyryl-CoA/acetate-CoA transferase (Cat or Ctf or BCoAT). The primers for *Pta* and *Ack* are degenerate primers that represent all the different base sequences encoding a single amino acid.

Table 2
Application of genetically engineered bacteria in improving butyric acid metabolism.

Genetically engineered strains	Recombinant gene source	Fermentation conditions (culture medium, temperature, pH, and fermentation methods)	Butyric acid			Reference
			C (g/L)	Y (g/g)	P (g/ L·h)	
C. tyrobutyricum	*Δpta*	CGM, 37 ℃, pH 6.0, fed-batch fermentation	32.5	0.38	0.63	[89]
	Δack	CGM, 37 ℃, pH 6.0, fed-batch fermentation in a FBB	41.7	0.42	0.25	[90]
	Δptb	CGM, 37 ℃, pH 6.0, batch fermentation	29.14	0.35	0.22	[60]
	GroESL ↑	CGM, 37 ℃, pH 6.0, fed-batch fermentation in a FBB	52.2	0.37	0.41	[91]
	GroESL ↑	CGM (corn straw as a major carbon source), 37 ℃, pH 6.0, fed-batch fermentation in a FBB	29.6	0.37	0.31	[62]
	GroESL ↑	CGM (rice straw as a major carbon source), 37 ℃, pH 6.0, fed-batch fermentation in a FBB	30.1	0.37	0.31	
	GroESL ↑; *sdr* ↑ (derived from *C. beijerinckii*)	CGM (corncob as a major carbon source), 37 ℃, pH 6.0, fed-batch fermentation in a FBB	32.8	0.36	0.29	[63]
	pykA ↑	CGM, 37 ℃, pH 6.0, fed-batch fermentation in a FBB	42.3	0.37	0.44	[92]
	pfkA ↑		38.6	0.36	0.40	
	pykA ↑; *pfkA* ↑		48.2	0.38	0.50	
	cat1 ↑	CGM, 37 ℃, pH 6.0, fed-batch fermentation in a FBB	38.2	0.39	0.40	[61]
	crt ↑		37.9	0.39	0.39	
	cat1 ↑; *crt* ↑		37.5	0.40	0.39	
	cat1 ↑; *crt* ↑; *pykA* ↑; *pfkA* ↑		46.8	0.39	0.83	
	scrA ↑, *scrB* ↑, *and scrK* ↑ (derived from *C.acetobutylicum*)	CGM (cane molasses as a major carbon source), 37 ℃, pH 6.0, fed-batch fermentation in a FBB	45.7	0.39	0.43	[64]
	galK ↑, *galE* ↑, *galT* ↑ *and galP* ↑ (derived from *C. acetobutylicum*)	CGM (coffee grounds as a major carbon source), 37 ℃, pH 6.0, fed-batch fermentation in a FBB	34.3	0.37	0.36	[65]
	xylT ↑, *xylA* ↑, *and xylB* ↑ (derived from *C. acetobutylicum*)	CGM (glucose and xylose as a major carbon source), 37 ℃, pH 6.0, batch fermentation in a stirred-tank bioreactors	37.5	0.34	0.72	[93]
		CGM (soybean hull as a major carbon source), 37 ℃, pH 6.0, batch fermentation in a stirred-tank bioreactors	29.7	0.35	0.67	
		CGM (sugarcane bagasse as a major carbon source), 37 ℃, pH 6.0, batch fermentation in a stirred-tank bioreactors	42.6	0.36	0.56	
	ΔhprK	CGM (glucose and xylose as a major carbon source), 37 ℃, pH 6.0	12.73	–	–	[43]
	ΔxylR		9.83	–	–	
	ΔhprK; *ΔxylR*		–	–	0.31	
	ΔhprK; *ΔxylR*	CGM (lignocellulosic hydrolysates as a major carbon source), 37 ℃, pH 6.0, batch fermentation	13.4	–	0.34	
	ΔfadR; *aceF+*; *adhE*; *adhR*; *atoC*; *Ptet-atoB*; *ΔldhR*	Glucose, 37 ℃, aerobic cultivation	3.1	–	–	[67]
	ΔfadR; *aceF+*; *adhE*; *adhR*; *atoC*; *Ptet-atoB*; *ΔldhR*	Glucose, 37 ℃, semianaerobic cultivation	11.4	–	–	
	ΔfadR; *aceF+*; *adhE*; *adhR*; *atoC*; *Ptet-atoB*; *Δpta*	Glucose, 37 ℃, aerobic cultivation	3.5	–	–	
	ΔfadR; *aceF+*; *adhE*; *adhR*; *atoC*; *Ptet-atoB*; *Δpta*	Glucose, 37 ℃, semianaerobic cultivation	5.8	–	–	
	ΔfrdA; *ΔldhA*; *Δpta*; *ΔadhE*; *hbd* ↑ *and crt* ↑ (derived from *C. acetobutylicum*); *ter* ↑ (derived from *T. denticola*)	TB (glucose as a major carbon source), 37 ℃, fed-batch fermentation and semianaerobic cultivation	7.2	–	–	[66]
E. coli	*phaA* ↑ (derived from *C. necator*); *ter* ↑ (derived from *C. acetobutylicum*)	TB (glucose as a major carbon source), 37 ℃, fed-batch fermentation and semianaerobic cultivation	10.0	–	–	[68]
	ΔldhA; *ΔadhE*; *ΔackA*; *Δpta*; *ΔfrdC*	TB (glucose as a major carbon source), 37 ℃, pH 7.0, fed-batch and anaerobic cultivation	7.47	–	0.7	[69]
	tesBT ↑ (derived from *T. mimeticus*)	TB (glucose as a major carbon source), 37 ℃, pH 7.0, fed-batch fermentation and semianaerobic cultivation	14.3	0.11	1.29	[70]
	phaA ↑ and *phaB* ↑ (derived from *R. eutropha*); *phaJ* ↑ (derived from *A. caviae*); *ter* ↑ (derived from *T. denticola*);	TB (glucose as a major carbon source), 37 ℃, fed-batch fermentation and semianaerobic cultivation	12.3	–	0.228	[71]
	ΔldhA; *ΔfrdABCD*; *atoB* ↑; *hbd* ↑, *crt* ↑, *ptb* ↑, *buk*↑ (derived from *C. acetobutylicum*); *ter* ↑ (derived from *T. denticola*)	TB (glucose as a major carbon source), 37 ℃, pH 7.0, fed-batch and anaerobic cultivation	33.1	0.37	0.89	[72]

