Bacillus spp. as potential probiotics: promoting piglet growth by improving intestinal health

Xiaopeng Tang[1]*, Yan Zeng[2], Kangning Xiong[1]* and Jinfeng Zhong[3]

[1]State Engineering Technology Institute for Karst Desertfication Control, School of Karst Science, Guizhou Normal University, Guiyang, China, [2]Key Laboratory for Information System of Mountainous Areas and Protection of Ecological Environment, Guizhou Normal University, Guiyang, China, [3]Hunan Polytechnic of Environment and Biology, College of Biotechnology, Hengyang, China

The application of *Bacillus* spp. as probiotics in the swine industry, particularly for piglet production, has garnered significant attention in recent years. This review aimed to summarized the role and mechanisms of *Bacillus* spp. in promoting growth and maintaining gut health in piglets. *Bacillus* spp. can enhance intestinal barrier function by promoting the proliferation and repair of intestinal epithelial cells and increasing mucosal barrier integrity, thereby reducing the risk of pathogenic microbial invasion. Additionally, *Bacillus* spp. can activate the intestinal immune system of piglets, thereby enhancing the body's resistance to diseases. Moreover, *Bacillus* spp. can optimize the gut microbial community structure, enhance the activity of beneficial bacteria such as *Lactobacillus*, and inhibit the growth of harmful bacteria such as *Escherichia coli*, ultimately promoting piglet growth performance and improving feed efficiency. *Bacillus* spp. has advantages as well as challenges as an animal probiotic, and safety evaluation should be conducted when using the newly isolated *Bacillus* spp. This review provides a scientific basis for the application of *Bacillus* spp. in modern piglet production, highlighting their potential in improving the efficiency of livestock production and animal welfare.

KEYWORDS

Bacillus spp., growth performance, gut microbial, intestinal health, piglets

Introduction

Weaning is one of the key events in the life cycle of pigs. Changes in diet and environment during weaning usually result in reduced feed intake, intestinal inflammation, and imbalances in intestinal microbial composition, leading to diarrhea in weaned piglets (1–3). In practical production, the addition of antibiotics (4, 5) or high doses of zinc oxide (ZnO) (2, 6–8) to the diets has been found to effectively alleviate diarrhea in piglets after weaning and promote their growth and development. However, while acknowledging the positive effects of antibiotics and high doses of ZnO on weaned piglets, it is also necessary to recognize their negative effects. Long-term or excessive of antibiotics in swine will lead to the imbalance of pig intestinal microbiota, the increase of antibiotic-resistant pathogens, and the residue of antibiotics in pork, which will directly or indirectly harm human health (9, 10). Excessive or long-term use of high doses of ZnO may lead to dull skin and coarse hair, inhibit the growth and development

of pigs, reduce the bioavailability of ZnO, and may also lead to an increase in the percentage of intestinal bacteria resistant to multiple drugs, causing waste of zinc resources and environmental pollution (11–13). The prohibition or restriction of dietary antibiotics, and the reduction of ZnO in feed have formed a broad consensus in the world. Therefore, the search for green, safe and efficient non-antibiotic additives and ZnO substitutes, such as essential oils (14), organic acids (15), prebiotics (16), and probiotics (17), and so on to improve intestinal health and reduce diarrhea of weaned piglets has become a hot field of animal nutrition.

Probiotics are live bacteria that can improve the beneficial flora of the host with single or multiple bacteria (18). Previous studies have demonstrated that probiotics can improve the immune function, enhance the ability to resist bacterial infection, regulate the balance of intestinal flora, and thus improve production performance of piglets (19–22). There are many kinds of probiotics, including *Bifidobacteria*, *Lactobacillus*, yeast, *Bacillus* spp., etc. Among these probiotics, *Bacillus* spp. has gained increasing attention as an alternative to antibiotics or ZnO due to its advantages such as heat resistance during feed granulation, resistance to low pH in the stomach, and stability at ambient temperatures (23, 24).

Bacillus spp. are Gram-positive aerobic or facultative anaerobic bacteria capable of producing resistant endospores (25). *Bacillus* spp. can adapt to a variety of environments and are widely distributed around the world because their spores are highly resistant to ultraviolet radiation, high temperatures, strong acids, ionizing radiation, and many toxic chemicals (26, 27), making it widely used in industrial, agricultural, medical and other fields (25, 28–30). This is also the advantage of *Bacillus* spp. as a probiotic for animals. In the field of animal nutrition and feed science, compared with other types of probiotics, *Bacillus* spp. has more advantages. First of all, in the process of feed processing, *Bacillus* spp. can resist high temperature because of the presence of spores, and therefore, *Bacillus* spp. can maintain biological activity after processing, and its spore-forming ability is beneficial for long-term storage compared to that of non-pore-forming bacteria (31, 32). For example, Amerah et al. (33) showed that Bacillus spores are resistant to pelleting temperatures of up to 90 ° C, and more than 90% of spores are still alive in feed samples. Second, because of the strong stress resistance of Bacillus spores, they can tolerate cholate and stomach acid after entering the gastrointestinal tract, and successfully colonize and play their role in the intestine (23, 24). For example, Dong et al. (34) showed that *Bacillus licheniformis* (*B. licheniformis*) WBL009 had strong acid and bile salt resistance. The survival rate of *B. licheniformis* WBL009 was 35.8% after 2 h treatment in artificial gastric juice with pH value of 2.5, and 51.6% after 12 h treatment with bile salt concentration of 0.3%.

Numerous studies have shown that *Bacillus* spp. can produce extracellular enzymes such as glycoenzyme, protease and lipase, and also has antibacterial and immune regulation effects, thereby improving the nutrient digestibility, reducing the colonization of harmful bacteria such as, *Escherichia coli* and *Clostridium perfringens* of piglets, and thus improving the growth performance of piglets (35–40). There are many types of *Bacillus* spp., including *Bacillus subtilis* (*B. subtilis*), *B. licheniformis*, *Bacillus coagulans* (*B. coagulans*), *Bacillus megaliformis* (*B. megaliformis*), *Bacillus cereus* (*B. cereus*), and so on. However, not all *Bacillus* spp. are effective probiotics, some strains cannot be used because of limitations such as poor colonization effect or virulence factors, and some *Bacillus* spp. strains have been identified as opportunistic pathogens that causing food spoilage and toxin release in the hosts (41). Therefore, although most of the *Bacillus* spp. are generally recognized as safe for animal consumption, it is necessary to summarize the application effect of different *Bacillus* spp. on piglets. In this paper, the effects of *Bacillus* spp. in piglet production were summarized from the perspective of growth promotion and health benefits, so as to provide a reference for the rational utilization of *Bacillus* spp. in swine production.

Growth promoting effect of *Bacillus* spp. on piglets

Early weaning technology is the production method commonly used in most intensive pig farms at present. The weaning process of piglets is accompanied by a series of changes in diet, society and environment, which can easily cause weaning stress (3). The transition from high digestibility liquid milk to non-digestible solid feed is a great challenge for the immature gastrointestinal tract of the piglets, which usually causes diarrhea due to indigestion (42). Due to the combination of sudden separation from the sow, social factors, and other influences, piglets experience anxiety, leading to a reduction in feed intake or even refusal to eat (3, 42, 43). It is generally believed that the production performance of the whole pig farm is determined by the production performance of the piglets, and diarrhea is the culprit leading to the low production performance of the piglets. Therefore, the control of post-weaning diarrhea of piglets is of great significance to improve the economic benefits of pig production.

Previous studies have shown that the addition of *Bacillus* spp. can improve the intestinal health of pigs by altering the intestinal flora, thereby inhibiting the growth of pathogens, enhancing immune function, improving nutrient utilization and digestibility, reducing the incidence of diarrhea, and ultimately improving the growth performance of piglets (44–48). The growth promoting effect of single *Bacillus* spp. strains on piglets were summarized in Table 1. These studies indicated that the addition of single *Bacillus* spp. strains, including *B. subtilis* (35, 45–47, 49–61), *B. coagulans* (62–66), *B. amyloliquefaciens* (44, 67–71), *B. licheniformis* (72–76), *B. halotolerans* (77), B. toyonensis (78), *B. cereuscould* (79), and so on could promote the growth performance and effectively prevent the occurrence of diarrhea of piglets.

In addition to the addition of single strains of *Bacillus* spp., the combination of *Bacillus* spp. with other probiotics or functional substances has shown a synergistic effect in promoting the growth performance of piglets. For example, Liu et al. (35) showed that the combination of *B. subtilis* QST713 (100 mg/kg) with β-mannanase (150 mg/kg) effectively decreased the feed conversion ratio (FCR) of piglets throughout the trial period. Jiao et al. (37) demonstrated that compared to the control group, the combination of *Bacillus* spp. (1.3×10^9 CFU/kg; *B. licheniformis* and *B. subtilis* with the ratio of 1: 1) with medium-chain fatty acid (0.588 g/kg) significantly increased the average daily gain (ADG) and dry matter intake of piglets in phase 1 (days 1 to 9), and significantly increased the ADG of piglets in the whole period (days 1 to 36). Liu et al. (80) showed that the combination of *B. subtilis* with *Lactobacillus plantarum* (*L. plantarum*) jointly increased average daily feed intake (ADFI) and ADG of weaned piglets in d 14 ~ 28 and d 28 ~ 42. Pu et al. (81) showed that piglets fed a diet added with benzoic acid (3,000 g/t) and *B. coagulans* (400 g/t)

TABLE 1 Growth promoting effect of *Bacillus* spp. on piglets.

Bacillus spp.	Piglet age	Optimal added amount	Experimental period	Growth performance	References
B. subtilis	25-days-old	100 mg/kg or 200 mg/kg	42 days	Diarrhea rate↓	Liu et al. (35)
B. subtilis	28-days-old	300 g/t	42 days	FBW↑, ADG↑, G/F↑	Hu and Kim (45)
B. subtilis	26 ± 2 days-old	4×10^9 CFU/kg or 2×10^{10} CFU/kg	28 days	Diarrhea index↓, ADG↑, G/F↑	Hu et al. (46)
B. subtilis	21-days-old	2.56×10^9 CFU/kg	28 days	Diarrhea score↓, ADG↑, G/F↑	He et al. (47)
B. subtilis	45-days-old	0.1%	28 days	FBW↑, ADG↑	Deng et al. (49)
B. subtilis	25-days-old	1.44×10^9 CFU/kg	28 days	ADG↑, diarrhea rate↓	Tian et al. (50)
B. subtilis	25-days-old	500 mg/kg	42 days	ADG↑, F/G↓	Tang et al. (51)
B. subtilis	28-days-old	1×10^9 CFU/kg	42 days	FBW↑, ADG↑, F/G↓	Wang et al. (52)
B. subtilis	26.9 ± 2.0 days-old	1.875×10^5 CFU/g	14 days	FBW↑, ADG↑	Duddeck et al. (53)
B. subtilis	28-days-old	1.0×10^9 CFU/kg	Not mentioned	FBW↑, nitrogen deposition rate↑	He et al. (54)
B. subtilis	21-days-old	1×10^8 CFU/mL	42 days	ADG↑, ADFI↑	Jia et al. (55)
B. subtilis	21-days-old	500 mg/kg	28 days	FBW↑, ADG↑, diarrhea rate↓	Júnior et al. (56)
B. subtilis	25 ± 2 days-old	1.0×10^7 CFU/kg	14 days	ADG↑, F/G↓	Li et al. (57)
B. subtilis	21-days-old	0.05%	21 days	G/F↑, diarrhea rate↓	Park et al. (58)
B. subtilis	Not mentioned	1.28×10^9 CFU/kg or 2.56×10^9 CFU/kg	19 days	ADG↑	Kim et al. (59)
B. subtilis	21-days-old	1.0×10^9 CFU/kg	28 days	FBW↑, ADG↑, diarrhea index↓	He et al. (60)
B. subtilis	Not mentioned	2.0×10^9 CFU/kg	28 days	ADG↑, FCR↓, diarrhea index↓	Sudan et al. (61)
B. coagulans	26-days-old	600 g/t	28 days	ADG↑, F/G↓, diarrhea index↓	Sun et al. (62)
B. coagulans	7-days-old	1.0×10^8 CFU/kg	7 days	ADFI↑	Zhang et al. (63)
B. coagulans	24-days-old	400 mg/kg	28 days	ADG↑	Fu et al. (64)
B. coagulans	21-days-old	2×10^6 CFU/kg or 1×10^7 CFU/kg	21 days	Diarrhea rate↓	Wu et al. (65)
B. coagulans	21-days-old	2×10^6 CFU/kg	14 days	Diarrhea rate↓	Wu et al. (66)
B. amyloliquefaciens	28-days-old	5.0×10^9 CFU/kg	14 days	FBW↑, ADG↑, F/G↓, diarrhea rate↓	Du et al. (67)
B. amyloliquefaciens	21-days-old	2.0 g/kg	28 days	ADG↑, G/F↑, feed efficiency↑	Li et al. (68)
B. amyloliquefaciens	28-days-old	1.0×10^9 CFU/kg	32 days	ADG↑, diarrhea rate↓	Ji et al. (69)
B. amyloliquefaciens	42-days-old	2.0×10^8 CFU/kg	28 days	ADG↑, ADFI↑	Wang et al. (70)
B. amyloliquefaciens	21-days-old	1.0×10^8 CFU/kg	42 days	ADG↑, FBW↑, F/G↓, diarrhea rate↓	Wang et al. (71)
B. licheniformis	28-days-old	500 mg/kg	14 days	FBW↑, ADG↑, F/G↓, diarrhea rate↓	Yu et al. (72)
B. licheniformis	21 ± 1-days-old	1×10^9 CFU/kg or 1×10^{10} CFU/kg	28 days	ADG↑, F/G↓, diarrhea rate↓	Cao et al. (73)
B. licheniformis	28-days-old	500 mg/kg or 1,000 mg/kg	28 days	ADG↑, diarrhea rate↓	Yu et al. (74)
B. licheniformis	21 ± 1-days-old	1,000 g/t	14 days	FBW↑, ADG↑, diarrhea incidence↓	Sun et al. (75)

