Biological Functions of Clostridium butyricum and Its Applications in Animal Production: Postprint

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Abstract

Clostridium butyricum, as a novel microecological preparation, exhibits characteristics such as heat resistance, acid tolerance, and tolerance to multiple antibiotics. The addition of Clostridium butyricum preparation in livestock and poultry production not only promotes animal growth, regulates the microecological balance of intestinal flora, and exerts multiple probiotic effects on the host, but also reduces the use of antibiotic products in feed, thereby decreasing drug residues in meat products. The use of Clostridium butyricum can reduce bacterial drug resistance, thus safeguarding animal health. This paper primarily reviews the biological functions of Clostridium butyricum and its applications in animal production, aiming to provide references for its utilization in healthy animal breeding and safe production.

Full Text

Biological Functions of Clostridium butyricum and Its Application in Animal Production

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Abstract: As a novel microecological preparation, *Clostridium butyricum* exhibits characteristics such as heat resistance, acid tolerance, and resistance to multiple antibiotics. Supplementing livestock and poultry diets with *C. butyricum* preparations not only promotes animal growth and regulates intestinal microflora balance to confer various probiotic benefits to the host, but also reduces the use of antibiotic feed additives, thereby decreasing drug residues in animal products. Utilizing *C. butyricum* can reduce bacterial resistance and safeguard animal health. This review summarizes the biological functions of

C. butyricum and its applications in animal production, aiming to provide a reference for healthy and safe animal breeding practices.

Keywords: *Clostridium butyricum*; microecological preparation; biological function; animal production; application

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Clostridium butyricum, also known as butyric acid bacteria or butyric acid clostridia, was first discovered and reported in 1933 by Dr. Miyoshi Miyairi from Chiba Medical University in Japan, hence it is also called Miyairi bacteria [1]. Taxonomically classified under the genus *Clostridium*, it is an anaerobic Gram-positive spore-forming bacillus isolated from the intestines of healthy humans and animals. It can colonize the animal intestinal tract and produces short-chain fatty acids, primarily butyric acid and lactic acid, as beneficial metabolites. As a new generation of spore-forming probiotic preparation, *C. butyricum* possesses superior biological characteristics compared to non-spore probiotics, including heat resistance, acid tolerance, and resistance to multiple antibiotics. In July 2009, China' s Ministry of Agriculture approved *C. butyricum* preparations for use as microbial feed additives [2]. When used as a feed additive, *C. butyricum* exhibits multiple biological functions in regulating animal intestinal health, primarily by promoting the growth of beneficial bacteria (such as *Lactobacillus*, *Bacillus*, and *Bifidobacterium*), regulating animal intestinal microflora balance, stabilizing gastrointestinal function, enhancing immune stress response, and thereby maintaining animal health and promoting growth. As a microecological additive, *C. butyricum* can be used either alone or in combination with other beneficial bacteria to achieve mutual enhancement among microbial communities.

1 Biological Functions of *Clostridium butyricum*

Clostridium butyricum is a symbiotic bacterium in human and animal intestines that can colonize the host intestinal tract through the gastrointestinal environment. It belongs to anaerobic or facultative anaerobic spore-forming bacilli and is unaffected by gastric acid and bile acids. Its primary metabolic product, butyric acid, serves as the main nutritional substance for regeneration and repair of intestinal epithelial tissue cells.

1.1 Growth Promotion

Clostridium butyricum promotes the proliferation and development of beneficial intestinal flora (such as *Bifidobacterium* and *Lactobacillus*) while inhibiting the growth and reproduction of harmful and putrefactive bacteria, thereby correcting intestinal flora disorders and reducing enterotoxin production. Additionally, dietary supplementation with *C. butyricum* can effectively degrade antinutritional factors in feed, eliminate their antinutritional effects, improve feed nutritional value, and promote digestion and decomposition of nutrients, consequently enhancing feed utilization efficiency. Similar to other aerobic bacilli, *C.*

