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Review Article

Exopolysaccharides from lactic acid bacteria, as an alternative to antibiotics, on regulation of intestinal health and the immune system

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ABSTRACT

Over-use or misuse of antibiotics in livestock and poultry production contributes to the rising threat of antibiotic resistance in animals and has negative ecological effects. Exopolysaccharides from lactic acid bacteria (LAB-EPS) are a class of biological macromolecules which are secreted by lactic acid bacteria to the outside of the cell wall during their growth and metabolism. Numerous studies demonstrated that LAB-EPS have anti-inflammatory and antimicrobial activities and are able to regulate intestinal health and the immune system in livestock. They are biodegradable, nontoxic and bio-compatible, which are considered as ideal alternatives to antibiotics. This review aims to discuss and summarize recent research findings of LAB-EPS on regulation of intestinal health and the immune system in animals, and thus provide scientific justification for commercial applications of LAB-EPS in livestock.

1. Introduction

The lactic acid bacteria (LAB) constitute a group of Gram-positive bacteria, which produce lactic acid as the major end product of carbohydrate metabolism (Axelsson, 2004). LAB are among the most important microbes that are used in animal diets (Zhou et al., 2019a). They have been found to confer several health benefits to animals, including regulating intestinal microbiota, enhancing intestinal peristalsis, maintaining intestinal micro-ecological balance, reducing inflammation and improving intestinal function (Deng et al., 2022; Wang et al., 2020; Zhou et al., 2019a). In addition, LAB can inhibit growth of spoilage bacteria as well as enhance intestinal mucosal immunity of animals, which help to improve animal intestinal health, improve feed conversion ratio and promote animal growth (Deng et al., 2022; Wang et al., 2020; Xie et al., 2015). Therefore, LAB are considered as an alternative to antibiotics for enhancing the gastrointestinal immunity of animals (Deng et al., 2022; Kim et al., 2017).

Studies have shown that exopolysaccharides (EPS), which are the secondary metabolites produced by LAB, might play an important role in the functions of LAB mentioned above (Castro-Bravo et al., 2018; Lynch et al., 2018; Werning et al., 2022; Xiu et al., 2020; Zeidan et al., 2017). EPS are carbohydrate polymers secreted from LAB into their extracellular environment. Approximately 30 species of LAB have been confirmed to synthesize EPS (Xu et al., 2019b). Previous studies reported that addition of EPS from lactic acid bacteria (LAB-EPS) in the diet has significant beneficial effects on animal gastrointestinal health (Fig. 1). LAB-EPS are involved in regulation of intestinal barrier function and intestinal mucosal immune responses and thus modulate the host immune response (Castro-Bravo et al., 2018; Patten et al., 2014; Vinderola et al., 2006). The purposes of this review are to provide more aggregated information on the structure and classification of LAB-EPS, and discuss and summarize the latest research findings of using LAB-EPS in animal feeding to improve animals' intestinal health and regulate their immunity. In addition, this review aims to provide a scientific basis of applying LAB-EPS in commercial feed manufacturing.

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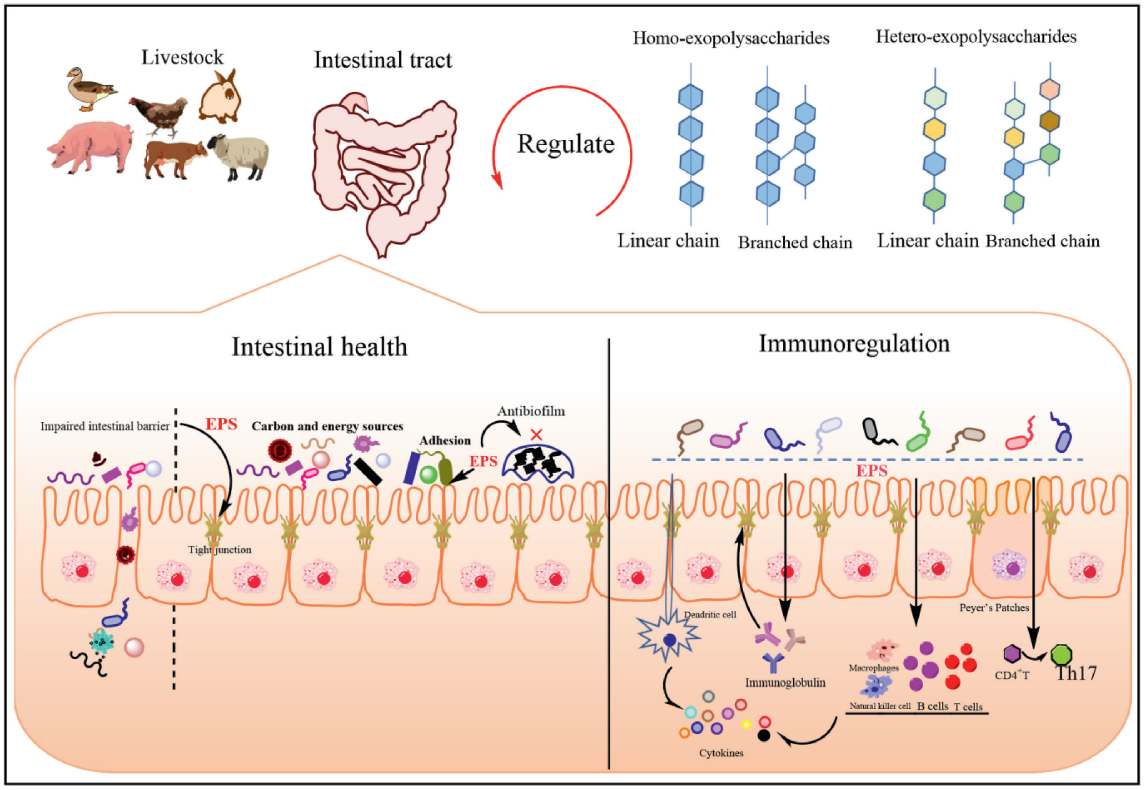