(Continued)

TABLE 1 (Continued)

Bacillus spp.	Piglet age	Optimal added amount	Experimental period	Growth performance	References
B. licheniformis	21-days-old	2×10^9 CFU/kg	17 days	Diarrhea index score↓	Xu et al. (76)
B. halotolerans	25-days-old	1×10^9 CFU/kg	42 days	Diarrhea rate↓	Li et al. (77)
B. toyonensis	24 ± 3-days-old	500 mg/kg or 1,000 mg/kg	44 days	FBW↑, ADG↑, diarrhea rate↓	Kantas et al. (78)
B. cereus	28 ± 3-days-old	1×10^9 CFU/kg	35 days	FBW↑, ADG↑, FCR↓, diarrhea score↓	Papatsiros et al. (79)

ADFI, average daily feed intake; ADG, average daily gain; FBW, final body weight; FCR, feed conversion ratio; F/G, feed in-take to gain ratio; G/F, gain to feed intake ratio. "↑" stands for increase, and "↓" stands for decrease.

mixture significantly increased the final body weight (FBW) and ADG, and decreased FCR compared with piglets fed a basal diet. Phaengphairee et al. (82) showed that dietary supplementation of full-fat black soldier fly larvae in combination with multi-probiotics (*B. subtilis*, *B. licheniformis*, and *Saccharomyces cerevisiae*) significantly increased the ADG, gain-to-feed ratio (G/F), and nutrient digestibility of weaned piglets.

Although numerous studies have confirmed the growth-promoting effect of *Bacillus* spp. alone or in combination with other additives on weaned piglets, some studies have shown that *Bacillus* spp. has no effect on the growth performance of weaned piglets. For example, Jiao et al. (37), Huting et al. (38), Kritas and Morrison (83), Luise et al. (84), and Ding et al. (85) showed that dietary supplementation with *Bacillus* spp. alone has no effect on the growth performance of piglets. The different effects of *Bacillus* spp. on alleviating diarrhea and improving growth performance of weaned piglets may be closely related to the different types of *Bacillus* spp., dosage, adding stage, use mode, diet composition and physiological health status of piglets. Although the effect of *Bacillus* spp. on the growth performance of weaned piglets is inconsistent, it is certain that *Bacillus* spp. has some other benefits, such as promoting intestinal health.

Intestinal health benefits of *Bacillus* spp. on piglets

Weaning is the transitional period when piglets shift from relying on maternal milk nutrition to solid feed. During this time, the digestive system of the piglets faces significant challenges, making them susceptible to intestinal dysfunction and diseases (86, 87). Promoting intestinal development and intestinal health of weaned piglets is the key to cope with weaning stress. In previous studies, there are many ways to promote the intestinal health of piglets, among which probiotics, especially *Bacillus* spp., are prominent in promoting the intestinal health of piglets.

Bacillus spp. promotes intestinal development

The gut is the main site for the digestion and absorption of nutrients in animals and serves as a selective barrier for the body to prevent exogenous harmful substances from entering the circulatory system (3, 87). The gastrointestinal digestive system of piglets is still immature, and external stress will further damage the intestinal structure of piglets, such as intestinal villi shedding, crypt hyperplasia, intestinal mucosal atrophy, etc., and then destroy the intestinal mucosal barrier function and digestive absorption capacity (3, 88, 89). Previous studies have shown that *Bacillus* spp. has good nutritional physiological effects on intestinal development of piglets, including the maintenance of intestinal morphology and structure (50, 71, 73, 75, 85, 90), promoting the secretion of intestinal digestive enzymes (49, 75, 91, 92), and reducing intestinal permeability (59, 90, 91).

Bacillus spp. have a positive effect on the intestinal morphological structure and function of piglets, and can improve the intestinal damage caused by various stresses, so as to maintain the integrity of intestinal mucosa (65, 66). *Bacillus* spp. can increase the intestinal villus height (VH), decrease the intestinal crypt depth (CD), increase the intestinal villus height to crypt depth ratio (VCR) and reduce the apoptosis of intestinal mucosal epithelial cells of piglets (50, 67, 68, 92). For instance, Du et al. (67) showed that dietary supplementation of *B. amyloliquefaciens* (5.0×10^9 CFU/kg) significantly increased intestinal VH and VCR, while decreased intestinal CD. Hu et al. (92) reported that dietary supplementation with *B. subtilis* PB6 can improve intestinal damage of suckling piglets caused by intrauterine growth retardation (IUGR), as shown by higher VH and VCR, and lower CD than that of IUGR piglets without *B. subtilis* supplementation. Du et al. (93) showed that dietary supplementation with *B. amyloliquefaciens* SC06 (2.0×10^8 CFU/kg) significantly increased intestinal villus length, and intestinal villi morphology was improved. Wang et al. (36) showed that the combination of *B. subtilis* with *B. licheniformis* significantly increased the ileum VH and the jejunum and ileum VCR, and decreased the jejunum CD of piglets. The increase in VH and the decrease in CD on the intestinal mucosa effectively increase the surface area of the intestinal tract in contact with the digested food, thus improving the absorption efficiency of nutrients.

Most *Bacillus* spp. have a strong enzyme production capacity and can produce a variety of extracellular enzymes, such as cellulase, xylanase, amylase, protease, lipase (94–98), which can assist animals to digest feed and improve nutrient absorption. In addition to the exogenous enzymes secreted by themselves, *Bacillus* spp. can also stimulate the secretion of various digestive enzymes in the intestines of piglets (49, 75, 91, 92). For example, Deng et al. (49) showed that *B. subtilis* supplementation significantly increased ileum lipase, amylase, lactase, and maltase activities compared to control group, and significantly increased ileum lactase, and maltase activities compared to antibiotic group. Hu et al. (91) showed that replacement of aureomycin with *B. amyloliquefaciens* (2×10^8 cfu/Kg) significantly

improved intestinal amylase, disaccharides and Na^+/K^+-ATPase activities of piglets, and half replacement of aureomycin with *B. amyloliquefaciens* (1×10^8 cfu/Kg), the activity of intestinal chymotrypsin was significantly improved. Many studies have shown that dietary addition of *Bacillus* spp. can significantly improve the nutrient digestibility of piglets (38, 45, 99–101), which may be related to the secretion of intestinal digestive enzymes. For instance, Huting et al. (38) showed that dietary addition of multi-strains of *Bacillus* spp. significantly improved the apparent total tract digestibility (ATTD) of dry matter (DM) and organic matter (OM) of piglets. Hu and Kim (45) showed that dietary *B. subtilis* C-3102 supplementation significantly improved the apparent the ATTD of DM, crude protein (CP), and energy of piglets. Cai et al. (99) and Lewton et al. (100) showed that piglets fed with *Bacillus* spp. based direct-fed microbial had a higher protein utilization, as indicated by increased the ATTD of nitrogen. Cui et al. (101) showed that the ether extract (EE) and phosphate digestibility was increased when piglets fed with *B. subtilis*.

When exposed to stress conditions such as weaning stress, *Escherichia coli* (*E. coli*) infection, IUGR and lipopolysaccharide (LPS) stimulation, piglets are susceptible to intestinal mucosal injury, potentially leading to increased intestinal permeability (3, 87, 102–104). In general, intestinal permeability is assessed by quantifying the passage of small molecules like horseradish peroxidase (59), monitoring plasma levels of D-lactic acid, endotoxins, and diamine oxidase (DAO) (37, 44, 105), as well as by measuring trans-epithelial electrical resistance (TEER) in intestinal tissues (106). Studies have shown that *Bacillus* spp. can reduce intestinal permeability of piglets. Kim et al. (59) demonstrated that a high dosage of *B. subtilis* (2.56×10^9 CFU/kg) supplementation lowered both intestinal intercellular and intracellular permeability in *E. coli* infected pigs. Hu et al. (91) showed that substituting aureomycin with *B. amyloliquefaciens* (2×10^8 cfu/Kg) preserved intestinal integrity, as evidenced by significantly decreased DAO activity. Xie et al. (107) showed that a combination of *Lactobacillus acidophilus* and *B. subtilis* reduced serum DAO level in piglets. In an IPEC-J2 cell model of deoxynivalenol (DON)-induced injury, Gu et al. (108) reported that TEER was higher in cells treated with *B. subtilis* compared to non-*B. subtilis* treated cells.

Bacillus spp. promotes intestinal mucosal barrier

The intestinal epithelium serves as the primary barrier, facilitating nutrient breakdown and absorption via brush border enzymes and various transport proteins at the apical and basolateral membranes, while also protecting against antigenic invasion (3). Tight junctions are vital components of the intestinal mucosal barrier against harmful pathogens, predominantly made up of membrane protein complexes, including zonula occludens proteins (ZOs), occludin, and claudins (109). Alterations in the structure and function of tight junctions may directly impair the integrity of the intestinal mucosal barrier, leading to the infiltration of pathogens and other undesirable substances through the epithelial layer (110). Previous studies have confirmed the regulatory role of *Bacillus* spp. on intestinal tight junction proteins in piglets (35, 75, 105, 111–113). For instance, in a study using an IPEC-J2 cells model infected with *E. coli*, Sudan et al. (37) demonstrated that *B. subtilis* significantly upregulated the expression

of *ZO-1*, *claudin-1*, and *occludin* genes. Similarly, Li et al. (77) found that *B. halotolerans* markedly increased both the gene and protein expression levels of ZO-1, claudin-1, and Occludin in weaned piglets suffering from *E. coli*-induced diarrhea. Sun et al. (75) showed that dietary inclusion of *B. licheniformis* significantly promoted the expression of Occludin and ZO-1 in jejunum mucosa of piglets. Additionally, Fu et al. (105) demonstrated that piglets supplemented with *B. licheniformis*, either alone or in combination with *Clostridium butyricum* significantly up-regulated the protein expression of ZO-1 and Occludin in the jejunum, as well as ZO-1, Claudin-1 and Occludin in the ileum of piglets. In addition, Yang et al. (114) showed that dietary supplementation with a low dose (3.9×10^8 CFU/day) of a mixture of *B. licheniformis and B. subtilis* significantly up-regulated the protein expression of ZO-1 in the jejunum of piglets infected with *E. coli*, thereby preventing the loss of intestinal epithelial barrier integrity.