Note: ↑ Gene overexpression; ↓ Gene knockdown; Δ Gene knockout; Unlabeled are strains with their own genes, labeled are strains from which the genes originated. CGM means *Clostridium* growth medium containing 60 g/L glucose, 5 g/L yeast extract, 5 g/L peptone, 3 g/L $(NH_4)_2SO_4$, 0.6 g/L $MgSO_4 \cdot 7H_2O$, and 0.03 g/L $FeSO_4 \cdot 7H_2O$; TB means terrific broth containing 12 g/L tryptone, 24 g/L yeast extract, 2.31 g/L KH_2PO_4, 12.54 g/L K^2HPO_4, and 4 mL/L glycerol; M9: 0 6 g/L Na_2HPO_4,3 g/L KH_2PO_4, 0.5 g/L NaCl, 2 g/L NH_4Cl, 1 g/L $(NH_4)_2SO_4$, 241 mg/L $MgSO_4$, and 14.7 g/L $CaCl_2$; minimal means mineral salt medium. In all culture media, apart from the carbon source specifically indicated, other media primarily use glucose as the carbon source. C means concentration; Y means yield; P means productivity. "–" indicates that no relevant information is provided in the original study.

Butyric acid production in *Clostridium* fermentation is commonly associated with the coupling of butyric acid to acetic acid[43]. It was shown that the overexpression of *cat1* and *crt* genes in *C. tyrobutyricum* significantly increased the conversion of acetyl-CoA to butyric acid. During the fed-batch fermentation, ATCC 25755/cat1 + crt produced butyric acid/acetic acid ratio up to 15.76 g/g, which was 2.24 times higher than that of the wild-type strain[61].

The range of fermentation substrates efficiently utilized by *C. tyrobutyricum* is limited, but this substrate spectrum can be broadened through the constructive recombination of genes from other *Clostridia*. For instance, the overexpression of sucrose metabolism genes (*scrA*, *scrB*, and *scrK*) from *C. acetobutylicum* ATCC 824 in ATCC 25755 has been demonstrated to impart the ability to co-utilize sucrose, fructose, and glucose in sugarcane molasses. This modification leads to a notable decrease in feedstock costs for butyric acid fermentation by approximately 47% (untreated sugarcane molasses vs. conventional glucose fermentation)[64]. Likewise, the overexpression of galactose catabolic genes (*galK*, *galE*, *galT*, and *galP*) from *C. acetobutylicum* ATCC 824 in ATCC 25755 facilitated the effective utilization of galactose derived from spent coffee grind hydrolysate. This not only prevented the occurrence of glucose-induced inhibition of CCR but also increased butyric acid concentration and yield by 78.6% and 56.5%, respectively, compared to the wild-type strain[65]. Furthermore, a recent study revealed that CCR in *C. tyrobutyricum* can be eliminated by concurrent down-regulation of the bifunctional HPr kinase/phosphorylase (*hPrK*) and *xylR* genes, which encode the xylose transcriptional regulator[43].

Overall, genetic engineering can directionally modify the metabolic pathways and metabolic capacities of butyric acid-producing strains, which in turn endows the target strains with excellent characteristics such as stronger tolerance (e.g., acid tolerance, substrate, and product inhibition tolerance), higher yield, and wider substrate source. This approach stands as one of the primary methods and strategies for developing commercial strains in the future.

4.2.2 Application of genetic engineering in E. coli