Fig. 1. Exopolysaccharides from lactic acid bacteria (LAB-EPS) have probiotic effects on the intestinal health and immune system in livestock. Homopolysaccharides and heteropolysaccharides are two categories of LAB-EPS, which can be used as carbon and energy sources by intestinal microbiota to support the growth of beneficial bacteria and fight against pathogenic bacteria in the intestine. LAB-EPS can improve intestinal health by adhering to intestinal epithelial cells, restoring impaired intestinal barrier function and inhibiting biofilm formation by bacterial pathogens. LAB-EPS also have been found to regulate the immune system by boosting the proliferation of T/B lymphocytes, natural killer cell tumoricidal activity, mitogenic activity, mononuclear cell phagocytic capacity and inducing cytokines, therefore enhancing the host immune response to pathogens.

2. Structure and classification of LAB-EPS

LAB-EPS, an important metabolic by-product of many LAB species, are not permanently attached to the surface of the LAB cells and are released into the extracellular medium during growth as loose slime (Lynch et al., 2018; Zhou et al., 2019b). LAB-EPS are highly viscous, water-soluble and high molecular weight carbohydrate polymers that vary in their physiochemical characteristics and composition (Lynch et al., 2018; Zhou et al., 2019b). They are composed of either a single type or multiple types of monosaccharide that range in degree of branching, from linear molecules to highly branched molecules (Ikeda et al., 2019; Rajoka et al., 2020). Microscopic images of LAB-EPS showed distributed spherical shaped particles with small holes on the surfaces. The images also revealed that in an aqueous solution, the LAB-EPS molecules were not completely dissociated but remained in three-dimensional network-like structures (Ikeda et al., 2019; Nachtigall et al., 2020).

LAB-EPS are categorized into homopolysaccharides (HoPS) and heteropolysaccharides (HePS) depending on their chemical composition and mechanisms of synthesis (Table 1). HoPS consist of only a single type of monosaccharide with repeating subunits, such as glucose or fructose, and are classified into α-D-glucans, ß-D-glucans (Fig. 2), fructans and polygalactans (Angelin and Kavitha, 2020; Rajoka et al., 2020). HePS are composed of 3 to 8 repeated monosaccharides subunits, typically D-glucose, D-galactose, L-rhamnose and rare monosaccharides including fucose, arabinose, mannose, N-acetylgalactosamine, N-acetylglucosamine and glucuronic acid (Angelin and Kavitha, 2020; Lynch et al., 2018). The average molecular weight of HoPS is greater than 10^6 Da, which is generally higher than the molecular mass of HePS (within the range of 10^4 to 10^6 Da) (Sanalibaba and Çakmak, 2016). A variety of LAB genera including *Streptococcus, Lactobacillus, Oenococcus, Leuconostoc,* and *Weissella* are capable of producing HoPS, while HePS are mainly produced by mesophilic and thermophilic LAB including *Streptococcus, Lactobacillus, Lactococcus,* and *Bifidobacterium* (Lynch et al., 2018; Rajoka et al., 2020). The production yields and composition of LAB-EPS are affected by LAB strain and growth conditions including medium composition, pH, temperature, oxygen level, turbidity and the growth phase (Angelin and Kavitha, 2020; Gerwig, 2019). HoPS are secreted in greater quantities than HePS in LAB due to their biosynthetic pathways (Gerwig, 2019; Nwodo et al., 2012).

3. Biosynthetic pathways of LAB-EPS

The extracellular synthesis pathway and Wzx/Wzy-dependent pathway are the two universal pathways for LAB-EPS biosynthesis (Rajoka et al., 2020; Zhou et al., 2019b). Generally, HoPS are only synthesized by the extracellular biosynthetic pathway following a two-step process as shown in Fig. 3. The first step is polymerization whereby monosaccharide is added to the growing polysaccharide chains. This process is assisted by specific extracellular glycosyltransferases and fructosyltransferases. Next, polymerized HoPS chains are released to the extracellular surroundings (Angelin and Kavitha, 2020; Wu et al., 2022; Zhou et al., 2019b).

HePS are mainly produced by the Wzx/Wzy-dependent pathway, which is more complex and involves more enzymes and reacting sites than the extracellular synthesis pathway. Hence, HePS have a greater variability in structure than HoPS. The Wzx/Wzy-dependent pathway consists of five main steps as shown in

Table 1
Main differences between homopolysaccharides (HoPS) and heteropolysaccharides (HePS) produced by lactic acid bacteria (LAB).