Another important molecule in the intestinal mucosal barrier is mucins (MUCs), which are secreted by intestinal goblet cells (87, 115). As principal constituents of the mucus layer, MUCs facilitate gut lubrication and form the initial line of defense within the mucosal barrier, which can promote the colonization of symbiotic bacteria, inhibit the attachment of pathogens, and maintain the homeostasis of the intestinal environment (116, 117). Therefore, the differentiation and proliferation of intestinal goblet cells, along with the normal secretion of MUCs, are crucial for maintaining intestinal health. Stress commonly disrupts these processes in piglets, leading to compromised cellular functions. For example, studies by Li et al. (77), Zhang et al. (118), and Xu et al. (119) showed that the number of intestinal goblet cells decreased significantly when piglets were infected with *E. coli*. Conversely, *Bacillus* spp. have been shown to enhance the maturation and differentiation of intestinal goblet cells and the secretion of MUCs in piglets (60, 68, 80, 93, 107, 118). Specifically, research by He et al. (60) revealed that dietary inclusion of *B. pumilus* significantly increased the number of goblet cells in duodenal villi, while supplementation of *B. subtilis* significantly up-regulated the expression of *MUC2* gene in the jejunum mucosa of piglets. Liu et al. (80) showed that the combination of *B. subtilis* with *L. plantarum* at dosage of 1 kg/t significantly increased the number of colonic goblet cells of weaned piglets. Zhang et al. (118) demonstrated that dietary supplementation a dosage of 7.8×10^8 CFU/kg *Bacillus* spp. probiotics mixture significantly increased the number of ileal goblet cells, and up-regulated the expression of *MUC2* gene in the ileum of piglets infected with *E. coli*. In conclusion, the protective effect of *Bacillus* spp. on intestinal mucosal barrier of piglets is achieved by promoting the expression of intestinal tight junction proteins and the secretion of MUCs.

Bacillus spp. promotes intestinal immune function and inhibits inflammatory response

The intestine is not only the most important digestive organ in animals but also the largest immune organ. The gut immune system recognizes and combats pathogens, such as bacteria, viruses, and parasites, that traverse the gut surface to prevent them from entering the bloodstream and causing systemic infections, thus playing a pivotal role in maintaining overall health (3). The intestinal

development of piglets is still immature, and piglets will encounter many pathogenic and non-pathogenic challenges due to physiological and psychological factors during weaning, leading to the destruction of intestinal immune barrier function (3, 120). Numerous *in vitro* and *in vivo* studies have confirmed that *Bacillus* spp. can enhance the intestinal immune function of piglets by promoting the proliferation of immune cells, enhancing immune function, and inhibiting inflammatory responses (44, 47, 59, 114, 121).

Initially, *Bacillus* spp. enhances the intestinal immune capacity of piglets by stimulating the activity and proliferation of immune cells. The intestinal immune system of piglets is not fully matured in their early stages, and *Bacillus* spp. can stimulate immune cells in the intestinal submucosa, particularly T cells and B cells in the gastrointestinal-associated lymphoid tissue (GALT), to promote their proliferation and differentiation (114, 122, 123). This proliferation helps to establish a stronger and more active immune defense network, thereby enhancing the ability to resist pathogenic invasions. For instance, Xie et al. (107) showed that dietary supplementation with *L. acidophilus* and *B. subtilis* mixture increased CD4+ T cells and sIgA+ cells in intestinal mucosa of piglets, and Zhang et al. (121) showed that oral administration of *B. subtilis* significantly increased the number of IgA secreting cells and CD3+ T cells in intestinal tract of piglets, which suggests a direct influence on the mucosal immune response. Yang et al. (114) demonstrated that dietary supplementation with high-dose (7.8×10^8 CFU/day) of *B. licheniformis* and *B. subtilis* mixture significantly increased the percentage of CD4⁻CD8⁻T cells in the inflamed intestine of piglets challenged with *E. coli*. Similarly, Zhou et al. (122) showed that adding a combination of *B. licheniformis* and *B. subtilis* to the diet notably increased the percentage of CD4＋Foxp3+ T regulatory cells among the intraepithelial lymphocytes. It also increased the presence of CD4＋IL-10+ T cells in the Peyer's patches and the lamina propria of small intestines in *E. coli*-infected piglets.

Secondly, *Bacillus* spp. enhances the intestinal immune function of piglets by producing a variety of antimicrobial substances, such as antimicrobial peptides (AMPs) and short-chain fatty acids (SCFAs), which directly inhibit the growth of pathogens, thereby protecting piglets from infections (77, 105, 107, 124). For example Xie et al. (107) observed that dietary inclusion of a combination of *L. acidophilus* and *B. subtilis* upregulated the gene expression of AMPs, including porcine beta defensin-2 (*PBD-2*), *PBD-3* and regenerating islet-derived IIIγ (*RegIIIγ*), and short-chain fatty acid receptors including *GPR43*, *GPR41*and *GPR109A* in the ileum mucosa of piglets. Fu et al. (105) showed that the use of *B. licheniformis* alone had no effect on the expression of AMPs genes, while the combination of *B. licheniformis* and *Clostridium butyricum* could significantly increase the expression of intestinal AMPs genes, including *PBD-1*, *PBD-2*, *PBD-3* and *PR-39* in piglets. At the same time, the use of *B. licheniformis* alone or in combination with *Clostridium butyricum* significantly increased the content of acetic, propionic acid, butyric acid, and total acid in the ileal contents of piglets.

Finally, *Bacillus* spp. also plays an important role in inhibiting inflammation by regulating the production of inflammatory cytokines, such as reducing the release of pro-inflammatory factors (such as TNF-α, IL-1β, and IL-6), while increasing the production of anti-inflammatory cytokines (such as IL-10, and IL-22), thereby preventing intestinal damage caused by excessive immune activity (47, 59, 72, 80,

105, 107). For example, Yu et al. (72) showed that dietary *B. licheniformis* supplementation significantly increased anti-inflammatory factors (IL-10), and reduced pro-inflammatory factors (TNF-α and IL-6) levels in the jejunal mucosa of piglets challenged with LPS. Li et al. (77) showed that *B. halotolerans* can inhibit the expression of various inflammatory factors in intestines of piglets by suppressed the activation of the toll-like receptor (TLR)2/TLR4-myeloid differentiation factor 88 (MyD88)-nuclear transcription factor-κB (NF-κB) pathway. Xie et al. (107) demonstrated that dietary inclusion of a combination of *L. acidophilus* and *B. subtilis* upregulated the expression of IL-22 in the ileum mucosa of piglets. In an *E. coli*-infected IPEC-1 model, Ji et al. (69) showed that *B. amyloliquefaciens* can inhibit the mRNA expression of *IL-1α*, *IL-6*, *IL-8*, and *TNF-α* by suppression of mitogen-activated protein kinase (MAPK) signaling pathways. In addition, *Bacillus* spp. can promote intestinal immune function by promoting the secretion of intestinal secretory immunoglobulin A (sIgA), which is also an important mechanism for suppressing excessive immune responses and inflammation (44, 77, 80, 107).

Bacillus spp. inhibits pathogenic bacteria and regulates intestinal flora homeostasis

The gastrointestinal tract of animals is inhabited by a large number of microorganisms, and these microorganisms and their metabolites play an important role in host health in terms of nutrition, intestinal barrier and immunity through interaction with intestinal mucosa (125, 126). Changes in diet, environment, and other factors impact (3, 87). The optimization of intestinal microbial structure of piglets by nutritional strategies is one of the current research hotspots (127–129). Among which, the use of probiotics including *Bacillus* spp. have a significant effect on the regulation of intestinal microbial homeostasis in piglets (76, 130–133). For example, Wang et al. (36) showed that dietary supplementation with *B. subtilis* and *B. licheniformis* mixture decreased the abundance of *Blautia* and *Clostridium*, while increased the abundances of *Bacteroidetes* and *Ruminococcaceae*. Hu et al. (46), Wang et al. (52), and Li et al. (77) showed that piglets fed *Bacillus* spp. significantly increased the number of intestinal *Lactobacillus* and decreased the number of *E. coli*. In which, *Bacteroidetes* is benefits for the degradation of proteins and carbohydrates (134), and the activation of host's immune system (135). *Ruminococcaceae* are associated with energy production and can ferment cellulose and hemicellulose to produce SCFAs (136). *Clostridium* is associated with diarrhea, and high abundance of intestinal *Clostridium* increases the risk of diarrhea in piglets (137). The abnormal increase of *E. coli* abundance can cause intestinal oxidative damage, reduce immune function and destroy intestinal integrity, making it a primary pathogen causing diarrhea of piglets (77, 138). *Lactobacillus* plays an important role in intestinal health, such as preventing diarrhea and intestinal infections, so it is considered to be a beneficial bacterium to maintain the balance of intestinal flora (46). Previous studies shown that an important sign of piglet diarrhea is primarily distinguished by the increased number of *E. coli* and the decreased number of *Lactobacillus* in intestines (46, 77, 138).

Therefore, the potential mechanisms by which *Bacillus* spp. modulate the gut microbial composition of piglets involve the

TABLE 2 Beneficial effects of *Bacillus* spp. on the intestinal health of piglets.

Bacillus spp.	Optimal added amount	Intestinal health benefits	References
B. subtilis	100 mg/kg or 200 mg/kg	E-cadherin, ZO-1, and occludin↑, *E. coli* and *Clostridium perfringens*↓	Liu et al. (35)
B. subtilis and *B. licheniformis* mixture	4×10⁹ CFU/g	VH and VCR↑, CD↓, TLR-4 and TNF-α↓, *Bacteroidetes* and *Ruminococcaceae*↑, *Blautia* and *Clostridium*↓	Wang et al. (36)
B. subtilis and *B. licheniformis* mixture	1.3×10⁹ CFU/kg	Intestinal permeability↓, Shannon and Simpson index↑, *Treponema*↑	Jiao et al. (37)
B. amyloliquefaciens	1.0× 10⁹ CFU/kg	Intestinal permeability↓, IL-1β and IFN-γ↓, T-AOC, IL-10 and sIgA↑, *Bacteroides, Phascolarctobacterium* and *Desulfovibrio*↑, *Streptococcus, Tyzzerella, Vellionella* and *paraeggerthella*↓	Jiang et al. (44)
B. subtilis	4×10⁹ or 2×10¹⁰ CFU/kg	*E. coli*↓, *Lactobacillus*↑	Hu et al. (46)
B. subtilis	2.56×10⁹ CFU/kg	Claudin 1↓, IL-6 and PTGS2↓	He et al. (47)
B. amyloliquefaciens	2.56×10⁹ CFU/kg	Firmicutes and *Bifidobacterium*↑, Actinomycetota, Bacteroidota and *Clostridium sensustricto* 1↓	Jinno et al. (48)
B. subtilis	0.1%	Lipase, amylase, and maltase activities↑, VCR↑, Firmicutes↑, *E. coli*↓	Deng et al. (49)
B. subtilis	1.44× 10⁹ CFU/kg	VH and VCR↑, *Anaerovibrio* and *Bulleidia*↑, *Clostridium* and *Coprococcus*↓, colonic SCFAs↑	Tian et al. (50)
B. subtilis	500 mg/kg	VH and VCR↑, propionic acid and butyric acid↑, *occludin, EGF,* and *IGF-1R*↑, *Bacillus* and *Bifidobacterium*↑	Tang et al. (51)
B. subtilis	1×10⁹ CFU/kg	*Lactobacillus*↑, *E. coli*↓	Wang et al. (52)
B. subtilis	1.875×10⁵ CFU/g	*E. coli*↓, butyrate↑	Duddeck et al. (53)
B. subtilis	1.0× 10⁹ CFU/kg	Pedicoccus, Collinella, Turiciator, Veillonella, Clostridium, and Escherichia in jejunum↑, Olsenella and Pediococcus in ileum↑	He et al. (54)
B. subtilis	1×10⁸ CFU/mL	Intestinal permeability↓, GPx and SOD↑, MDA and H₂O₂↓, *Occludin*↑	Jia et al. (55)
B. subtilis	500 mg/kg	VH and VCR↑, intestinal permeability↓	Júnior et al. (56)
B. subtilis	1.0× 10⁷ CFU/kg	Serum IFN-γ and TNF-α↓, serum IL-10, IgA and IgG↑	Li et al. (57)
B. subtilis	0.05%	VH and VCR↑	Park et al. (58)
B. subtilis	1.28×10⁹ CFU/kg or 2.56×10⁹ CFU/kg	Intestinal permeability↓, *MUC2*↑, IL-1α and IL-6↓	Kim et al. (59)
B. subtilis	1.0× 10⁹ CFU/kg	Total coliforms in mesenteric lymph nodes↓, *MUC2*↑, *PTGS-2* and *IL-1β*↓, *Lachnospiraceae, Peptostreptococcaceae* and *Pasteurellaceae*↓	He et al. (60)
B. subtilis	2.0×10⁹ CFU/kg	lactic acid bacteria and *Bacillus* spp.↑, *E. coli* and total *coliforms*↓, IL-8↓, MUC-1 and occludin↑	Sudan et al. (61)
B. coagulans	600 g/t	Bacteria diversity↑, *Ruminococcaceae_UCG-014*↑, *Prevotella_9, unclassified_f__Lachnospiraceae,* and *Anaerovibrio*↓	Sun et al. (62)