butyricum produces highly active extracellular enzymes during its resuscitation and development in the digestive tract, including proteases, lipases, amylases, glycosidases, and various other enzymes. It also produces enzymes that degrade non-starch polysaccharides in plant feed, such as pectinase, cellulase, and glucanase. These active enzymes directly act on the "enzyme pool" of the animal digestive tract, degrading corresponding nutrients in feed and improving nutrient digestibility, thereby enhancing the utilization of crude protein and energy and reducing feed conversion ratio. *Clostridium butyricum* possesses protease I, protease II, and lipase, which can improve animal digestion and absorption of fat and crude protein [3]. Furthermore, *C. butyricum* functions as an amino acid carrier that can transport but not decompose amino acids, which benefits animal growth. Uyeno et al. [4] reported that feeding calves a mixture of *C. butyricum* and cellooligosaccharide improved intestinal microflora structure, maintained intestinal microecological health, reduced diarrhea incidence, and enhanced production performance.

1.2 Regulation of Intestinal Microflora Balance

The establishment of animal intestinal microflora begins during the birth process when newborn animals acquire bacteria from the skin and milk of the mother, surrounding objects, and ambient air. Due to the immature physiological functions of newborn animals, disease incidence is relatively high, including malnutrition, premature birth, and diarrhea-related diseases. *Clostridium butyricum* can accelerate the growth of intestinal mucosal epithelial cells and promote the maturation of young animal intestines, enhance intestinal peristalsis, restore intestinal motility, and prevent or correct intestinal microflora imbalance. Studies have shown that *C. butyricum* exhibits significant repair effects on symptoms such as damaged intestinal mucosal integrity, villus destruction, and epithelial cell swelling and shedding caused by antibiotics [5]. Intestinal microflora imbalance can lead to various diseases, but *C. butyricum* can both prevent abnormal proliferation of pathogenic and putrefactive bacteria in the intestine and promote the proliferation and development of beneficial intestinal flora, thereby correcting microflora disorders and reducing enterotoxin production.

Hossain et al. [6] fed broiler chickens a compound probiotic preparation made of *C. butyricum*, *Bacillus subtilis*, and *Lactobacillus*, then measured microbial flora in ileal and cecal contents after slaughter. The results showed that probiotic feeding significantly increased *Lactobacillus* and *Bifidobacterium* populations while markedly inhibiting *Escherichia coli* and *Clostridium perfringens* growth, with the 0.2% high-dose group showing better inhibition effects than the 0.1% low-dose group, and both groups outperforming the antibiotic group.

Various factors including host neurological, functional, or organic changes, invasion by pathogenic bacteria, viruses, or molds, and antibiotic administration can cause normal flora imbalance, leading to conditions such as acute diarrhea, autumn-winter diarrhea, secondary diarrhea, viral diarrhea, antibiotic-associated diarrhea, and irritable bowel syndrome. Endogenous

infections caused by translocation of harmful bacteria and immune disorders triggered by congenital immune dysregulation can damage local intestinal mucosa, subsequently causing intestinal inflammation such as chronic terminal ileitis, ulcerative colitis, and necrotizing enterocolitis. Butyric acid produced by *C. butyricum* can inhibit histone deacetylase activity, while its produced folic acid participates in gene methylation and demethylation, regulating host gene expression to prevent and treat enteritis and diarrhea [1]. *Clostridium butyricum* is used as an anti-diarrheal probiotic in Japan. Diarrhea patients receiving *C. butyricum* treatment from the beginning or middle of trials showed 9% and 5% reductions in diarrhea, respectively. During antibiotic treatment, *C. butyricum* supplementation can increase anaerobic bacteria and prevent *Bifidobacterium* reduction [7]. Yao et al. [8] investigated the clinical efficacy of interval application of live *C. butyricum* powder and montmorillonite powder in treating pediatric persistent diarrhea. The trial divided patients into two groups: the observation group received live *C. butyricum* powder 2 hours after montmorillonite powder, while the control group received compound gastric protease powder 2 hours after montmorillonite powder. The results showed the observation group achieved a total effective rate of 100.00% compared to 72.58% in the control group, with statistically significant differences between groups, demonstrating that interval application of live *C. butyricum* powder and montmorillonite powder is remarkably effective for pediatric persistent diarrhea and worthy of clinical promotion, with similar reports available [9-11].