Item	HoPS	HePS	Reference
Type of monosaccharide	Contain one type of monosaccharide	Contain 3 to 8 types of monosaccharide	Rajoka et al. (2020); Lynch et al. (2018)
Main monosaccharide	Glucose or fructose	Glucose, galactose, and rhamnose	Angelin and Kavitha (2020)
Type of link	α or β link present	α and β links present	Rajoka et al. (2020); Lynch et al. (2018)
Structure	Typically linear or branched	Typically branched	Abarquero et al. (2022)
Molecular mass	greater than 10^6 Da	10^4 to 10^6 Da	Sanalibaba and Çakmak (2016); Angelin and Kavitha (2020)
Mainly produced genera	*Streptococcus, Lactobacillus, Oenococcus, Leuconostoc,* and *Weissella*	*Streptococcus, Lactobacillus, Lactococcus,* and *Bifidobacterium*	Rajoka et al. (2020); Lynch et al. (2018)
Biosynthesis precursors	Produced extracellularly from sucrose or starch	Produced from intracellular intermediates	Abarquero et al. (2022); Baruah and Goyal (2022)
Production level	Produced in relatively high amounts (g/L)	Produced in relatively low amounts (g/L)	Gerwig (2019); Nwodo et al. (2012)
Presence of noncarbohydrate groups	No noncarbohydrate groups present	Noncarbohydrate groups like acetyl or phosphates may be present	Baruah and Goyal (2022)
Charge	Typically carries no charge	Can contain charged groups	Abarquero et al. (2022); Lynch et al. (2018)
Health benefits	Primarily associated with prebiotic capacity	Primarily associated with immune modulation	Abarquero et al. (2022); Lynch et al. (2018)

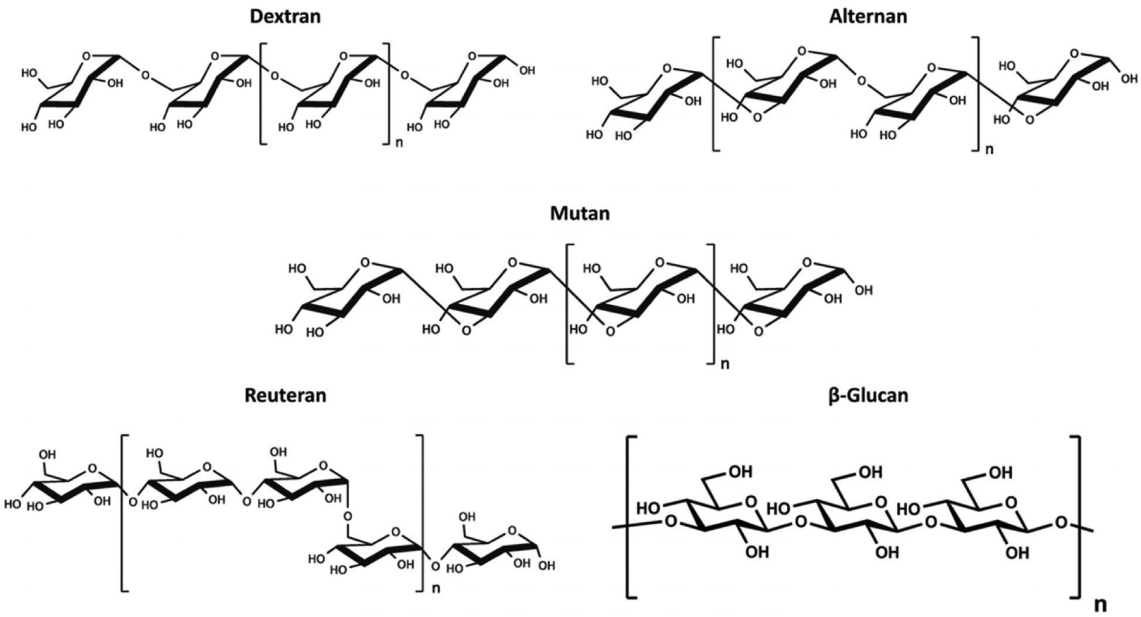

Fig. 2. Structures of the glucans produced by lactic acid bacteria. Glucans are classified into α-D-glucans and ß-D-glucans where α-D-glucans are subdivided into dextran, alternan, mutan and reuteran (Abarquero et al., 2022).

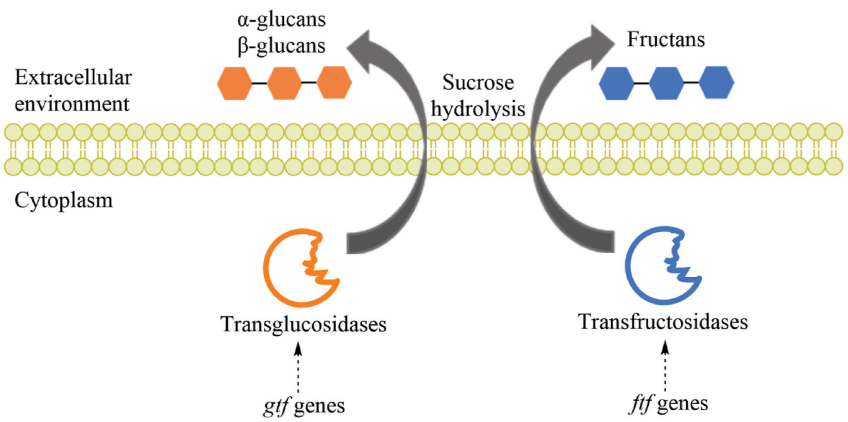

Fig. 3. The extracellular synthesis pathway for biosynthesis of homopolysaccharides (Wu et al., 2022).