(Continued)

TABLE 2 (Continued)

Bacillus spp.	Optimal added amount	Intestinal health benefits	References
B. coagulans	1.0×10^8 CFU/kg	VH and VCR↑, MPO and apoptosis of intestinal epithelial cells↓, *Enterococcus*, *Clostridium* and *Lactobacillus* in jejunum↓, *E. coli*, *Bifidobacterium* and *Lactobacillus* in colon↓	Zhang et al. (63)
B. coagulans	400 mg/kg	Serun C3, LZM and TNF-α↓, VCR↑, *ZO-1*↑, IL-1β↓, *TNF-α*↓	Fu et al. (64)
B. coagulans	2×10^6 CFU/kg or 1×10^7 CFU/kg	Intestinal permeability↓, VH ↑, CD↓, SOD and CAT↑, MDA and H_2O_2↓	Wu et al. (65)
B. coagulans	2×10^6 CFU/kg	Intestinal morphology↑, Occludin↑, GSH-Px↑, MDA↓, serum TNF-α, IL-1β↓, caspase-3↓	Wu et al. (66)
B. amyloliquefaciens	5.0×10^9 CFU/kg	*Lachnospiraceae*, *Peptococcaceae.rc4_4*, *Erysipelotrichaceae.L7A_E11*, and *Mollicutes.RF39*↑	Du et al. (67)
B. amyloliquefaciens	2.0 g/kg	VH and VCR↑, goblet cell↑, IL-10↑, TNF-α↓, *Lactobacillus* and *Bifidobacterium*↑, *E. coli*↓	Li et al. (68)
B. amyloliquefaciens	2.0×10^8 CFU/kg	MDA↓, T-AOC and GSH-Px↑, *SOD, CAT, GST* and *NQO1*↑, Nrf2↑	Wang et al. (70)
B. amyloliquefaciens	1.0×10^8 CFU/kg	Goblet cells↑, VH and VCR↑, IL-10↑, TNF-α↓, *Lactobacillus*↑	Wang et al. (71)
B. licheniformis	500 mg/kg	Serum IL-10↑, serum IL-1β and IL-6↓, acetic acid, propionic acid, butyric acid, isobutyric acid, and isovaleric acid↑, *Clostridium_sensu_stricto_1* and *Oscillospira*↑	Yu et al. (72)
B. licheniformis	1×10^9 CFU/kg or 1×10^{10} CFU/kg	Serum IgA, IgG, and IgM↑, VCR↑, propionic and isobutyric acid↑, *Prevotella*↑	Cao et al. (73)
B. licheniformis	500 mg/kg or 1,000 mg/kg	IgA and IgG↑, IL-1β↓, acetate and propionic acids↑	Yu et al. (74)
B. licheniformis	1,000 g/t	VH and VCR↑, *SOD1, Nrf2*, and *HO-1*↑, sIgA, occludin and ZO-1↑, *Lactobacillus*↑, *clostridium_sensu_stricto_1*↓	Sun et al. (75)
B. licheniformis	2×10^9 CFU/kg	*Aminopeptidase N, occludin, ZO-1*, and *SGLT1*↑, *Bacteroidetes*↓	Xu et al. (76)
B. halotolerans	1×10^9 CFU/kg	VH and VCR↑, intestinal permeability↓, ZO-1, claudin-1 and occludin↑, sIgA↑, *Lactobacillus*↑, *E. coli*↓	Li et al. (77)
B. toyonensis	500 mg/kg or 1,000 mg/kg	Lactic acid bacteria↑, enteric pathogens↓	Kantas et al. (78)
B. subtilis and *L. plantarum* mixture	1 kg/t, 1.5 kg/t, or 2 kg/t	VCR↑, goblet cells↑, intestinal permeability↓, IL-1β, TNF-α, and IL-2↓, sIgA↑, SOD↑, MDA↓, *Claudin-1, occludin, ZO-1* and *MUC1*↑, *Lactobacillus*↑	Liu et al. (80)
B. coagulans and benzoic acid mixture	400 g/t and 3,000 g/t, respectively	Serum TNF-α↓, *SGLT1, claudin-1, occludin* and *mucin2*↑, *Bifidobacterium* and *Bacillus*↑	Pu et al. (81)
B. subtilis	1.28×10^6 CFU/g	Villus mitotic index↑	Luise et al. (84)
B. subtilis	500 g/t	VH and VCR↑, butyrate, tryptamine, and cadaverine↑, skatole↓, Phylum Cyanobacteria and Proteobacteria↑, Genus *Actinobacter, Coprococcus, Enterococcus*, and *Dorea*↑	Ding et al. (85)

(Continued)

TABLE 2 (Continued)

Bacillus spp.	Optimal added amount	Intestinal health benefits	References
B. coagulans and *lactulose* mixture	2×10^9 CFU/kg and 10 g/kg, respectively	VH and VCR↑, apoptosis↓, ZO-2, Occludin and claudin-3 protein↑	Zheng et al. (90)
B. amyloliquefaciens	2×10^8 CFU/kg	Intestinal permeability↑, amylase, disaccharides and Na⁺/K⁺-ATPase↑, β-diversity of intestinal microbiota↑	Hu et al. (91)
B. amyloliquefaciens	2×10^8 CFU/kg	Villus length↑, VCR↑, goblet-cell number↑, tight junction proteins↑, *TNF-α* and IL-1α↓	Du et al. (93)
B. subtilis	2×10^9 CFU/kg	VCR↑, intestinal permeability↓, *ZO-1*, *occludin*, and *claudin-1*↑, protein carbonyl↓, CAT and SOD↑, *SOD1*, *CAT* and *Nrf2*↑, HO-1, SOD1, and Nrf2↑	Yun et al. (104)
B. licheniformis	1×10^9 CFU/kg	Intestinal permeability↓, ZO-1, claudin −1 and occludin↑, *Phascolarctobacterium*↓	Fu et al. (105)
B. subtilis and *L. acidophilus* mixture	0.1%	Intestinal permeability↑, ZO-1, Occludin, and MUC2↑, *PBD-2*, *PBD-3*, and *RegIIIγ*↑, CD4+ T cells and sIgA+ cells↑, sIgA↑, IL-22↑	Xie et al. (107)
L. johnsonii, *B. licheniformis* and *B. subtilis* mixture	1×10^8, 4×10, and 4×10^5 CFU/mL, respectively	Goblet cells↑, cell death↓, *Salmonella*↓	Liu et al. (112)
B. licheniformis and *B. subtilis* mixture	3.9×10^8 CFU/day or 7.8×10^8 CFU/day	CD4 − CD8 − T cells↑, IL-22 and IκBα↑	Yang et al. (114)
B. licheniformis and *B. subtilis* mixture	3.9×10^8, 7.8×10^8 or 3.9×10^9 CFU/day	*Lactobacillus gasseri* and *Lactobacillus salivarius* populations↑, goblet cells and MUC2 production↑	Zhang et al. (118)
B. amyloliquefaciens	1×10^8 CFU/kg	Intestinal barrier integrity and intestinal antioxidant capacity↑, inflammatory response and enterocyte apoptosis↓	Wang et al. (121)
B. subtilis	1×10^8 CFU/kg	IgA-secreting cells and CD3⁺ T cells↑, sIgA↑	Zhang et al. (122)
B. licheniformis and *B. subtilis* mixture	3.9×10^7 CFU/mL or 7.8×10^7 CFU/ mL	Foxp3(−)IL-10(+) T cells↑, CD4(+)Foxp3(+) Treg cells↑, CD4(+)IL-10(+) T cells↑	Zhou et al. (123)
B. subtilis	2.56×10^9 CFU/kg	*Actinomycetaceae* and *Lachnospiraceae*↓, *Veillonellaceae*, *Bifidobacteriaceae*, Lactobacillaceae↑	Jinno et al. (131)
B. coagulans	1×10^7 CFU/g	VH↑, CD↓, IL-10↑, IL-6↓, *Lactobacillus*↑, *E. coli*↓	Liu et al. (142)
C. butyricum, *B. subtilis,* and *B. licheniformis* mixture	5.0×10^{10}, 5.0×10^9, and 5.0×10^9 CFU/ kg, respectively	Ileal apoptotic cells↓, TNF-α and IL-1β↓, butyrate and valerate↑, *Bacillus*↑, intestinal morphology↑	Cao et al. (143)

C3, complement 3; CAT, catalase; CD, crypt depth; GSH-Px, Glutathione peroxidase; GST, glutathione S-transferase; HO-1, heme oxygenase-1; IκBα, NF-kappa-B inhibitor alpha; NQO1, NAD (P)H, quinone oxidoreductase 1; IFN-γ, interferon-γ; IL-1β, Interleukin-1β; IL-6, Interleukin-6; IL-10, Interleukin-10; LZM, lysozyme; MDA, malondialdehyde; MUC2, mucin 2; Nrf2, Nuclear factor erythroid 2-related factor 2; PBD-2, porcine beta defensin-2; RegIIIγ, regenerating islet-derived IIIγ; SGLT1, sodium-glucose cotransporter1; sIgA, secretory immunoglobulin A; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TNF-α, tumor necrosis factor α; VCR, villus height to crypt depth ratio; VH, villus height; ZO-1, zonula occludens-1; "↑" stands for increase, and "↓" stands for decrease.

promotion of beneficial bacterial proliferation and the suppression of pathogenic bacterial growth. On one hand, *Bacillus* spp. competes with opportunistic pathogens for adhesion sites and nutrients, thereby inhibiting the attachment and colonization of harmful microbes in the intestinal tract (139). On the other hand, *Bacillus* spp. ferments carbohydrates to produce a large amount of L-lactic acid, which reduces intestinal pH value, forms an anaerobic acidic environment conducive to the growth of beneficial bacteria such as *Lactobacillus* and *bifidobacterium*, and prevents the invasion of aerobic and eosinophilic pathogens (140, 141). In addition, *Bacillus* spp. secretes a variety of antibacterial substances, such as MUCs, AMPs, sIgA, and SCFAs, which exhibit marked antagonistic activity against various pathogens, thereby regulating the balance of intestinal microbiota (80, 107, 118).

The beneficial effects of *Bacillus* spp. on the intestinal health of piglets are summarized in Table 2. A review of these literatures revealed that *Bacillus* spp. exert a positive influence on intestinal health of piglets. Firstly, *Bacillus* spp. facilitates intestinal development and reduces gut permeability in piglets. Secondly, *Bacillus* spp. enhances the function of the intestinal mucosal barrier by upregulating tight junction proteins and stimulating MUCs secretion. Thirdly, *Bacillus* spp. enhances intestinal immunity by activating immune cells within the gut, modulating the secretion of both pro-inflammatory and anti-inflammatory cytokines, and increasing the production of secretory immunoglobulins. Lastly,

Bacillus spp. helps maintain a dynamic balance of the intestinal microbiota by encouraging the growth of beneficial microorganisms and suppressing the proliferation of pathogenic bacteria.

Numerous studies have shown that a healthy gut can promote the digestion and absorption of nutrients, thus promoting animal growth (3). Analysis of the existing literatures found that *Bacillus* spp. had good regulatory effects on the intestinal tract of piglets which can promote intestinal development, promote intestinal mucosal barrier, promote intestinal immune function, inhibit inflammatory response, inhibit pathogenic bacteria and regulate intestinal flora homeostasis. Moreover, most of the Bacillus can promote the growth of piglets. Given the close connection between intestinal health and animal growth, we can speculate that *Bacillus* spp. promotes growth by promoting intestinal health of piglets.