1.3 Immune Modulation

Intestinal barrier function is primarily achieved through the intestinal mucosal barrier, which includes mechanical, biochemical, chemical, and immunological barriers, as well as the molecular sieve of the intestinal mucosal lamina propria. The mechanical barrier comprises intestinal mucosal epithelial cells, intercellular junctions on the lateral sides of epithelial cells, epithelial basement membrane, and cell coats on the epithelial surface, which constitute the first line of defense for the immune system. Therefore, the integrity of intestinal mucosal epithelial structure is closely related to intestinal mechanical barrier function and overall immune function.

Butyric acid, the product of *C. butyricum*, stimulates intestinal mucosal immune activity and promotes immune function to some extent through regulation of intestinal microecology. The interaction between the intestinal immune system and intestinal microorganisms jointly maintains intestinal homeostasis [12]. *Clostridium butyricum* is well-known for treating intestinal inflammatory diseases. Gao et al. [13] investigated the ability of *C. butyricum* to inhibit *E. coli*-induced apoptosis in chicken embryo intestinal cells. The results showed that *C. butyricum* and its culture supernatant significantly inhibited *E. coli* growth and markedly suppressed apoptosis induced by regulating the expression of X-linked inhibitor of apoptosis protein (XIAP), B-cell lymphoma-extra large (Bcl-xL), Fas, B-cell lymphoma-2 (Bcl-2), Bcl-2-associated X protein (Bax), tumor pro-

tein 53 (P53), and through activation of caspase-9 and caspase-3. *Clostridium butyricum* can prevent intestinal diseases caused by enterohemorrhagic *E. coli*. Under the challenge of *E. coli* K88, dietary supplementation with *C. butyricum* can promote broiler growth performance, improve immune function, and exert beneficial effects on cecal microflora [14-15]. Preventive treatment with *C. butyricum* increased the activities of superoxide dismutase (SOD) and catalase (CAT) in mouse serum while significantly reducing malondialdehyde (MDA) levels in untreated acute liver injury (ALI) mice compared with normal control mice [16]. *Clostridium butyricum* also significantly reduced lung resistance in asthmatic mice; oral administration inhibited airway inflammation, mast cell degranulation, airway remodeling, and the expression of ovalbumin-specific immunoglobulin E and ovalbumin-specific immunoglobulin G1, reversed T helper cell 1 (Th1)/T helper cell 2 (Th2) imbalance, and increased the expression of the anti-inflammatory cytokine interleukin-10 (IL-10) [17]. Meanwhile, medical research reports indicate that oral administration of *C. butyricum* combined with pediatric antidiarrheal granules shows good efficacy in children and can optimize their immune function, making it worthy of clinical promotion [18]. *Clostridium butyricum* has varying effects from health to pathogenicity. While non-toxic strains have validated probiotic properties in clinical practice, other strains have been associated with pathological conditions such as infant botulism or necrotizing enterocolitis (NEC) in preterm infants. Since both toxigenic and non-toxigenic clostridia form part of the normal reproductive bacterial population, understanding the triggers for beneficial or pathogenic factor expression remains a challenge [19].

Clostridium butyricum can activate immune responses and promote the development of the immune system. Its secreted butyrate and hydrogen (H) can enhance antioxidant enzyme activity, reduce oxidative stress, and inhibit inflammation. Supplementing broiler diets with different doses of *C. butyricum* and measuring antioxidant indicators in duodenal, jejunal, and ileal mucosa at day 21 and day 42 showed that *C. butyricum* could enhance antioxidant enzyme activity and reduce oxidative damage to intestinal epithelial tissue through butyrate and H production [20]. Wang et al. [21] reported that *C. butyricum* significantly reduced gastric mucosal damage area and improved pathological conditions of gastric mucosa. In all gastric ulcer model studies, *C. butyricum* not only reduced SOD and CAT activities but also decreased MDA levels; pretreatment with *C. butyricum* reduced the accumulation of interleukin-1 (IL-1), tumor necrosis factor- (TNF-), and leukotriene B4 (LTB4) while increasing 6-keto-prostaglandin F1 expression.