Fig. 4 (Rajoka et al., 2020; Wu et al., 2022; Zhou et al., 2019b). The first step is transportation and phosphorylation of monosaccharide and disaccharides. In this step, the phosphotransferase system-assisted pathway and permease-assisted pathway are the two alternative pathways for importing monosaccharides or disaccharides (Rajoka et al., 2020; Zhou et al., 2019b). The second step is formation of sugar nucleotides. In this step, glucose-6-phosphate and galactose-1-phosphate are converted into glucose-1-phosphate with the aid of phosphoglucomutase and galactose-1-phosphate uridylytransferase. Next, various enzymes including UDP-galactose 4-epimerase, UDP-glucose pyrophosphorylase, dTDP-glucose pyrophosphorylase, dTDP-glucose 4,6-dehydratase or dTDP-4-dehydrorhamnose 3,5-epimerase mediate the transition from glucose-1-phosphate to UDP-glucose, UDP-galactose and dTDP-rhamnose (Laws et al., 2001; Zhou et al., 2019b). The third step is the synthesis of repeating units. Single repeating units, which are associated with an undecaprenol diphosphate anchor in the inner membrane surface, are continuously accumulated through the glycosyltransferases (Nachtigall et al., 2020; Nguyen et al., 2020; Schmid et al., 2015). Step four is the translocation of repeating units. In this step, the repeating units are transferred from the intracellular membrane to the outer membrane or periplasmic place with the assistance of flippase (Becker, 2015; Wu et al., 2022). Step five is the polymerization of repeating units by

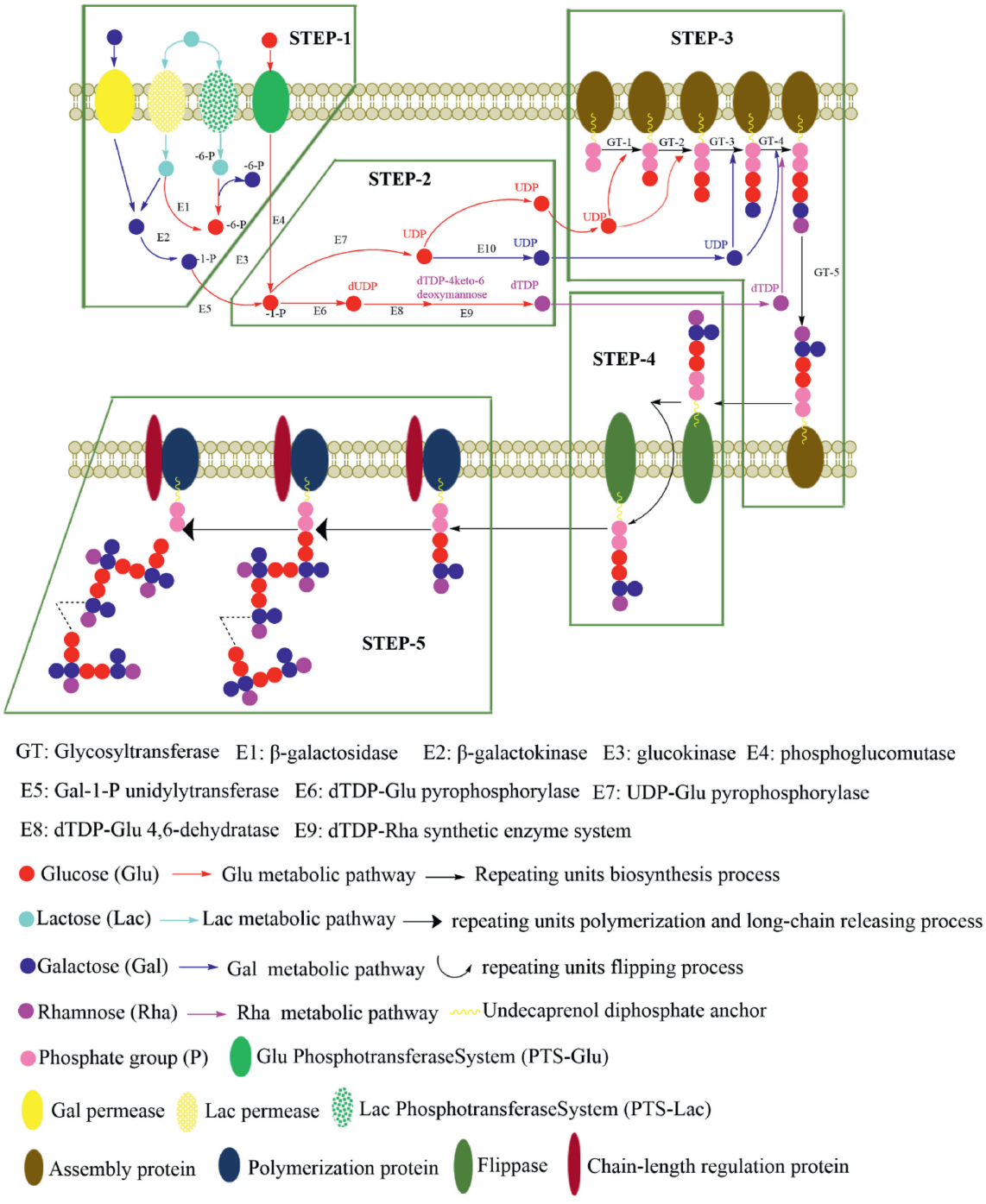

Fig. 4. The Wzx/Wzy-dependent pathway for biosynthesis of heteropolysaccharides (Wu et al., 2022).

an outer membrane polymerization protein, then the long polymer chains are exported into the extracellular space (Deo et al., 2019).

4. Regulation of intestinal health by LAB-EPS

4.1. Adhesion to intestinal epithelial cells

LAB-EPS have cross-linked three-dimensional network structures and film-forming properties, which may be used as bioactive agents such as adhesins in the feed industry (Almalki, 2020; Daba et al., 2021). EPS are important adhesins of LAB that positively affect their ability to colonise the intestinal epithelium. EPS are able to easily adhere to intestinal epithelial cells that allow LAB to colonise the gastrointestinal tract and prevent the rapid removal of LAB by intestinal peristalsis (Abarquero et al., 2022; Caggianiello et al., 2016). In addition, EPS play an important role in the formation of LAB biofilm, which facilitates colonization of the gastrointestinal tract by LAB and increases bacterial survival (Konieczna et al., 2018). It has been suggested that EPS increase the hydrophobicity of the LAB cell surface and promote bacterial adhesion to the intestinal mucosa (Dertli et al., 2015). The adhesion of LAB to the intestinal mucosa can impede the adhesion of pathogens and inhibit the growth of pathogenic intestinal bacteria due to the ability of LAB to competitively exclude enteropathogens (Werning et al., 2022). The in vitro and in vivo experimental models studying the potential roles of LAB-EPS in regulating intestinal health are listed in Table 2.