Potential risks and concerns of *Bacillus* spp. as probiotics

Over the past decade, concerns about the overuse of antibiotics have shifted attention to probiotics in the animal feed industry as they can improve growth performance and reduce disease risk (32, 144). The spore-forming *Bacillus* spp. has received extensive scientific and commercial attention, and their beneficial effects have been widely reported and acknowledged (32, 41). However, with a better understanding of their positive role, many questions have been raised about their safety and the relevance of spore formation in the practical application of this class of microorganisms. The first is the safety concerns of the *Bacillus* spp. Some strains of *Bacillus* can cause infections such as bacteremia and endocarditis when they enter the bloodstream (27, 41). For example, A study by Deng et al. (145) who had collected 50 commercial probiotic products and isolated bacillus from the products, which showed that 34 probiotic products (68%) exhibited hemolysis, including 19 human probiotics, 9 animal probiotics, and 6 plant probiotics. 19 of 28 *B. cereus* isolates maintained to exhibit hemolysis after heat treatment. Secondly, pathogenic potential. Some species within the *Bacillus* genus are known to be pathogenic. For example, *Bacillus cereus* is notorious for causing food poisoning, which causes great harm to food safety and animal health by producing enterotoxin and vomitoxin (146, 147). For example, Li et al. (146) showed that piglets received *Bacillus cereus* caused diarrhea, weight loss, and reduced IgG titers of swine fever virus (CSFV) and porcine epidemic diarrhea (PED). The potential for probiotic strains to switch from a beneficial to a pathogenic state is a concern. The third risk factor is antimicrobial and antibiotic resistance issues. There is a worry that the use of *Bacillus* spp. as probiotics could contribute to the spread of antibiotic resistance genes, particularly in the context of their extensive use in the food industry and as a biological control agent in agriculture (41, 148). For example, Deng et al. (145) showed that all 48 *Bacillus* spp. isolates exhibited resistance to lincomycin, and 5 were resistant to tetracycline. Zhu et al. (149) evaluated the safety of 15 strains of *Bacillus cereus* and found that nearly half of the strains carried the antimicrobial resistance gene tet (45). In one strain, tet (45) is located on a mobile genetic element that encodes site-specific recombination mechanisms and is transferred to *Staphylococcus aureus* and *B. subtilis* by electrical transformation. Zhai et al. (150) tested the antimicrobial resistance and antibiotic resistance of 114 isolates of *Bacillus* spp., and the antibiotic susceptibility tests showed that the intrinsic resistance rates of *Bacillus* to ampicillin and penicillin were 80 and 86%, respectively. *Bacillus* strains with acquired antibiotic resistance may spread antibiotic resistance between Bacillus and other clinical pathogens through horizontal gene transfer. Fourth, strain-specificity concerns. As probiotics, the action of *Bacillus* spp. appears to be strain-specific, which means that not all strains will have the same benefits or risks. This adds a layer of complexity to their use and regulation (41). Fifth, lack of standardization. There is a lack of standardization in the production and use of *Bacillus* spp. probiotics, which can lead to variability in efficacy and safety (27). Lastly, regulatory challenges. The regulatory framework for probiotics, including *Bacillus* spp., varies by country and is not always clear, leading to challenges in ensuring the safety and quality of probiotic products (27). In conclusion, *Bacillus* spp. has advantages as well as challenges as an animal probiotic, and safety evaluation should be conducted when using the newly isolated *Bacillus* spp.

Conclusion

The use of *Bacillus* spp. as probiotics in piglets offers a promising approach to promote growth and enhance intestinal health. The mechanisms by which *Bacillus* spp. exert their beneficial effects include improving growth performance, enhancing intestinal mucosal barrier function, improving intestinal immune function and producing antimicrobial compounds, as well as modulating gut microbiota. Further research is needed to identify the most effective strains and optimal application strategies to maximize the benefits of *Bacillus* spp. as probiotics in piglets.

Author contributions

XT: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. YZ: Writing – original draft, Formal analysis. KX: Writing – review & editing, Funding acquisition. JZ: Writing – review & editing, Software, Resources.

Funding

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Upadhaya SD, Kim IH. The impact of weaning stress on gut health and the mechanistic aspects of several feed additives contributing to improved gut health function in weanling piglets-a review. *Animals*. (2021) 11:2418. doi: 10.3390/ani11082418

2. Su W, Li Z, Gong T, Wang F, Jin M, Wang Y, et al. An alternative ZnO with large specific surface area: preparation, physicochemical characterization and effects on growth performance; diarrhea, zinc metabolism and gut barrier function of weaning piglets. *Sci Total Environ*. (2023) 882:163558. doi: 10.1016/j.scitotenv.2023.163558

3. Tang X, Xiong K, Fang R, Li M. Weaning stress and intestinal health of piglets: a review. *Front Immunol*. (2022) 13:1042778. doi: 10.3389/fimmu.2022.1042778

4. Barton MD. Impact of antibiotic use in the swine industry. *Curr Opin Microbiol*. (2014) 19:9–15. doi: 10.1016/j.mib.2014.05.017

5. Hu Q, Liu C, Zhang D, Wang R, Qin L, Xu Q, et al. Effects of low-dose antibiotics on gut immunity and antibiotic Resistomes in weaned piglets. *Front Immunol*. (2022) 11:903. doi: 10.3389/fimmu.2020.00903

6. Ming D, Wang J, Yin C, Chen Y, Li Y, Sun W, et al. Porous zinc oxide and plant polyphenols as a replacement for high-dose zinc oxide on growth performance, diarrhea incidence, intestinal morphology and microbial diversity of weaned piglets. *Animals*. (2024) 14:523. doi: 10.3390/ani14030523

7. Schokker D, Kar SK, Willems E, Bossers A, Dekker RA, Jansman AJM. Dietary supplementation of zinc oxide modulates intestinal functionality during the post-weaning period in clinically healthy piglets. *J Anim Sci Biotechnol*. (2023) 14:122. doi: 10.1186/s40104-023-00925-1

8. Peng P, Deng D, Chen S, Li C, Luo J, Romeo A, et al. The effects of dietary porous zinc oxide supplementation on growth performance, inflammatory cytokines and tight Junction's gene expression in early-weaned piglets. *J Nutr Sci Vitaminol*. (2020) 66:311–8. doi: 10.3177/jnsv.66.311

9. Wang L, Wang C, Peng Y, Zhang Y, Liu Y, Liu Y, et al. Research progress on anti-stress nutrition strategies in swine. *Anim Nutr*. (2023) 13:342–60. doi: 10.1016/j.aninu.2023.03.006

10. Zhang M, Yang Z, Wu G, Xu F, Zhang J, Luo X, et al. Effects of probiotic-fermented feed on the growth profile, immune functions, and intestinal microbiota of Bamei piglets. *Animals*. (2024) 14:647. doi: 10.3390/ani14040647

11. Lei XJ, Liu ZZ, Park JH, Kim IH. Novel zinc sources as antimicrobial growth promoters for monogastric animals: a review. *J Anim Sci Technol*. (2022) 64:187–96. doi: 10.5187/jast.2022.e1

12. Ekhlas D, Sanjuán JMO, Manzanilla EG, Leonard FC, Argüello H, Burgess CM. Comparison of antimicrobial resistant *Escherichia coli* isolated from Irish commercial pig farms with and without zinc oxide and antimicrobial usage. *Gut Pathog*. (2023) 15:8. doi: 10.1186/s13099-023-00534-3

13. Bonetti A, Tugnoli B, Piva A, Grilli E. Towards zero zinc oxide: feeding strategies to manage post-weaning diarrhea in piglets. *Animals*. (2021) 11:642. doi: 10.3390/ani11030642

14. Liang C, Fu R, Chen D, Tian G, He J, Zheng P, et al. Effects of mixed fibres and essential oils blend on growth performance and intestinal barrier function of piglets challenged with enterotoxigenic *Escherichia coli* K88. *J Anim Physiol Anim Nutr*. (2023) 107:1356–67. doi: 10.1111/jpn.13866

15. Ma J, Piao X, Shang Q, Long S, Liu S, Mahfuz S. Mixed organic acids as an alternative to antibiotics improve serum biochemical parameters and intestinal health of weaned piglets. *Anim Nutr*. (2021) 7:737–49. doi: 10.1016/j.aninu.2020.11.018

16. Watthanaphansak S, Kim HB. Gut microbial shifts by synbiotic combination of Pediococcus acidilactici and lactulose in weaned piglets challenged with Shiga toxin-producing *Escherichia coli*. *Front Vet Sci*. (2023) 9:1101869. doi: 10.3389/fvets.2022.1101869

17. Xu X, Chang J, Wang P, Liu C, Liu M, Zhou T, et al. Combination of glycyrrhizic acid and compound probiotics alleviates deoxynivalenol-induced damage to weaned piglets. *Ecotoxicol Environ Saf*. (2023) 256:114901. doi: 10.1016/j.ecoenv.2023.114901

18. Ali MS, Lee EB, Hsu WH, Suk K, Sayem SAJ, Ullah HMA, et al. Probiotics and Postbiotics as an alternative to antibiotics: an emphasis on pigs. *Pathogens*. (2023) 12:874. doi: 10.3390/pathogens12070874

19. Vieira AM, Sessin AP, Soratto TAT, Pires PGDS, Cardinal KM, Wagner G, et al. Effect of functional oils or probiotics on performance and microbiota profile of newly weaned piglets. *Sci Rep*. (2021) 11:19457. doi: 10.1038/s41598-021-98549-w

20. Yang J, Qian K, Wang C, Wu Y. Roles of probiotic lactobacilli inclusion in helping piglets establish healthy intestinal inter-environment for pathogen defense. *Probiotics Antimicrob Proteins*. (2018) 10:243–50. doi: 10.1007/s12602-017-9273-y

21. Azizi AFN, Uemura R, Omori M, Sueyoshi M, Yasuda M. Effects of probiotics on growth and immunity of piglets. *Animals*. (2022) 12:1786. doi: 10.3390/ani12141786

22. Grandmont A, Rhouma M, Létourneau-Montminy MP, Thériault W, Mainville I, Arcand Y, et al. Characterization of the effects of a novel probiotic on Salmonella colonization of a piglet-derived intestinal microbiota using improved bioreactor. *Animals*. (2024) 14:787. doi: 10.3390/ani14050787

23. Tang X, Liu X, Liu H. Effects of dietary probiotic (*Bacillus subtilis*) supplementation on carcass traits, meat quality, amino acid, and fatty acid profile of broiler chickens. *Front Vet Sci*. (2021) 8:767802. doi: 10.3389/fvets.2021.767802

24. Li CL, Wang J, Zhang HJ, Wu SG, Hui QR, Yang CB, et al. Qi GH. Intestinal morphologic and microbiota responses to dietary Bacillus spp. in a broiler chicken model. *Front Physiol*. (2019) 9:1968. doi: 10.3389/fphys.2018.01968

25. Poveda J, González-Andrés F. Bacillus as a source of phytohormones for use in agriculture. *Appl Microbiol Biotechnol*. (2021) 105:8629–45. doi: 10.1007/s00253-021-11492-8

26. Polonca S. Environment shapes the intra-species diversity of *Bacillus subtilis* isolates. *Microb Ecol*. (2020) 79:853–64. doi: 10.1007/s00248-019-01455-y

27. Todorov SD, Ivanova IV, Popov I, Weeks R, Chikindas ML. Bacillus spore-forming probiotics: benefits with concerns? *Crit Rev Microbiol*. (2022) 48:513–30. doi: 10.1080/1040841X.2021.1983517

28. Contesini FJ, Melo RR, Sato HH. An overview of Bacillus proteases: from production to application. *Crit Rev Biotechnol*. (2018) 38:321–34. doi: 10.1080/07388551.2017.1354354

29. Adeniji AA, Loots DT, Babalola OO. *Bacillus velezensis*: phylogeny, useful applications, and avenues for exploitation. *Appl Microbiol Biotechnol*. (2019) 103:3669–82. doi: 10.1007/s00253-019-09710-5

30. Gupta AK, Maity C. Efficacy and safety of *Bacillus coagulans* LBSC in irritable bowel syndrome: a prospective, interventional, randomized, double-blind, placebo-controlled clinical study [CONSORT compliant]. *Medicine*. (2021) 100:e23641. doi: 10.1097/MD.0000000000023641

31. Cutting SM. Bacillus probiotics. *Food Microbiol*. (2011) 28:214–20. doi: 10.1016/j.fm.2010.03.007

32. Khalid F, Khalid A, Fu Y, Hu Q, Zheng Y, Khan S, et al. Potential of *Bacillus velezensis* as a probiotic in animal feed: a review. *J Microbiol*. (2021) 59:627–33. doi: 10.1007/s12275-021-1161-1