The primary constraint in the large-scale industrial butyric acid production through *Clostridium* fermentation is the requirement of maintaining an anaerobic environment. Currently, there are still major technical barriers to directly genetically modifying *Clostridium* into aerobic strains. However, utilizing genetic engineering techniques to introduce butyric acid synthesis-related genes from *Clostridium* into *E. coli* proves to be an effective strategy for overcoming the anaerobic requirements inherent in the *Clostridium* fermentation industry[66].

Several strategies have been implemented to engineer *E. coli* for butyric acid production. These approaches include the knockout of *E. coli*'s native genes, the introduction of exogenous genes, and the reduction of acetic acid coupled with the production of butyric acid by targeted gene knockouts. Seregina et al.[67] successfully constructed a butyric acid-producing *E. coli* mutant MG1655 by eliminating the *fadR*, *aceF*, *ldhA*, and *pta* genes from *E. coli*. Interestingly, this mutant strain did not introduce any heterologous genes, but had a notable butyric acid yield of 1.3 g/L in LB medium containing 3% glucose. To minimize the formation of by-products during fermentation, Baek et al.[66] constructed a genetically engineered *E. coli* for high butyric acid production by introducing the *hbd* and *crt* genes of *C. acetobutylicum* and the trans-enoyl-coenzyme A reductase (*Ter*) gene of *Treponema denticola*. After using synthetic scaffolding proteins to enhance the overall catalytic efficiency of the heterologous enzyme, this approach resulted in a significant 3-fold increase in butyric acid yield to 7.2 g/L[66]. Furthermore, the introduction of the *phaA* gene from *Cupriavidus necator* and the *ter* gene from *C. acetobutylicum* DSM792 in place of the *thl* and *bcd* genes will help to re-establish the butyric acid pathway in *E. coli*. This modification led to a butyric acid yield of 10 g/L and achieving a butyric acid/acetic acid ratio as high as 143 g/g[68]. It has been found that elimination of the *ldhA*, *adhE*, *ackA*, *pta*, and *frdC* genes in *E. coli* can reduce the formation of fermentation by-products (e.g., acetic acid) and increase butyric acid production, and that butyric acid productivity was initially high (0.7 g/L·h) but declined as fermentation progressed[69]. The researchers conducted a comparison of 3 thioesterases (*TesAT* from 4-radius anaerobic *Coccobacillus*, *TesBF* from *Bryonia formosana*, and *TesBT* from *Thetaiotaomicron mimeticus*) for SCFAs production in *E. coli*. Utilizing the natural fatty acid synthesis (FASII) pathway, *E. coli* strains expressing the *tesBT* gene exhibited the greatest butyric acid yield of 14.3 g/L[70]. Moreover, Kataoka et al.[71] successfully engineered a novel butyric acid synthesis pathway in *E. coli*. This pathway integrates acetyltransferase (*phaA*) and NADPH-dependent acetoacetyl-CoA reductase (*phaB*) from *Ralstonia eutropha*, (*R*)-specific enoyl-CoA hydratase (*phaJ*) from *Aeromonas caviae*, trans-enoyl-CoA reductase (*ter*) from *T. denticola*, and endogenous thioesterase. Notably, this pathway achieved a butyric acid yield of 12.3 g/L at 54 h of fed-batch fermentation under aerobic conditions[71]. In addition, the butyric acid yield of recombinant *E. coli* LW393 was up to 28.4 g/L during the batch fermentation in a mineral salt medium with glucose as a carbon source, which accounted for 90% of the total fermentation products[72].