Recent studies have showed that the presence or absence of EPS and their composition influence the adhesion of *Lactobacillus rhamnosus* KL 53 A and *Lactobacillus casei* Fyos to enterocytes. Enzymatic deglycosylation of the EPS from *L. rhamnosus* KL 53 A decreased the adhesion efficiency of *L. rhamnosus* KL 53 A to enterocytes (Konieczna et al., 2018). Similarly, Fanning et al. (2012) reported that the EPS synthesized by *Bifidobacterium breve* UCC2003 decreased the colonization levels of the intestinal pathogen *Citrobacter rodentium* in mice. Furthermore, the EPS from *L. paracasei* subsp. *paracasei* BGSJ2 were found to reduce the adherence of *Escherichia coli* to Caco-2 cells (Živković et al., 2016). Two types of HePS with molecular mass of 13,600 Da and 41,200 Da were secreted by *L. paracasei* subsp. *paracasei* BGSJ2, which was able to inhibit the adhesion of *E. coli* to Caco-2 cells. However, a mutant *L. paracasei* subsp. *paracasei* EPS7, which cannot secret the low molecular mass HePS, did not show the anti-adhesion effects against *E. coli* (Živković et al., 2016). Therefore, only one of the EPS from *L. paracasei* subsp. *paracasei* BGSJ2 played an important role for the inhibition of *E. coli* adherence. Enterotoxigenic *E. coli* (ETEC) can adhere to porcine intestinal mucosa and deliver toxins and cause fluid loss, the most common cause of diarrhea in piglets. Chen et al. (2014) and Yang et al. (2015) found that the EPS originated from *L. reuteri* inhibited ETEC adhesion to porcine intestinal mucosa and reduced fluid loss induced by ETEC infection. These results agree with the findings from Kšonžeková et al. (2016) and Tkáčiková et al. (2020) who indicated the EPS produced by *L. reuteri* DSM 17938 and *L. reuteri* strain L26 Biocenol prevented adhesion of ETEC to the porcine intestinal epithelial cell line (IPEC-1), therefore inhibiting proinflammatory cytokine gene expression induced by ETEC infection. These results suggested the role of LAB-EPS as a prophylactic agent in ETEC-induced diarrhea in piglets and the use of LAB-EPS as a feed additive to prevent gastrointestinal infections.

4.2. Regulation of intestinal microbiota

Intestinal microbiota is an integral part of the animal body and has evolved together with the host (Rinttilä and Apajalahti, 2013). The intestinal microbial community changes dynamically and is able to regulate metabolic, neurological and immunological functions in the body, thus playing an important role in animal health (Adak and Khan, 2019; Nieuwdorp et al., 2014; Rieder et al., 2017). The intestinal microbiota is essential for the maintenance of physiological balance and nutrient metabolism as well as the development of the immune system (Purchiaroni et al., 2013; Wang et al., 2018b). As high molecular weight polysaccharides, LAB-EPS can be used as carbon and energy sources by intestinal microbiota to support the growth of beneficial bacteria and fight against pathogenic bacteria in the intestine (Chaisuwan et al., 2020). LAB-EPS can be degraded by intestinal microbiota into short-chain fatty acids, for example acetic, propionic and butyric acids, which are important to stimulate the immune system, maintain intestinal epithelial function, modulate epithelial growth and reduce the incidence of inflammatory and immune diseases (Tang et al., 2019).

Experiments performed by Ashfaq et al. (2020) showed that EPS not only increased the abundance of beneficial LAB but also reduced the abundance of pathogenic bacteria including *E. coli*, *Salmonella* and *Enterococcus* species in poultry intestines, suggesting that EPS have the potential to be used in poultry feed as alternatives to antibiotics for inhibiting the growth of intestinal pathogens. Xu et al. (2019a) reported the EPS from *L. buchneri* TCP016 ameliorated intestinal dysbiosis and lipopolysaccharide/D-galactosamine-induced liver damage by inhibiting the growth of Helicobacteraceae, Lachnospiraceae and Enterobacteriaceae, and enhancing the growth of *Lactobacillus*, Rikenellaceae, Bacteroidaceae, and Prevotellaceae in mice. Zhang et al. (2017) demonstrated that the EPS secreted by *L. plantarum* YW11 were able to decrease the abundance of *Flexispira* and support the growth of *Blautia* and *Butyricicoccus* in aging mice, thus improving gut microbiota and enhancing animal health. Li et al. (2014a) and Mahdhi et al. (2017) investigated in vitro antimicrobial activity of EPS from *B. bifidum* and *L. plantarum* and reported that LAB-EPS showed a concentration-dependent inhibitory effect against tested pathogens including *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Candida albicans*, *Bacillus cereus*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Cronobacter sakazakii* and *Shigella sonnei*. Mahdhi et al. (2017) demonstrated that the EPS from *L. plantarum* had the highest antibacterial activity against *P. aeruginosa* with a minimal inhibitory concentration (MIC) value of 1 mg/mL. Whereas, those from *S. typhimurium* and *S. aureus* were inhibited with a MIC of 2 mg/mL and *L. monocytogenes* was inhibited with a MIC of 10 mg/mL. Similarly, Jeong et al. (2017) found the EPS secreted by *L. kefiranofaciens* DN1 exhibited strong antibacterial activity against *L. monocytogenes* ATCC 51776 and *S. enteritidis* 108. Also, the EPS from *L. reuteri* SHA101 and *L. vaginalis* SHA110 exerted marked bactericidal effects against *E. coli* ATCC 25922, *S. typhimurium* CMCC (B) 50,115 and *S. petrasii* subsp. *pragensis* KY196531 in vitro (Rajoka et al., 2019).