33. Amerah AM, Quiles A, Medel P, Sánchez J, Lehtinen MJ, Gracia MI. Effect of pelleting temperature and probiotic supplementation on growth performance and immune function of broilers fed maize/soy-based diets. *Anim Feed Sci Technol*. (2013) 180:55–63. doi: 10.1016/j.anifeedsci.2013.01.002

34. Dong W, Gong X, Zheng F, Liu Y, Zhang L, Zhang X, et al. Isolation, screening and biological characteristics of *Bacillus licheniformis* from pigs. *J Gansu Agric Univ*. (2021) 56:8–17. doi: 10.13432/j.cnki.jgsau.2021.05.002

35. Liu J, Ma X, Zhuo Y, Xu S, Hua L, Li J, et al. The effects of *Bacillus subtilis* QST713 and β-mannanase on growth performance, intestinal barrier function, and the gut microbiota in weaned piglets. *J Anim Sci*. (2023) 101:skad 257. doi: 10.1093/jas/skad257

36. Wang X, Tian Z, Azad MAK, Zhang W, Blachier F, Wang Z, et al. Dietary supplementation with Bacillus mixture modifies the intestinal ecosystem of weaned piglets in an overall beneficial way. *J Appl Microbiol*. (2021) 130:233–46. doi: 10.1111/jam.14782

37. Jiao S, Zheng Z, Zhuang Y, Tang C, Zhang N. Dietary medium-chain fatty acid and Bacillus in combination alleviate weaning stress of piglets by regulating intestinal microbiota and barrier function. *J Anim Sci*. (2023) 101:skac 414. doi: 10.1093/jas/skac414

38. Huting AMS, Lagos LV, Hansen LHB, Molist F. Effect of supplementing a Bacillus multi-strain probiotic to a post-weaning diet on nutrient utilisation and nitrogen retention of piglets. *Animals*. (2023) 13:3597. doi: 10.3390/ani13233597

39. Sudan S, Zhan X, Li J. A novel probiotic *Bacillus subtilis* strain confers Cytoprotection to host pig intestinal epithelial cells during Enterotoxic *Escherichia coli* infection. *Microbiol Spectr*. (2022) 10:e0125721. doi: 10.1128/spectrum.01257-21

40. Liu Y, Yue Z, Sun Z, Li C. Harnessing native Bacillus spp. for sustainable wheat production. *Appl Environ Microbiol*. (2023) 89:e0124722. doi: 10.1128/aem.01247-22

41. Elshaghabee FMF, Rokana N, Gulhane RD, Sharma C, Panwar H. Bacillus as potential probiotics: status, concerns, and future perspectives. *Front Microbiol*. (2017) 8:1490. doi: 10.3389/fmicb.2017.01490

42. Campbell JM, Crenshaw JD, Polo J. The biological stress of early weaned piglets. *J Anim Sci Biotechnol*. (2013) 4:19. doi: 10.1186/2049-1891-4-19

43. Heo JM, Opapeju FO, Pluske JR, Kim JC, Hampson DJ, Nyachoti CM. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. *J Anim Physiol Anim Nutr*. (2013) 97:207–37. doi: 10.1111/j.1439-0396.2012.01284.x

44. Jiang Z, Su W, Li W, Wen C, Du S, He H, et al. *Bacillus amyloliquefaciens* 40 regulates piglet performance, antioxidant capacity, immune status and gut microbiota. *Anim Nutr*. (2022) 12:116–27. doi: 10.1016/j.aninu.2022.09.006

45. Hu J, Kim IH. Effect of *Bacillus subtilis* C-3102 spores as a probiotic feed supplement on growth performance, nutrient digestibility, diarrhea score, intestinal microbiota, and excreta odor contents in weanling piglets. *Animals*. (2022) 12:316. doi: 10.3390/ani12030316

46. Hu Y, Dun Y, Li S, Zhao S, Peng N, Liang Y. Effects of *Bacillus subtilis* KN-42 on growth performance, diarrhea and Faecal bacterial Flora of weaned piglets. *Asian Australas J Anim Sci*. (2014) 27:1131–40. doi: 10.5713/ajas.2013.13737

47. He Y, Kim K, Kovanda L, Jinno C, Song M, Chase J, et al. *Bacillus subtilis*: a potential growth promoter in weaned pigs in comparison to carbadox. *J Anim Sci*. (2020) 98:skaa 290. doi: 10.1093/jas/skaa290

48. Jinno C, Wong B, Klünemann M, Htoo J, Li X, Liu Y. Effects of supplementation of *Bacillus amyloliquefaciens* on performance, systemic immunity, and intestinal microbiota of weaned pigs experimentally infected with a pathogenic enterotoxigenic *E. coli* F18. *Front Microbiol*. (2023) 14:1101457. doi: 10.3389/fmicb.2023.1101457

49. Deng B, Wu J, Li X, Zhang C, Men X, Xu Z. Effects of *Bacillus subtilis* on growth performance, serum parameters, digestive enzyme, intestinal morphology, and colonic microbiota in piglets. *AMB Express*. (2020) 10:212. doi: 10.1186/s13568-020-01150-z

50. Tian Z, Wang X, Duan Y, Zhao Y, Zhang W, Azad MAK, et al. Dietary supplementation with *Bacillus subtilis* promotes growth and gut health of weaned piglets. *Front Vet Sci*. (2021) 7:600772. doi: 10.3389/fvets.2020.600772

51. Tang W, Qian Y, Yu B, Zhang T, Gao J, He J, et al. Effects of *Bacillus subtilis* DSM32315 supplementation and dietary crude protein level on performance, gut barrier function and microbiota profile in weaned piglets 1. *J Anim Sci*. (2019) 97:2125–38. doi: 10.1093/jas/skz090

52. Wang H, Kim KP, Kim IH. Influence of *Bacillus subtilis* GCB-13-001 on growth performance, nutrient digestibility, blood characteristics, faecal microbiota and faecal score in weanling pigs. *J Anim Physiol Anim Nutr*. (2019) 103:1919–25. doi: 10.1111/jpn.13199

53. Duddeck KA, Petersen TE, Adkins HJ, Smith AH, Hernandez S, Wenner SJ, et al. Dose-dependent effects of supplementing a two-strain *Bacillus subtilis* probiotic on growth performance, blood parameters, fecal metabolites, and microbiome in nursery pigs. *Animals*. (2023) 14:109. doi: 10.3390/ani14010109

54. He F, Jin X, Sun K, Zhao L, Yang W, Zhang X, et al. *Bacillus subtilis* JATP-3 improves nitrogen metabolism by regulating intestinal Flora and AKG in weaned piglets. *Probiotics Antimicrob Proteins*. (2023). doi: 10.1007/s12602-023-10196-x [Epub ahead of print].

55. Jia R, Sadiq FA, Liu W, Cao L, Shen Z. Protective effects of *Bacillus subtilis* ASAG 216 on growth performance, antioxidant capacity, gut microbiota and tissues residues of weaned piglets fed deoxynivalenol contaminated diets. *Food Chem Toxicol*. (2021) 148:111962. doi: 10.1016/j.fct.2020.111962

56. Júnior DTV, de Amorim RG, Soares MH, Silva CB, Frank EO, Gonzalez-Vega JC, et al. Supplementation of *Bacillus subtilis* DSM 32540 improves performance and intestinal health of weaned pigs fed diets containing different fiber sources. *Livest Sci*. (2023) 270:105202. doi: 10.1016/j.livsci.2023.105202

57. Li H, Jiang X, Qiao J. Effect of dietary *Bacillus subtilis* on growth performance and serum biochemical and immune indexes in weaned piglets. *J Appl Anim Res*. (2021) 49:83–8. doi: 10.1080/09712119.2021.1877717

58. Park S, Lee JW, Jerez Bogota K, Francis D, González-Vega JC, Htoo JK, et al. Growth performance and gut health of *Escherichia coli*-challenged weaned pigs fed diets supplemented with a *Bacillus subtilis* direct-fed microbial. *Transl. Anim Sci*. (2020) 4:txaa 172. doi: 10.1093/tas/txaa172

59. Kim K, He Y, Xiong X, Ehrlich A, Li X, Raybould H, et al. Dietary supplementation of *Bacillus subtilis* influenced intestinal health of weaned pigs experimentally infected with a pathogenic *E. coli*. *J Anim Sci Biotechnol*. (2019) 10:52. doi: 10.1186/s40104-019-0364-3

60. He Y, Jinno C, Kim K, Wu Z, Tan B, Li X, et al. Dietary Bacillus spp. enhanced growth and disease resistance of weaned pigs by modulating intestinal microbiota and systemic immunity. *J Anim Sci Biotechnol*. (2020) 11:101. doi: 10.1186/s40104-020-00498-3

61. Sudan S, Fletcher L, Zhan X, Dingle S, Patterson R, Huber LA, et al. Comparative efficacy of a novel *Bacillus subtilis*-based probiotic and pharmacological zinc oxide on growth performance and gut responses in nursery pigs. *Sci Rep*. (2023) 13:4659. doi: 10.1038/s41598-023-31913-0

62. Sun T, Miao H, Zhang C, Wang Y, Liu S, Jiao P, et al. Effect of dietary *Bacillus coagulans* on the performance and intestinal microbiota of weaned piglets. *Animal*. (2022) 16:100561. doi: 10.1016/j.animal.2022.100561

63. Zhang Y, Tian X, Dong Y, Li R, Shen M, Yi D, et al. *Bacillus coagulans* prevents the decline in average daily feed intake in young piglets infected with enterotoxigenic

Escherichia coli K88 by reducing intestinal injury and regulating the gut microbiota. *Front Cell Infect Microbiol*. (2023) 13:1284166. doi: 10.3389/fcimb.2023.1284166

64. Fu R, Liang C, Chen D, Yan H, Tian G, Zheng P, et al. Effects of dietary Bacillus coagulans and yeast hydrolysate supplementation on growth performance, immune response and intestinal barrier function in weaned piglets. *J Anim Physiol Anim Nutr*. (2021) 105:898–907. doi: 10.1111/jpn.13529

65. Wu T, Zhang Y, Lv Y, Li P, Yi D, Wang L, et al. Beneficial impact and molecular mechanism of *Bacillus coagulans* on Piglets' intestine. *Int J Mol Sci*. (2018) 19:2084. doi: 10.3390/ijms19072084

66. Wu T, Zhang Q, Xu H, Li P, Zhao D, Wang L, et al. Protective effects of α-terpineol and *Bacillus coagulans* on intestinal function in weaned piglets infected with a recombinant *Escherichia coli* expressing heat-stable enterotoxin STa. *Front Vet Sci*. (2023) 10:1118957. doi: 10.3389/fvets.2023.1118957

67. Du H, Yao W, Kulyar MF, Ding Y, Zhu H, Pan H, et al. Effects of *Bacillus amyloliquefaciens* TL106 isolated from Tibetan pigs on probiotic potential and intestinal microbes in weaned piglets. *Microbiol Spectr*. (2022) 10:e0120521. doi: 10.1128/spectrum.01205-21

68. Li Y, Zhang H, Su W, Ying Z, Chen Y, Zhang L, et al. Effects of dietary *Bacillus amyloliquefaciens* supplementation on growth performance, intestinal morphology, inflammatory response, and microbiota of intra-uterine growth retarded weaning piglets. *J Anim Sci Biotechnol*. (2018) 9:22. doi: 10.1186/s40104-018-0236-2

69. Ji J, Hu S, Zheng M, Du W, Shang Q, Li W. *Bacillus amyloliquefaciens* SC06 inhibits ETEC-induced pro-inflammatory responses by suppression of MAPK signaling pathways in IPEC-1 cells and diarrhea in weaned piglets. *Livest Sci*. (2013) 158:206–14. doi: 10.1016/j.livsci.2013.09.017

70. Wang Y, Wu Y, Wang B, Cao X, Fu A, Li Y, et al. Effects of probiotic Bacillus as a substitute for antibiotics on antioxidant capacity and intestinal autophagy of piglets. *AMB Express*. (2017) 7:52. doi: 10.1186/s13568-017-0353-x

71. Wang Q, Wang F, Tang L, Wang Y, Zhou Y, Li X, et al. *Bacillus amyloliquefaciens* SC06 alleviated intestinal damage induced by inflammatory via modulating intestinal microbiota and intestinal stem cell proliferation and differentiation. *Int Immunopharmacol*. (2024) 130:111675. doi: 10.1016/j.intimp.2024.111675