Although genetically engineered *E. coli* strains can overcome the strictly anaerobic environment required for butyric acid fermentation in *Clostridium*, the butyric acid production of these genetically engineered strains has been reported to be lower than that of *Clostridium*. These disparities could be attributed to the comparatively lower butyric acid tolerance exhibited by the genetically engineered *E. coli*. Therefore, future research needs to improve the butyric acid tolerance of genetically engineered strains.

4.3 Interaction of prebiotics/probiotics with butyrate-producing bacteria to enhance their abundance andmetabolic capacity

Both prebiotics and probiotics are dietary supplements that play a role in regulating intestinal microbiota. For example, prebiotics can be selectively utilized by host microorganisms, especially beneficial microorganisms, which in turn provide health benefits to the host by partially regulating gut microbiota, such as increasing the relative abundance of butyric acid-producing bacteria[73]. Probiotics can modulate intestinal bacteria by producing bacteriostatic metabolites (e.g., organic acids and bacteriocins) or by competing with harmful microorganisms for nutrients and living space[74]. Therefore, maximizing the synergistic effects of prebiotics/probiotics with butyric acid-producing bacteria can stimulate the

growth, reproduction, and metabolism of butyric acid-producing bacteria in the host gastrointestinal tract, thereby targeting increasing the intestinal butyric acid level. Currently, studies investigating the interaction effects between prebiotics/probiotics and butyric acid-producing bacteria to increase the level of intestinal butyric acid include 1) the synergistic effects of prebiotics and butyric acid-producing bacteria; 2) the synergistic effects of probiotics and butyric acid-producing bacteria; and 3) the combined synergistic effects of prebiotics and probiotics on butyric acid-producing bacteria.

4.3.1 Interaction effects between prebiotics and butyric acid-producing bacteria

There is a diverse range of prebiotics, including polyphenols and various non-directly digestible carbohydrates such as gum, pectin, inulin, oligosaccharides, and resistant starch[73]. Supplementation of prebiotics has the potential to directly or indirectly enhance the levels of intestinal SCFAs[55]. On one hand, prebiotics can increase intestinal butyric acid levels by serving as a fermentation substrate for butyric acid-metabolizing bacteria. On the other hand, prebiotics can indirectly promote the growth and proliferation of intestinal butyric acid bacteria, thus elevating butyric acid levels. Recent studies have found that the different types of prebiotics can be targeted to increase the abundance of some specific butyric acid-producing bacteria (Table 3). For example, chitooligosaccharides (COS) elevated the relative abundance of *Akkermansia, Bacteroides*, and *Clostridium* in the gastrointestinal tract of normal rats. Meanwhile, *in vitro* fermentation studies revealed that COS undergoes degradation by the human intestinal microbiota, fostering the proliferation of *Clostridium* and leading to the significant production of SCFAs[75-76]. Additionally, inulin has been observed to enhance the abundance and metabolites of Bifidobacterium adolescentis, consequently increasing the abundance of *Faecalibaculum prausnitzi* along with elevated the concentration of its metabolite-butyric acid[77].