Clostridium butyricum induces intestinal macrophage proliferation to suppress colitis [22], and as a probiotic, it can induce bone marrow-derived macrophages to inhibit inflammation on inflammatory mucosa through Toll-like receptor (TLR) 2 [12]. Yang [23] demonstrated that teichoic acid from *C. butyricum* plays an important role in adhesion. Teichoic acid extracted and purified from *C. butyricum* can cause inflammatory responses in HT-29 cells but also modulates inflammation by increasing secretion of inflammatory inhibitory factors,

thereby preventing excessive inflammatory factor expression that could damage tissues, with *C. butyricum* and its teichoic acid showing similar trends. Other studies indicate that teichoic acid has some inhibitory effect when *E. coli* adheres to human colon cancer cells and intestinal cells [24], and the lipid fraction isolated from its spores can inhibit urokinase synthesis in leukemia lymphocytes, thymic carcinoma, and lung cancer cells to some extent [25].

1.4 Nutrient Provision

Clostridium butyricum can produce B vitamins, vitamin K, and other substances in the intestine, along with enzymes such as amylase, protease, glycosidase, cellulase, and phospholipid synthase. Through the action of these enzymes, it ferments to produce glucose, maltose, and other substances that aid digestion and absorption, while certain oligosaccharides produced also provide nutrients for other probiotics. A konjac glucomannan-degrading enzyme has been isolated and purified from anaerobic intestinal bacteria and the *C. butyricum-Clostridium beijerinckii* group. This enzyme consists of a single polypeptide chain with a molecular weight of 50,000-53,000 u and is a -mannan endonuclease that specifically acts on polysaccharides such as konjac glucomannan and coffee mannan to produce oligosaccharides and monosaccharides [26]. Additionally, the *C. butyricum-C. beijerinckii* group can produce endo- and exo-pectate lyases and pectin methylesterase, which are secreted into the human large intestine and act on pectin to produce 4,5-unsaturated digalacturonic acid, ultimately decomposing into volatile short-chain fatty acids [27].

1.5 Anticancer and Antitumor Effects

Clostridium butyricum improves clinical, histological, and biochemical manifestations in colitis-associated cancer (CAC) models, extends epithelial cell microvilli, and increases transmembrane resistance by reducing transepithelial permeability. It promotes miR-200c expression, thereby increasing proliferation rate, and can regulate the production of pro-inflammatory cytokines TNF- and interleukin-12 (IL-12) through miR-200c. It may participate in inflammation-related cancer processes by regulating epithelial barrier function through miR-200c [28]. Chen et al. [29] reported that *C. butyricum* and *Bacillus subtilis* could inhibit proliferation of colorectal cancer (CRC) cells induced by 1,2-dimethylhydrazine dihydrochloride (DMH), cause cell cycle arrest, and promote apoptosis, with molecular mechanisms involving reduced inflammation and improved immune homeostasis. Research indicates that *C. butyricum* MIYAIRI588 (CBM588) induces the release of endogenous tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) from polymorphonuclear neutrophils (PMNs) and bacillus Calmette-Guérin (BCG), with matrix metalloproteinase-8 (MMP-8) being one of the key factors responsible for this release [30]. The TLR2/4 signaling pathway has been identified as important for MMP-8-mediated TRAIL release. CBM588 is as effective as BCG against cancer cells by inducing apoptosis both in vivo and in vitro.