72. Yu X, Cui Z, Qin S, Zhang R, Wu Y, Liu J, et al. Effects of *Bacillus licheniformis* on growth performance, diarrhea incidence, antioxidant capacity, immune function, and fecal microflora in weaned piglets. *Animals*. (2022) 12:1609. doi: 10.3390/ani12131609

73. Cao G, Yang S, Wang H, Zhang R, Wu Y, Liu J, et al. Effects of *Bacillus licheniformis* on the growth performance, antioxidant capacity, Ileal morphology, intestinal short chain fatty acids, and colonic microflora in piglets challenged with lipopolysaccharide. *Animals*. (2023) 13:2172. doi: 10.3390/ani13132172

74. Yu X, Dai Z, Cao G, Cui Z, Zhang R, Xu Y, et al. Protective effects of *Bacillus licheniformis* on growth performance, gut barrier functions, immunity and serum metabolome in lipopolysaccharide-challenged weaned piglets. *Front Immunol*. (2023) 14:1140564. doi: 10.3389/fimmu.2023.1140564

75. Sun W, Chen W, Meng K, Cai L, Li G, Li X, et al. Dietary supplementation with probiotic *Bacillus licheniformis* S6 improves intestinal integrity via modulating intestinal barrier function and microbial diversity in weaned piglets. *Biology*. (2023) 12:238. doi: 10.3390/biology12020238

76. Xu H, Gong J, Lu P, Azevedo P, Li L, Yu H, et al. Functional evaluation of *Bacillus licheniformis* PF9 for its potential in controlling enterotoxigenic *Escherichia coli* in weaned piglets. *Transl Anim Sci*. (2024) 8:txae 050. doi: 10.1093/tas/txae050

77. Li M, Zhao D, Guo J, Pan T, Niu T, Jiang Y, et al. Bacillus halotolerans SW207 alleviates enterotoxigenic *Escherichia coli*-induced inflammatory responses in weaned piglets by modulating the intestinal epithelial barrier, the TLR4/MyD88/NF-κB pathway, and intestinal microbiota. *Microbiol Spectr*. (2024) 12:e0398823. doi: 10.1128/spectrum.03988-23

78. Kantas D, Papatsiros VG, Tassis PD, Giavasis I, Bouki P, Tzika ED. A feed additive containing Bacillus toyonensis (Toyocerin (®)) protects against enteric pathogens in postweaning piglets. *J Appl Microbiol*. (2015) 118:727–38. doi: 10.1111/jam.12729

79. Papatsiros VG, Tassis PD, Tzika ED, Papaioannou DS, Petridou E, Alexopoulos C, et al. Effect of benzoic acid and combination of benzoic acid with a probiotic containing *Bacillus cereus* var. Toyoi in weaned pig nutrition. *Pol J Vet Sci*. (2011) 14:117–25. doi: 10.2478/v10181-011-0017-8

80. Liu Y, Gu W, Liu X, Zou Y, Wu Y, Xu Y, et al. Joint application of Lactobacillus plantarum and *Bacillus subtilis* improves growth performance, immune function and intestinal integrity in weaned piglets. *Vet Sci*. (2022) 9:668. doi: 10.3390/vetsci9120668

81. Pu J, Chen D, Tian G, He J, Zheng P, Mao X, et al. Effects of benzoic acid, Bacillus coagulans and oregano oil combined supplementation on growth performance, immune status and intestinal barrier integrity of weaned piglets. *Anim Nutr*. (2020) 6:152–9. doi: 10.1016/j.aninu.2020.02.004

82. Phaengphairee P, Boontiam W, Wealleans A, Hong J, Kim YY. Dietary supplementation with full-fat *Hermetia illucens* larvae and multi-probiotics, as a substitute for antibiotics, improves the growth performance, gut health, and antioxidative capacity of weaned pigs. *BMC Vet Res*. (2023) 19:7. doi: 10.1186/s12917-022-03550-8

83. Kritas SK, Morrison RB. Evaluation of probiotics as a substitute for antibiotics in a large pig nursery. *Vet Rec*. (2005) 156:447–8. doi: 10.1136/vr.156.14.447

84. Luise D, Bertocchi M, Motta V, Salvarani C, Bosi P, Luppi A, et al. Bacillus sp. probiotic supplementation diminish the *Escherichia coli* F4ac infection in susceptible weaned pigs by influencing the intestinal immune response, intestinal microbiota and blood metabolomics. *J Anim Sci Biotechnol*. (2019) 10:74. doi: 10.1186/s40104-019-0380-3

85. Ding H, Zhao X, Azad MAK, Ma C, Gao Q, He J, et al. Dietary supplementation with Bacillus subtilis and xylo-oligosaccharides improves growth performance and intestinal morphology and alters intestinal microbiota and metabolites in weaned piglets. *Food Funct*. (2021) 12:5837–49. doi: 10.1039/d1fo00208b

86. Shin D, Chang SY, Bogere P, Won K, Choi JY, Choi YJ, et al. Beneficial roles of probiotics on the modulation of gut microbiota and immune response in pigs. *PLoS One*. (2019) 14:e0220843. doi: 10.1371/journal.pone.0220843

87. Tang X. Probiotic roles of *Clostridium butyricum* in piglets: considering aspects of intestinal barrier function. *Animals*. (2024) 14:1069. doi: 10.3390/ani14071069

88. Tang W, Liu J, Ma Y, Wei Y, Liu J, Wang H. Impairment of intestinal barrier function induced by early weaning via autophagy and apoptosis associated with gut microbiome and metabolites. *Front Immunol*. (2021) 12:804870. doi: 10.3389/fimmu.2021.804870

89. Wu Z, Yan Y, Li W, Li Y, Yang H. Expression profile of mi R-199a and its role in the regulation of intestinal inflammation. *Animals*. (2023) 13:1979. doi: 10.3390/ani13121979

90. Zheng W, Zhao Z, Yang Y, Ding L, Yao W. The synbiotic mixture of lactulose and *Bacillus coagulans* protects intestinal barrier dysfunction and apoptosis in weaned piglets challenged with lipopolysaccharide. *J Anim Sci Biotechnol*. (2023) 14:80. doi: 10.1186/s40104-023-00882-9

91. Hu S, Cao X, Wu Y, Mei X, Xu H, Wang Y, et al. Effects of probiotic Bacillus as an alternative of antibiotics on digestive enzymes activity and intestinal integrity of piglets. *Front Microbiol*. (2018) 9:2427. doi: 10.3389/fmicb.2018.02427

92. Hu L, Peng X, Chen H, Yan C, Liu Y, Xu Q, et al. Effects of intrauterine growth retardation and *Bacillus subtilis* PB6 supplementation on growth performance, intestinal development and immune function of piglets during the suckling period. *Eur J Nutr*. (2017) 56:1753–65. doi: 10.1007/s00394-016-1223-z

93. Du W, Xu H, Mei X, Cao X, Gong L, Wu Y, et al. Probiotic Bacillus enhance the intestinal epithelial cell barrier and immune function of piglets. *Benef Microbes*. (2018) 9:743–54. doi: 10.3920/BM2017.0142

94. Latorre JD, Hernandez-Velasco X, Wolfenden RE, Vicente JL, Wolfenden AD, Menconi A, et al. Evaluation and selection of Bacillus species based on enzyme production, antimicrobial activity, and biofilm synthesis as direct-fed microbial candidates for poultry. *Front Vet Sci*. (2016) 3:95. doi: 10.3389/fvets.2016.00095

95. Parrado J, Rodriguez-Morgado B, Tejada M, Hernandez T, Garcia C. Proteomic analysis of enzyme production by *Bacillus licheniformis* using different feather wastes as the sole fermentation media. *Enzym Microb Technol*. (2014) 57:1–7. doi: 10.1016/j.enzmictec.2014.01.001

96. Olmos SJ. Bacillus probiotic enzymes: external auxiliary apparatus to avoid digestive deficiencies, water pollution, diseases, and economic problems in marine cultivated animals. *Adv Food Nutr Res*. (2017) 80:15–35. doi: 10.1016/bs.afnr.2016.11.001

97. Akhtar N, Cai HY, Kiarie EG, Li J. A novel Bacillus sp. with rapid growth property and high enzyme activity that allows efficient fermentation of soybean meal for improving digestibility in growing pigs. *J Appl Microbiol*. (2022) 133:3–17. doi: 10.1111/jam.15268

98. Vojnovic S, Aleksic I, Ilic-Tomic T, Stevanovic M, Nikodinovic-Runic J. Bacillus and Streptomyces spp. as hosts for production of industrially relevant enzymes. *Appl Microbiol Biotechnol*. (2024) 108:185. doi: 10.1007/s00253-023-12900-x

99. Cai L, Indrakumar S, Kiarie E, Kim IH. Effects of a multi-strain Bacillus species-based direct-fed microbial on growth performance, nutrient digestibility, blood profile, and gut health in nursery pigs fed corn-soybean meal-based diets. *J Anim Sci*. (2015) 93:4336–42. doi: 10.2527/jas.2015-9056

100. Lewton JR, Woodward AD, Moser RL, Thelen KM, Moeser AJ, Trottier NL, et al. Effects of a multi-strain *Bacillus subtilis*-based direct-fed microbial on weanling pig growth performance and nutrient digestibility. *Transl Anim Sci*. (2021) 5:txab 058. doi: 10.1093/tas/txab058

101. Cui K, Lv X, Diao Q, Zhang N. Effects of dietary supplementation with Bacillus subtilis and yeast culture on growth performance, nutrient digestibility, serum indices and faeces microbiota of weaned piglet. *J Anim Feed Sci*. (2019) 28:328–36. doi: 10.22358/jafs/114238/2019

102. Cao S, Zhang Q, Wang C, Wu H, Jiao L, Hong Q, et al. LPS challenge increased intestinal permeability, disrupted mitochondrial function and triggered mitophagy of piglets. *Innate Immun*. (2018) 24:221–30. doi: 10.1177/1753425918769372

103. Che L, Xu Q, Wu C, Luo Y, Huang X, Zhang B, et al. Effects of dietary live yeast supplementation on growth performance, diarrhoea severity, intestinal permeability and immunological parameters of weaned piglets challenged with enterotoxigenic *Escherichia coli* K88. *Br J Nutr*. (2017) 118:949–58. doi: 10.1017/S0007114517003051

104. Yun Y, Ji S, Yu G, Jia P, Niu Y, Zhang H, et al. Effects of *Bacillus subtilis* on jejunal integrity, redox status, and microbial composition of intrauterine growth restriction suckling piglets. *J Anim Sci*. (2021) 99:skab 255. doi: 10.1093/jas/skab255

105. Fu J, Wang T, Xiao X, Cheng Y, Wang F, Jin M, et al. *Clostridium Butyricum* ZJU-F1 benefits the intestinal barrier function and immune response associated with its modulation of gut microbiota in weaned piglets. *Cells*. (2021) 10:527. doi: 10.3390/cells10030527

106. Tang X, Xiong K. Effects of epidermal growth factor on glutamine and glucose absorption by IPEC-J2 cells challenged by lipopolysaccharide using the ussing chamber system. *Pak J Zool*. (2021) 53:417–22. doi: 10.17582/journal.pjz/20200117080156

107. Xie Z, Li M, Qian M, Yang Z, Han X. Co-cultures of Lactobacillus acidophilus and *Bacillus subtilis* enhance mucosal barrier by modulating gut microbiota-derived short-chain fatty acids. *Nutrients*. (2022) 14:4475. doi: 10.3390/nu14214475

108. Gu MJ, Song SK, Park SM, Lee IK, Yun CH. *Bacillus subtilis* protects porcine intestinal barrier from Deoxynivalenol via improved zonula Occludens-1 expression. *Asian Australas J Anim Sci*. (2014) 27:580–6. doi: 10.5713/ajas.2013.13744

109. Suzuki T. Regulation of the intestinal barrier by nutrients: the role of tight junctions. *Anim Sci J*. (2020) 91:e13357. doi: 10.1111/asj.13357

110. Zhao Z, Sun M, Cui X, Chen J, Liu C, Zhang X. *Bacillus coagulans* MZY531 alleviates intestinal mucosal injury in immunosuppressive mice via modulating intestinal barrier, inflammatory response, and gut microbiota. *Sci Rep*. (2023) 13:11181. doi: 10.1038/s41598-023-38379-0