Table 3
Prebiotics and/or probiotics targeted to increase the relative abundance of butyric acid-producing bacteria.

Interaction modes	Prebiotics/probiotics	Sources	Study models	Butyric acid-producing bacteria	Reference
Pre-B	Hesperetin (HT)	Citrus fruits	Normal mice on a commercial chow diet were fed 0.5% HT for 3 weeks	*Clostridium subcluster* XIVa	[94]
	Inulin	Oliggo-Fiber	Batch anaerobic fermentation was carried out under controlled conditions with two 250 mL fermenters at 37 °C for 12 days and pH 6.9	*Roseburia* and *F. prausnitzii*	[95]
	Gellan gum oligosaccharides (GGOS)	Gellan gum	Anaerobic fermentation with adult fecal flora under controlled conditions with 0.3% xanthan gum oligosaccharides (XGOS) as carbon source at 37 °C for 24 h	*Clostridia*, Clostridiales and Lachnospiraceae	[96]
	Fish oil (FO)	Fish	Execution assay of mice without specific pathogens infected with *Vibrio parahaemolyticus* one day after feeding FO at different concentrations for 14 days	*Clostridium*, *Akkermansia*, and *Roseburia*	[97]
	3,5,6,7,8,3′,4′-Heptameth oxyflavone (HMF)	Citrus peel	6-Week-old male mice were fed a HFFD supplemented with HMF for 10 weeks	*Firmicutes*	[98]
	Rehmannia glutinosa oligosaccharides (RGO)	*R. glutinosa*	Lipopolysaccharide-induced intestinal inflammation and barrier damage model in mice, followed by administration of different doses of RGO for 3 consecutive weeks	*Akkermansia*	[99]
	Rosmaric acid (RA)	–	Normal healthy mice were separately perfused with DSS and DSS + RA	norank_f_norank_o_Clostridia_UCG-014	[100]
	COS	–	The neonatal necrotizing enterocolitis model rats were infused with 45 mg/kg COS daily	*Akkermansia* and *Clostridium sensu stricto* 1	[101]
Pro-B	*Bacillus* sp. SGP1	Waste water sludge	Batch anaerobic fermentation was conducted using 60 g/L sucrose in a 1 L RCM medium at 37 °C for a duration of 72 h	*C. tyrobutyricum* ATCC	[78]
	E. faecium SF68	–	Male C57BL/6J mice were fed with HFD and treated with *E. faecium* SF68 (10^8 CFU/day) for 8 weeks	Firmicutes	[80]
	C. butyricum CBX 2021	Adult pigs	Antibiotic broad-spectrum compound antibiotics (ABX) feeding for 10 days induced intestinal ecological dysbiosis model in mice after supplementation for 10 days with *C. butyricum* CBX 2021	*Butyricicoccus*, *Roseburia,* norank_f_norank_o_Clostridia_UCG-014, and *Clostridium sensu stricto* 13	[82]
	C. butyricum	–	*C. butyricum* supplementation in spontaneously hypertensive rats	*Akkermansia*	[81]
	B. longum	–	Treatment with 1 × 10^6 CFU/mL *B. longum* for 4 days in the DSS-induced ulcerative colitis and TNBS-induced Crohn's disease zebrafish	*Faecalibaculum*	[83]
Pre-Pro-B	Oligofructose/inulin and *B. adolescentis*	Adult human stool	Anaerobic fermentation was carried out with 7.5 mL of YCFA medium at 37 °C for 24 h	*F. prausnitzii*	[86]
	Fructo-oligosaccharides and *B. longum*	–	The fermentations were co-cultured in mMCB medium with oligofructose or inulin as the sole carbon source	*F. prausnitzii* DSM 17677	[102]
	B. subtilis DSM 32315 and *L*-alanyl-*L*-glutamine	–	Subjects were supplemented with the study product within 4 weeks	*Clostridium* group XIVa	[87]
	COS and *Lactiplantibacillus plantarum*	–	After continuous gastric administration of COS and *L. plantarum* for 2 weeks, mice were induced with enteritis by replacing drinking water with DSS for 7 consecutive days	*Akkermansia*	[103]