In brief, LAB-EPS have exhibited significant inhibitory activity against pathogenic bacteria in the intestine. The EPS produced by some LAB strains such as *B. bifidum* and *L. plantarum* display strong broad-spectrum antibacterial properties against enteric bacteria. Therefore, EPS should have great potential application in the feed industry as an excellent replacement for antibiotics to modulate intestinal microbiota and inhibit pathogen colonization, hence improving animal health.

4.3. Restoration of intestinal barrier

A complex mucosal barrier serves as the first line of defence in the intestinal tract and is important for protecting the host against invading pathogens (Patten et al., 2014; Soenen et al., 2016). Numerous in vitro or in vivo studies in mice have demonstrated that LAB-EPS are able to restore impaired intestinal barrier function and thus enhance gastrointestinal health in livestock (Table 2). A

Table 2
In vitro and in vivo models to study the regulation of intestinal health by LAB-EPS.

In vitro model	Microorganism strain	Main sugar composition	Highlights	Reference
Caco-2 cells	*Lactobacillus rhamnosus* KL 53A *Lactobacillus casei* Fyos	Arabinose, glucose, galactose and maltose Glucose, galactose, arabinose and ribose	Enzymatic deglycosylation of *L. rhamnosus* KL 53A decreased the adhesion efficiency of *L. rhamnosus* KL 53 A to enterocytes	Konieczna et al. (2018)
Caco-2 cells	*Lactobacillus paracasei* subsp. *paracasei* BGSJ2 *Lactobacillus paracasei* subsp. *paracasei* EPS7	–	Only *L. paracasei* subsp. *paracasei* BGSJ2 was found to reduce the adherence of *Escherichia coli* to Caco-2 cells due to its high molecular mass heteropolysaccharides	Živković et al. (2016)
Jejunal segments from 5-week-old piglets	*Lactobacillus reuteri* TMW1.656 *Lactobacillus reuteri* LTH5794	α-D-glucans (reuteran) Fructose (levan)	Inhibiting enterotoxigenic *Escherichia coli* (ETEC) adhesion to intestinal mucosa and reducing fluid loss induced by ETEC infection	Chen et al. (2014)
IPEC-1 cells	*Lactobacillus reuteri* DSM 17938 *Lactobacillus reuteri* L26 Biocenol	Glucose	Inhibiting ETEC adhesion on IPEC-1 cells	Kšonžeková et al. (2016)
IPEC-1 cells	*Lactobacillus reuteri* L26 Biocenol	–	Inhibiting ETEC adhesion on IPEC-1 cells	Tkáčiková et al. (2020)
IPEC-J2 cells	*Lactobacillus rhamnosus* GG	–	Reducing the H_2O_2-induced oxidative damage in IPEC-J2 cells and increasing the survival rates of H_2O_2-damaged IPEC-J2 cells	Li et al. (2021)
Caco-2 cells	*Streptococcus thermophilus* MN-BM-A01	Rhamnose, glucose, galactose, and mannose	Enhancing the expression of tight junction proteins and restoring intestinal mucosal barrier function	Chen et al. (2019)
Cronobacter sakazakii, *Escherichia coli*, *Listeria monocytogenes*, *Staphyloccocus aureus*, *Candida albicans*, *Bacillus cereus*, *Salmonella typhimurium*, *Shigella sonnei*	*Bifidobacterium bifidum* WBIN03 *Lactobacillus plantarum* R315	– –	Exhibiting antibacterial activities against tested pathogens	Li et al. (2014a)
Staphylococcus aureus ATCC 25923, *Listeria monocytogenes* ATCC 19115, *Pseudomonas aeruginosa* ATCC 33787, *Salmonella typhimurium* ATCC 14028	*Lactobacillus plantarum* YW32	–	Exhibiting antibacterial activities against tested pathogens	Mahdhi et al. (2017)
Listeria monocytogenes ATCC 51776, *Salmonella enteritidis* 108	*Lactobacillus kefiranofaciens* DN1	Mannose, arabinose, glucose, galactose, and rhamnose	Exhibiting antibacterial activities against tested pathogens	Jeong et al. (2017)
Escherichia coli ATCC 25922, *Salmonella typhimurium* CMCC (B) 50,115, *Staphylococcus petrasii* subsp.*pragensis* KY196531	*Lactobacillus reuteri* SHA101 *Lactobacillus vaginalis* SHA110	–	Exhibiting antibacterial activities against tested pathogens	Rajoka et al. (2019)
Mice	*Bifidobacterium breve* UCC2003	–	Decreasing the colonization levels of the intestinal pathogen *Citrobacter rodentium*	Fanning et al. (2012)
Weaning piglet	*Lactobacillus reuteri* TMW1.656 *Lactobacillus reuteri* LTH5794	α-D-glucans (reuteran) Fructose (levan)	Reducing the colonization levels of ETEC in swine intestine	Yang et al. (2015)
Day old chick	*Leuconostoc fallax* *Bacillus paralicheniformis*	Glucose (dextran) Fructose (levan)	Increasing the abundance of lactic acid bacteria and reducing the abundance of *Escherichia coli*, *Salmonella* and *Enterococcus* species in poultry intestine	Ashfaq et al. (2020)
Mice	*Lactobacillus buchneri* TCP016	Rhamnose, glucosamine, galactose, glucose, xylose, galacturonic acid, glucuronic acid and mannose	Ameliorating intestinal mucosal injury, inhibiting the growth of Helicobacteraceae, Lachnospiraceae and Enterobacteriaceae, and enhancing the growth of *Lactobacillus*, Rikenellaceae, Bacteroidaceae, and Prevotellaceae	Xu et al. (2019a)
Mice	*Lactobacillus plantarum* YW11	–	Decreasing the abundance of *Flexispira* and supporting the growth of *Blautia* and *Butyricicoccus*	Zhang et al. (2017)
Mice	*Lactobacillus plantarum* NCU116	–	Alleviating the dextran sodium sulfate-induced colitis, enhancing the expression of tight junction proteins and improving intestinal epithelial barrier function	Zhou et al. (2018)