111. Zong X, Wang TH, Lu ZQ, Song DG, Zhao J, Wang YZ. Effects of *Clostridium butyricum* or in combination with *Bacillus licheniformis* on the growth performance, blood indexes, and intestinal barrier function of weanling piglets. *Livest Sci*. (2019) 220:137–42. doi: 10.1016/j.livsci.2018.12.024

112. Liu X, Xia B, He T, Li D, Su JH, Guo L, et al. Oral Administration of a Select Mixture of Lactobacillus and Bacillus alleviates inflammation and maintains mucosal barrier integrity in the ileum of pigs challenged with Salmonella Infantis. *Microorganisms*. (2019) 7:135. doi: 10.3390/microorganisms7050135

113. Li Q, Li L, Chen Y, Yu C, Azevedo P, Gong J, et al. *Bacillus licheniformis* PF9 improves barrier function and alleviates inflammatory responses against enterotoxigenic *Escherichia coli* F4 infection in the porcine intestinal epithelial cells. *J Anim Sci Biotechnol*. (2022) 13:86. doi: 10.1186/s40104-022-00746-8

114. Yang GY, Zhu YH, Zhang W, Zhou D, Zhai CC, Wang JF. Influence of orally fed a select mixture of Bacillus probiotics on intestinal T-cell migration in weaned MUC4 resistant pigs following *Escherichia coli* challenge. *Vet Res*. (2016) 47:71. doi: 10.1186/s13567-016-0355-8

115. Yang S, Yu M. Role of goblet cells in intestinal barrier and mucosal immunity. *J Inflamm Res*. (2021) 14:3171–83. doi: 10.2147/JIR.S318327

116. Gustafsson JK, Johansson MEV. The role of goblet cells and mucus in intestinal homeostasis. *Nat Rev Gastroenterol Hepatol*. (2022) 19:785–803. doi: 10.1038/s41575-022-00675-x

117. Li G, Gao M, Zhang S, Dai T, Wang F, Geng J, et al. Sleep deprivation impairs intestinal mucosal barrier by activating endoplasmic reticulum stress in goblet cells. *Am J Pathol*. (2024) 194:85–100. doi: 10.1016/j.ajpath.2023.10.004

118. Zhang W, Zhu YH, Zhou D, Wu Q, Song D, Dicksved J, et al. Oral Administration of a Select Mixture of Bacillus probiotics affects the gut microbiota and goblet cell function following *Escherichia coli* challenge in newly weaned pigs of genotype MUC4 that are supposed to be Enterotoxigenic *E. coli* F4ab/ac receptor negative. *Appl Environ Microbiol*. (2017) 83:e02747–16. doi: 10.1128/AEM.02747-16

119. Xu J, Jia Z, Xiao S, Long C, Wang L. Effects of Enterotoxigenic *Escherichia coli* challenge on Jejunal morphology and microbial community profiles in weaned crossbred piglets. *Microorganisms*. (2023) 11:2646. doi: 10.3390/microorganisms11112646

120. Bailey M, Haverson K, Inman C, Harris C, Jones P, Corfield G, et al. The development of the mucosal immune system pre-and post-weaning: balancing regulatory and effector function. *Proc Nutr Soc*. (2005) 64:451–7. doi: 10.1079/pns2005452

121. Wang Q, Wang F, Zhou Y, Li X, Xu S, Jin Q, et al. *Bacillus amyloliquefaciens* SC06 relieving intestinal inflammation by modulating intestinal stem cells proliferation and differentiation via AhR/STAT3 pathway in LPS-challenged piglets. *J Agric Food Chem*. (2024) 72:6096–109. doi: 10.1021/acs.jafc.3c05956

122. Zhang P, Huang L, Zhang E, Yuan C, Yang Q. Oral administration of *Bacillus subtilis* promotes homing of CD3+ T cells and IgA-secreting cells to the respiratory tract in piglets. *Res Vet Sci*. (2021) 136:310–7. doi: 10.1016/j.rvsc.2021.03.006

123. Zhou D, Zhu YH, Zhang W, Wang ML, Fan WY, Song D, et al. Oral administration of a select mixture of Bacillus probiotics generates Tr1 cells in weaned F4ab/ac R-pigs challenged with an F4+ ETEC/VTEC/EPEC strain. *Vet Res*. (2015) 46:95. doi: 10.1186/s13567-015-0223-y

124. Xu J, Zhong F, Zhang Y, Zhang J, Huo S, Lin H, et al. Construction of *Bacillus subtilis* strain engineered for expression of porcine β-defensin-2/cecropin P 1 fusion antimicrobial peptides and its growth-promoting effect and antimicrobial activity. *Asian Australas J Anim Sci*. (2017) 30:576–84. doi: 10.5713/ajas.16.0207

125. Park W. Gut microbiomes and their metabolites shape human and animal health. *J Microbiol*. (2018) 56:151–3. doi: 10.1007/s12275-018-0577-8

126. Barathan M, Ng SL, Lokanathan Y, Ng MH, Law JX. The profound influence of gut microbiome and extracellular vesicles on animal health and disease. *Int J Mol Sci*. (2024) 25:4024. doi: 10.3390/ijms25074024

127. Fan MZ, Cheng L, Wang M, Chen J, Fan W, Jashari F, et al. Monomodular and multifunctional processive endocellulases: implications for swine nutrition and gut microbiome. *Anim Microbiome*. (2024) 6:4. doi: 10.1186/s42523-024-00292-w

128. Galli GM, Andretta I, Levesque C, Stefanello T, Carvalho CL, Perez Pelencia JY, et al. Using probiotics to improve nutrient digestibility and gut-health of weaned pigs: a comparison of maternal and nursery supplementation strategies. *Front Vet Sci*. (2024) 11:1356455. doi: 10.3389/fvets.2024.1356455

129. Aderibigbe AS, Park CS, Johnson T, Velayudhan DE, Vinyeta E, Adeola O. Efficacy of a novel multi-enzyme feed additive on growth performance, nutrient digestibility, and gut microbiome of weanling pigs fed corn–wheat or wheat–barley-based diet. *J Anim Sci*. (2024) 102:skae 064. doi: 10.1093/jas/skae064

130. Sampath V, Cho S, Jeong J, Mun S, Lee CH, Hermes RG, et al. Dietary Bacillus spp. supplementation to both sow and progenies improved post-weaning growth rate, gut function, and reduce the pro-inflammatory cytokine production in weaners challenged with *Escherichia coli* K88. *Anim Microbiome*. (2024) 6:3. doi: 10.1186/s42523-024-00290-y

131. Jinno C, Li X, Liu Y. Dietary supplementation of *Bacillus subtilis* or antibiotics modified intestinal microbiome of weaned pigs under enterotoxigenic *Escherichia coli* infection. *Front Microbiol*. (2022) 13:1064328. doi: 10.3389/fmicb.2022.1064328

132. Cuevas-Gómez I, de Andrés J, Cardenas N, Espinosa-Martos I, Jiménez E. Feed supplementation with Ligilactobacillus salivarius PS21603 optimises intestinal morphology and gut microbiota composition in weaned piglets. *Benef Microbes*. (2024) 15:195–210. doi: 10.1163/18762891-bja00001

133. Poulsen AR, Jonge N, Nielsen JL, Højberg O, Lauridsen C, Cutting SM, et al. Impact of Bacillus spp. spores and gentamicin on the gastrointestinal microbiota of suckling and newly weaned piglets. *PLoS One*. (2018) 13:e0207382. doi: 10.1371/journal.pone.0207382

134. Thomas F, Hehemann JH, Rebuffet E, Czjzek M, Michel G. Environmental and gut bacteroidetes: the food connection. *Front Microbiol*. (2011) 2:93. doi: 10.3389/fmicb.2011.00093

135. Bousbaine D, Fisch LI, London M, Bhagchandani P, Rezende de Castro TB, Mimee M, et al. A conserved Bacteroidetes antigen induces anti-inflammatory intestinal T lymphocytes. *Science*. (2022) 377:660–6. doi: 10.1126/science.abg5645

136. Liang J, Kou S, Chen C, Raza SHA, Wang S, Ma X, et al. Effects of *Clostridium butyricum* on growth performance, metabonomics and intestinal microbial differences of weaned piglets. *BMC Microbiol*. (2021) 21:85. doi: 10.1186/s12866-021-02143-z

137. Rist VT, Weiss E, Sauer N, Mosenthin R, Eklund M. Effect of dietary protein supply originating from soybean meal or casein on the intestinal microbiota of piglets. *Anaerobe*. (2014) 25:72–9. doi: 10.1016/j.anaerobe.2013.10.003

138. Li J, Feng S, Wang Z, He J, Zhang Z, Zou H, et al. Limosilactobacillus mucosae-derived extracellular vesicles modulates macrophage phenotype and orchestrates gut homeostasis in a diarrheal piglet model. *NPJ Biofilms Microbiomes*. (2023) 9:33. doi: 10.1038/s41522-023-00403-6

139. Nakano MM, Zuber P. Anaerobic growth of a "strict aerobe" (*Bacillus subtilis*). *Ann Rev Microbiol*. (1998) 52:165–90. doi: 10.1146/annurev.micro.52.1.165

140. Cao J, Yu Z, Liu W, Zhao J, Zhang H, Zhai Q, et al. Probiotic characteristics of Bacillus coagulans and associated implications for human health and diseases. *J Funct Foods*. (2020) 64:103643. doi: 10.1016/j.jff.2019.103643

141. Suzuki H, Fujiwara Y, Thongbhubate K, Maeda M, Kanaori K. Spore-forming lactic acid-producing bacterium *Bacillus coagulans* synthesizes and excretes spermidine into the extracellular space. *J Agric Food Chem*. (2023) 71:9868–76. doi: 10.1021/acs.jafc.3c02184

142. Liu C, Xiao M, Zhao P, Li Q, Bai H, Cheng Z. Effects of dietary *Bacillus coagulans* on growth performance and intestinal health of weaned piglets challenged with enterotoxigenic *Escherichia coli* K88. *Chinese J Anim Nutr*. (2021) 33:4373–83. doi: 10.3969/j.issn.1006-267x.2021.08.018

143. Cao G, Tao F, Hu Y, Li Z, Zhang Y, Deng B, et al. Positive effects of a *Clostridium butyricum*-based compound probiotic on growth performance, immune responses, intestinal morphology, hypothalamic neurotransmitters, and colonic microbiota in weaned piglets. *Food Funct*. (2019) 10:2926–34. doi: 10.1039/c8fo02370k

144. Mingmongkolchai S, Panbangred W. Bacillus probiotics: an alternative to antibiotics for livestock production. *J Appl Microbiol*. (2018) 124:1334–46. doi: 10.1111/jam.13690

145. Deng F, Chen Y, Sun T, Wu Y, Su Y, Liu C, et al. Antimicrobial resistance, virulence characteristics and genotypes of Bacillus spp. from probiotic products of diverse origins. *Food Res Int*. (2021) 139:109949. doi: 10.1016/j.foodres.2020.109949

146. Li X, Li Q, Wang Y, Han Z, Qu G, Shen Z, et al. Gastric ulceration and immune suppression in weaned piglets associated with feed-borne Bacillus cereus and *Aspergillus fumigatus*. *Toxins*. (2020) 12:703. doi: 10.3390/toxins12110703

147. Zuo Z, Li Q, Guo Y, Li X, Huang S, Hegemann JH, et al. Feed-borne *Bacillus cereus* exacerbates respiratory distress in chickens infected with *Chlamydia psittaci* by inducing haemorrhagic pneumonia. *Avian Pathol*. (2020) 49:251–60. doi: 10.1080/03079457.2020.1716940

148. Zhang Y, Overbeck TJ, Skebba VLP, Gandhi NN. Genomic and phenotypic safety assessment of probiotic *Bacillus coagulans* strain JBI-YZ6.3. *Probiotics Antimicrob Proteins*. (2024). doi: 10.1007/s12602-024-10305-4 [Epub ahead of print].

149. Zhu K, Hölzel CS, Cui Y, Mayer R, Wang Y, Dietrich R, et al. Probiotic *Bacillus cereus* strains, a potential risk for public health in China. *Front Microbiol*. (2016) 7:718. doi: 10.3389/fmicb.2016.00718

150. Zhai Z, Cui C, Li X, Yan J, Sun E, Wang C, et al. Prevalence, antimicrobial susceptibility, and antibiotic resistance gene transfer of Bacillus strains isolated from pasteurized milk. *J Dairy Sci*. (2023) 106:75–83. doi: 10.3168/jds.2022-22199