Note: Pre-B means prebiotics and butyric acid-producing bacteria; Pro-B means probiotics and butyric acid-producing bacteria; Pre-Pro-B means prebiotics, probiotics, and butyric acid-producing bacteria. DSS, dextran sulphate sodium; TNBS, 6-trinitro-benzenesulfonicacid; HFFD, high-fat and fructose diet; HFD, high-fat diet. "–" indicates that no relevant information is provided in the original study.

4.3.2 Interaction effects between probiotics and butyric acid-producing bacteria

Both *in vivo* and *in vitro* studies have confirmed that probiotics can regulate intestinal microbiota and can interact with butyric acid-producing bacteria to improve their butyric acid metabolism (Table 3). It was found that *C. tyrobutyricum* cannot metabolize sucrose independently. Nevertheless, when co-cultured with sucrase-producing *Bacillus* sp. SGP1 using sucrose as a substrate, it exhibited a decrease in acetic acid production and an increase in butyric acid production. Under the optimal conditions, this co-culture system achieves a remarkable butyric acid concentration of 34.2 g/L and a yield of 0.35 g/g (butyric acid/sucrose)[78]. In addition to significantly enhancing the metabolic capacity of butyric acid-producing bacteria in *in vitro* co-culture, probiotics also can elevate the host intestinal butyric acid level by modulating the host intestinal microbiota. Numerous studies have demonstrated that dietary intervention with probiotics can increase the abundance of butyric acid-producing bacteria in the intestine, consequently leading to an elevated level of intestinal butyric acid. For instance, supplementation with *F. prausnitzii* can ameliorate host microbial ecological dysbiosis by increasing the relative abundance of *Faecalibaculum*, *Dubosiella*, and *Streptococcus*, thereby contributing to increased SCFAs levels[79]. Similarly, the administration of *Enterococcus faecium* SF68 has been observed to augment the abundance of *Faecalibaculum* and *Bifidobacterium*. Furthermore, it enhances the expression of the intestinal butyrate transporter, specifically the sodium-coupled monocarboxylate transporter 1, thereby facilitating the transport and utilization of butyric acid[80]. The supplementation of *C. butyricum* has been found to facilitate the colonization of more butyric acid-producing bacteria, including *Akkermansia muciniphila*, *Lactobacillus amylovorus*, and *Agthobacter rectalis*. This, in turn, accelerates the production of butyrate[81-82]. In addition, supplementation with *Bifidobacterium longum* BL986 has demonstrated the ability to increase the abundance of *Faecalibaculum* and elevate the level of butyrate[83].