Currently, probiotic-based tumor prevention and treatment has become a novel therapeutic approach, based on the principle that *C. butyricum* can selectively proliferate in tumors and antagonize tumor cell growth [31]. Due to this selective colonization of tumor tissue, genetically modified *C. butyricum* can secrete therapeutic proteins such as cytosine deaminase and TNF- , with even greater specificity if expression is controlled by radiation-inducible promoters, making tumor treatment more precise [32]. Metabolites of *C. butyricum* are considered to have anticancer potential, as its produced butyric acid can not only alter intestinal physiological conditions but also eliminate effects of some carcinogens, such as inhibiting the carcinogenic activity of nitrosamines [33]. Additionally, reports indicate that *C. butyricum* has neuromodulatory effects. Sun et al. [34] investigated whether *C. butyricum* attenuates cerebral ischemia/reperfusion (I/R) injury and its possible mechanisms. The results showed that I/R caused neurological deficits, increased caspase-3 and Bax protein expression levels, and decreased the Bcl-2/Bax ratio. *Clostridium butyricum* treatment significantly improved neurological deficits, alleviated pathological changes, reduced MDA content, and increased SOD activity in I/R mice. After *C. butyricum* pretreatment, caspase-3 and Bax expression were significantly reduced, the Bcl-2/Bax ratio was markedly increased, and butyric acid content in the brain was significantly elevated. Another study reported [35] that *C. butyricum* reversed I/R-induced reduction in phosphorylated AKT protein (p-Akt) expression and increased caspase-3 expression, thereby inhibiting neuronal apoptosis. *Clostridium butyricum* also restored the reduced fecal microbial community diversity and altered microbial community composition induced by I/R. These findings demonstrate that *C. butyricum* exerts neuroprotective effects through antioxidant and anti-apoptotic mechanisms in I/R mice, and that reversing the reduction of butyric acid content in mouse brain may be involved in this protection.

2 Application Progress of *Clostridium butyricum* in Animal Production

Clostridium butyricum can be used as a feed additive either alone or in combination with probiotics such as *Lactobacillus*, *Bacillus*, and *Bifidobacterium*, and even jointly with certain antibiotics to avoid hazards from antibiotic abuse. *Clostridium butyricum* microecological preparations can improve the intestinal microenvironment, enhance immune function, and reduce intestinal diseases such as diarrhea in animals [1, 36]. Its main metabolic product, butyric acid, is a non-toxic, amphipathic four-carbon short-chain fatty acid that can penetrate both Gram-positive and Gram-negative bacterial cell membranes, promote proliferation of beneficial bacteria, inhibit growth of harmful bacteria, and thus maintain overall intestinal health [37]. As a rapid energy source for intestinal cells, butyric acid possesses physiological functions including regulation of intestinal microbial flora, promotion of intestinal development, and enhancement of immune and antioxidant capacity.

2.1 Application in Poultry

One of the most important characteristics of probiotics is reducing pathogen proliferation by inhibiting pathogen adhesion, which is also a crucial indicator for measuring antibacterial properties. The adhesion inhibition effect of probiotics on pathogens is highly specific and often depends on the characteristics of both the probiotic and pathogen strains. Studies have shown that *C. butyricum* can replace antibiotics in broiler chickens [35]. After feeding broilers with different concentrations of *C. butyricum*, serum immunoglobulin M content increased [20], serum antioxidant capacity improved, immune function was enhanced, protein metabolism was promoted, and ammonia emission was reduced [38]. These effects indicate that *C. butyricum* can improve broiler immune performance, possibly because it can regulate immune pathways in the body. *Clostridium butyricum* can also promote immune responses in broilers challenged with *E. coli* K88 and improve intestinal barrier function and digestive enzyme activity [39]. Regarding specific regulatory mechanisms, research suggests that *C. butyricum* may alleviate inflammation in chickens by downregulating the TLR4, myeloid differentiation factor 88 (MyD88), and nuclear factor- B (NF- B)-dependent pathways [40]. Liao et al. [41] reported that dietary *C. butyricum* supplementation increased SOD activity in chick liver tissue and decreased MDA content, increased concentrations of total polyunsaturated fatty acids including C20:1n-9, C20:2n-6, C20:3n-6, C20:3n-3, C20:4n-6, C20:5n-3, and C22:6n-3 in breast muscle, as well as the ratio of total polyunsaturated to saturated fatty acids, and increased contents of C18:2 t-9, C18:2 t-12, C20:3n-6, C20:3n-3, and C20:5n-3 in thigh muscle.

Clostridium butyricum promotes proliferation of beneficial bacteria and inhibits growth of harmful bacteria to regulate intestinal microflora balance and improve the intestinal microenvironment. It enhances digestion and absorption capacity, maintains normal mucosal barrier function, improves immune status, increases intestinal volatile fatty acid content and antioxidant capacity [42], regulates nitrogen metabolism, improves intestinal morphology, and balances cecal microflora in broilers [43].