(*continued on next page*)

Table 2 (*continued*)

In vitro model	Microorganism strain	Main sugar composition	Highlights	Reference
Mice	*Streptococcus thermophilus* MN-BM-A01	Rhamnose, glucose, galactose, and mannose	Enhancing the expression of tight junction proteins and restoring intestinal mucosal barrier function	Chen et al. (2019)
Mice	*Lactobacillus helveticus* KLDS1.8701	–	Enhancing the expression of tight junction proteins and mucin, restoring intestinal mucosal barrier function	Liu et al. (2021)

cell culture study from Li et al. (2021) showed that the EPS produced by *L. rhamnosus* GG could reduce H_2O_2-induced oxidative damage in intestinal porcine epithelial (IPEC-J2) cells and significantly increased the survival rates of H_2O_2-damaged IPEC-J2 cells by up-regulating intracellular tight junction-related proteins. The lack of tight junctions can lead to an increase in the permeability of the intestinal barrier, which may facilitate the invasion of pathogens and potentially harmful antigens and therefore promote intestinal inflammation (Liu et al., 2021). Zhou et al. (2018) found the EPS from *L. plantarum* NCU116 could effectively alleviate dextran sodium sulfate-induced colitis in mice. In addition, EPS enhanced the expression of tight junction proteins and improved intestinal epithelial barrier function by activating signal transducer and activator of transcription 3 (STAT3) to bind to the promoter of zonula occludens protein-1 (ZO-1) and occludin. This resulted in an increased expression of ZO-1 and occludin in the intestinal epithelial cells. A similar result was found by Chen et al. (2019) on EPS from *S. thermophilus* MN-BM-A01, that the EPS could restore and maintain intestinal mucosal barrier function in vitro and in vivo in mice and enhance the expression of tight junction proteins such as claudin-1, occludin, and E-cadherin. These findings also agree with the previous study by Liu et al. (2021) who found the EPS produced by *L. helveticus* KLDS1.8701 significantly up-regulated the mRNA expression levels of claudins, occludin, ZO-1, and mucin proteins in mice, thus improving intestinal barrier function.

5. Regulation of the immune response by LAB-EPS

LAB-EPS can modulate systemic and mucosal immune responses in livestock, and in turn improve animal health (Laiño et al., 2016; Saadat et al., 2019). Many LAB and their EPS have been found to regulate the immune system by boosting the proliferation of T/B lymphocytes, natural killer (NK) cell tumoricidal activity, mitogenic activity and mononuclear cell phagocytic capacity, inducing cytokines, and therefore enhance the host immune response to pathogens (Angelin and Kavitha, 2020). LAB-EPS can affect the production of cytokines by macrophages, including tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), interleukin 2 (IL-2), interleukin 6 (IL-6), interleukin 10 (IL-10) and interleukin 12 (IL-12) (Saadat et al., 2019; Wang et al., 2018a; You et al., 2020). In addition, LAB-EPS are reported to induce the differentiation of dendritic cells, which in combination with the cytokines, can induce the naive T cells to differentiate into regulatory T cells, thus suppressing the excessive response of effector T cells and inducing immune homeostasis (Saadat et al., 2019). Furthermore, LAB-EPS can promote the secretion of host immunoglobulin A (IgA), which stimulates the mucosal immune system and strengthens the intestinal mucosal barrier (Wang et al., 2018a; Yılmaz and Şimşek, 2020). The in vitro and in vivo experimental models investigating the immunomodulatory potential of LAB-EPS are presented in Table 3.

Wang et al. (2018a) demonstrated that the EPS synthesized by *L. plantarum* JLK0142 could enhance immunomodulatory activity in cyclophosphamide-induced immunosuppressive mice by increasing the intestinal IgA content and promoting secretion of the cytokines TNF-α and IL-2. Nowak et al. (2020) investigated the influence of EPS produced by *L. rhamnosus* KL37 on T-cell functions and found that the *L. rhamnosus* EPS inhibited T-cell proliferation in vitro and in vivo. In addition, the *L. rhamnosus* EPS significantly reduced the secretion of interferon (IFN)-γ, suggesting the anti-inflammatory potential of EPS (Nowak et al., 2020). Ren et al. (2020) showed that the EPS from *L. casei* ATCC 393 could enhance the intestinal mucosa immunity in vitro and in vivo in mice by inducing the differentiation of CD4 T lymphocytes ($CD4^+T$) cells of Peyer's Patches into T-helper 17 (Th17) cells. The EPS produced by *B. breve* UCC2003 and JCM7017 strains were able to prevent maturation of dendritic cells (DCs) (Hickey et al., 2021). In addition, the EPS could inhibit the antigen-specific activation of $CD4^+T$ cells by DCs. These results indicated the *B. breve* EPS played an important role in immune evasion of adaptive immunity by *B. breve*, thus promoting host-microbe mutualism (Hickey et al., 2021).