4.3.3 Interaction effects between prebiotics-probiotics and butyric acid-producing bacteria

The strategy of increasing gut butyric acid levels through dietary interventions relies on the intricate interplay between prebiotics, probiotic communities, and butyric acid-producing bacteria (Table 3). For instance, when cultured independently with starch or oligofructose as the carbon source, *E. hallii*, and *A. caccae* demonstrated an inability to grow and produce butyric acid. However, in a co-cultured environment with *B. adolescentis*, they exhibited the capacity to generate butyric without any detectable lactate production from *B. adolescentis*[84-85]. Likewise, co-culture systems of *F. Prausnitzii* and *B. adolescentis* with starch or oligofructose as a carbon source resulted in similar results, but higher levels of butyrate were observed in the co-culture with oligofructose[86]. This result suggests that lactic acid generated by *B. adolescentis* during the metabolism of oligofructose promotes the growth, proliferation, and metabolic activities of butyric acid-producing bacteria like *E. hallii*, *A. caccae*, and *F. prausnitzii*. Furthermore, recent findings indicate that the simultaneous addition of *Bacillus subtilis* and *L*-alanyl-*L*-glutamine significantly boosts the abundance of butyric acid-producing bacteria, such as *F. prausnitzii*, along with an increase in host butyric acid level[87]. In summary, the progress in high-throughput DNA sequencing and molecular analysis techniques has made it imperative to screen for more high-yielding butyric acid-producing bacteria. However, the strains identified through screening may not be universally suitable for industrial production in all aspects. Genetic engineering provides a relatively simple and effective approach to customize these strains according to human requirements. Nevertheless, the current genetic modifications are limited to a small number of genes, making it difficult to obtain strains that fully meet the desired demands of industrial production. In contrast, dietary supplementation with prebiotics and/or probiotics provides a simpler, more direct, and effective approach to increase the abundance of intestinal butyric acid-producing bacteria and enhance their ability to metabolize butyric acid. Despite the potential benefits, there remains a considerable gap in our understanding of the intricate interactions between various prebiotics/probiotics and specific butyric acid-producing bacteria within the gut microbiota. Precisely targeting the abundance and metabolic capacities of intestinal butyric acid-producing strains through dietary interventions proves challenging, particularly given the diverse gut microenvironment in different individuals. Despite these challenges, dietary supplementation with prebiotics/probiotics to regulate intestinal butyric acid levels holds great promise. Future research in the targeted enhancement of intestinal butyric acid levels could focus extensively on analyzing the interactions between diverse prebiotics/probiotics and specific butyric acid-producing bacteria within the intestinal microbiota. Additionally, investigating the impact of different microecological environments on the dynamics between prebiotics/probiotics and intestinal butyric acid-producing bacteria could provide valuable insights for precision interventions.

5. Conclusion and future perspectives

The level of intestinal butyric acid stands out as a potential indicator of organismal health. First, it functions as a regulatory agent in cellular inflammation by stimulating GPRs (such as GPR41, GPR43, and GPR109A). Second, in tumor cells, the accumulation of butyric acid results in HDACs inhibition. This inhibitory effect on tumor cell proliferation manifests as an anti-tumor mechanism.

Currently, the industrial production of butyric acid is primarily dependent on the oxidative synthesis of propylene derived from fossil raw materials. However, growing environmental concerns and the depletion of fossil resources have spurred efforts to explore alternative production methods, particularly those utilizing biosynthesis from renewable feedstocks. Among these methods, utilizing *C. tyrobutyricum* as a production strain, employing bio-enhanced reactors for fermentation methods, and incorporating agricultural waste as substrates show the most promising industrial prospects. However, it is noteworthy that current research efforts still face challenges in fully meeting the demands of industrial-scale production.

Butyric acid can also be generated through the metabolism of gut microbiota in the human body. However, its proportion is typically low and subject to various influencing factors. To augment the biosynthesis of butyric acid, high-yielding strains

can be identified through high-throughput screening methods or modified through genetic engineering to meet production demands. Furthermore, regulating the composition of gut microbiota through dietary supplementation provides a relatively straightforward approach to maintaining intestinal health and boosting butyric acid production.

Successfully producing butyric acid from renewable feedstocks requires the acquisition of high-yielding strains that are both acid-tolerant and unaffected by inhibitors such as furfural and phenolic compounds present in lignocellulosic hydrolysates. Additionally, further research is also needed to understand the interactions between prebiotics and probiotics and to develop systems that combine both for disease therapy through *in vitro* and clinical studies.

Disclosure statement

The authors declare that they have no conflict of interest.

Acknowledgments

This research was supported by National Natural Science Foundation of China (32402055), and the Yunnan Fundamental Research Projects (202401CF070092 and 202301AW070015).

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