2.2 Application in Swine

Dietary probiotic supplementation can significantly improve intestinal immune response and pathogen adhesion. In terms of immune response, downregulated proteins such as transmembrane glycoprotein NMB and -galactoside -2,6-sialyltransferase 1 are involved in intestinal diseases and innate immune responses, while upregulated proteins participate in antigen presentation and complement activation pathways, such as ficolin-2 and inverted formin-2. Regarding cell growth and proliferation, downregulated proteins are involved in cell proliferation (e.g., methyl-CpG-binding domain protein 2), cell morphology (e.g., hexokinase-2), and tissue morphology (e.g., nuclear distribution protein nudE homolog 1) [44]. Compound *C. butyricum* probiotic preparations show significant effects in preventing piglet diarrhea and can be further promoted as green

additives [45]. Reports indicate that adding *C. butyricum* to early-weaned piglet diets can significantly increase intestinal microbial diversity, markedly reduce diarrhea rate, and improve weight gain and feed conversion efficiency [46-48]. Pang et al. [49] found that *C. butyricum* significantly reduced weaned piglet diarrhea rate and crypt depth, significantly increased villus height to crypt depth ratio, significantly decreased serum endotoxin and D-lactic acid concentrations, improved intestinal mucosal morphology, reduced intestinal permeability, and significantly increased occludin mRNA expression in the ileum and colon of weaned piglets, thereby upregulating tight junction protein gene expression and protecting intestinal mucosal barrier function.

Additionally, scholars have reported that *C. butyricum* can stimulate TLR2 expression at the mRNA level, while transcription levels of TLR4, TLR5, TLR9, and MyD88 remain unchanged. *Clostridium butyricum* significantly increased NF- B, interleukin-8 (IL-8), and TNF- expression levels, indicating that it can sensitize HT-29 cells [50]. However, in the absence of TLR2 expression, NF- B, IL-8, interleukin-6 (IL-6), and TNF- levels were significantly reduced, suggesting that TLR2 is required for *C. butyricum* recognition. Therefore, *C. butyricum* participates in activating TLR2-mediated, MyD88-independent signaling pathways in epithelial cells [51].

2.3 Application in Aquaculture

Duan et al. [52] conducted a 56-day acute high-temperature stress trial to evaluate the effects of dietary *C. butyricum* probiotic on growth performance and intestinal antioxidant capacity in kuruma shrimp (*Marsupenaeus japonicus*). The results showed that dietary supplementation with 100 mg/g *C. butyricum* improved growth performance and reduced feed conversion ratio. Hematoxylin-eosin (HE) staining revealed that *C. butyricum* increased intestinal epithelial cell height in kuruma shrimp. Dietary *C. butyricum* supplementation improved oxygen production capacity and MDA content, increased total antioxidant capacity (T-AOC), CAT and peroxidase (POD) activities, and enhanced expression levels of heat shock protein 70 (hsp70) and metallothionein (MT) genes. Studies on the antagonistic effects of *C. butyricum* against *Vibrio harveyi* and its effects on growth performance, digestibility, and immune response in freshwater shrimp showed that shrimp body weight and growth rate were significantly higher than in the control group. The probiotic *C. butyricum* hindered pathogen growth and promoted shrimp growth, protease, and amylase activities [53]. Different dietary concentrations of *C. butyricum* can improve growth performance in white shrimp, increase intestinal short-chain fatty acid content and body crude protein content, regulate intestinal digestive capacity, and enhance intestinal immune function against ammonia stress in *Litopenaeus vannamei* [54].

3 Summary

Clostridium butyricum in animal production can increase feed conversion rate, reduce feed-to-weight ratio, enhance animal immunity, and improve intestinal

microecological environment. As a microecological preparation, it has low toxicity, leaves no residues, demonstrates good safety, meets consumer expectations, and holds substantial market potential. However, *C. butyricum* preparations are not yet widely used as probiotic agents in animal feed, possibly due to its pungent odor affecting palatability and immature low-cost production processes. Meanwhile, the probiotic principles and mechanisms of action of *C. butyricum* and its metabolites in maintaining microecological balance and treating inflammation and cancer, the mechanisms of host cell receptor recognition, and the downstream immune signaling pathways leading to these benefits remain unclear and require further in-depth research.

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