You et al. (2020) and Xiu et al. (2020) showed that the EPS secreted from *L. helveticus* LZ-R-5 and *L. kiferi* WXD029 had potent immunomodulatory activity by promoting proliferation of RAW264.7 macrophages and production of nitric oxide and cytokines (TNF-α, IL-6, IL-1β and IL-10) in macrophages. In addition, these LAB-EPS could enhance macrophage phagocytosis and increase acid phosphatase activity, which might be used as a potential immunomodulatory agent. Zhu et al. (2019) indicated that the EPS produced by *L. plantarum* RS20D could stimulate nitric oxide release in macrophage RAW264.7 in vitro and increase the mRNA expression of cytokines including inducible nitric oxide synthetase (iNOS), TNF-α, and IL-6.

Dinić et al. (2018) found that the EPS synthesized by *L. paraplantarum* BGCG11 could reduce inflammatory pain and paw swelling in rats by reducing the mRNA expression of pro-inflammatory cytokines IL-1β and iNOS and increase the expression of anti-inflammatory cytokines IL-6 and IL-10 in rat's paw tissue. These results demonstrated the analgesic and anti-edematous potential of LAB-EPS due to the suppression of the inflammatory response. Chen et al. (2019) reported that the EPS from *S. thermophilus* MN-BM-A01 could significantly alleviate acute colitis in mice by reducing pro-inflammatory cytokine secretion (IFN-γ, IL-6, and TNF-α). Also, Liu et al. (2021) showed that the EPS from *L. helveticus* KLDS1.8701 had good anti-colitic effects by decreasing the levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and increasing the levels of anti-inflammatory cytokines (IL-10). Kšonžeková et al. (2016) found the EPS from *L. reuteristrain* DSM 17938 and *L. reuteri* L26 Biocenol have potential as a prophylactic agent for gastrointestinal infections and reduced ETEC-induced gene expression of proinflammatory cytokines IL-1β and IL-6. According to the previous study of Kanmani et al. (2018) on EPS from *L. delbrueckii* OLL1073R-1, LAB-EPS also could protect against intestinal viruses, including rotavirus, and promote the intestinal innate immune response. The *L. delbrueckii* EPS was able to modulate the innate antiviral response triggered by TLR3 activation

Table 3
In vitro and in vivo models to study the immunomodulatory activity and anti-biofilm property of exopolysaccharides from lactic acid bacteria (LAB-EPS).

In vitro model	Microorganism strain	Main sugar composition	Highlights	Reference
Lymph node or spleen cells of mice	*Lactobacillus rhamnosus* KL37	–	Inhibiting T-cell proliferation	Nowak et al. (2020)
Marrow cells of mice	*Lactobacillus casei* ATCC 393	Galactose, glucose, mannose, xylose, arabinose, rhamnose, and ribose	Promoting the differentiation of $CD4^+$ T cells into Th17 cells	Ren et al. (2020)
Murine macrophage cell line RAW264.7	*Lactobacillus helveticus* LZ-R-5	Glucose and galactose	Promoting proliferation of RAW264.7 macrophages and production of nitric oxide and cytokines (TNF-α, IL-6, IL-1β and IL-10) in macrophages, enhancing phagocytosis and acid phosphatase activity	You et al. (2020)
Murine macrophage cell line RAW264.7	*Lactobacillus kiferi* WXD029	Glucose, galactosamine and glucosamine	Promoting the proliferation and phagocytic activity, enhancing the production of nitric oxide and TNF-α, IL-6 and IL-1β in macrophages	Xiu et al. (2020)
Murine macrophage cell line RAW264.7	*Lactobacillus plantarum* RS20D	Glucose, galactose and glucosamine	Stimulating nitric oxide release on macrophage and increasing the expression of cytokines (iNOS, TNF-α, and IL-6)	Zhu et al. (2019)
IPEC-1 cells	*Lactobacillus reuteri* DSM 17938 *Lactobacillus reuteri* L26 Biocenol	Glucose Glucose	Reducing the ETEC-induced gene expression of proinflammatory cytokines IL-1β and IL-6	Kšonžeková et al. (2016)
Porcine intestinal epithelial cells	*Lactobacillus delbrueckii* OLL1073R-1	–	Modulating the innate antiviral response triggered by TLR3 activation, upregulating the expression of IFN-α and IFN-β and the antiviral factors MxA and RNase L	Kanmani et al. (2018)
Head kidney cells of fish	*Lactococcus lactis* Q-9	–	Promoting proliferation and phagocytosis, increasing the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), anti-inflammatory cytokines (IL-10, TGF-β) and nitic oxide	Feng et al. (2020a)
Head kidney cells of fish	*Lactococcus lactis* Z-2	Rhamnose, xylose, mannose, glucose and galactose	Promoting proliferation and phagocytosis, increasing the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), anti-inflammatory cytokines (IL-10, TGF-β) and nitic oxide	Feng et al. (2020b)
Bacillus cereus RSKK 863, *Listeria monocytogenes* ATCC 7644, *Enterococcus fecalis* ATCC 25175, *Pseudomonas aeruginosa* ATCC 72853	*Lactobacillus fermentum* LB-69 *Lactobacillus rhamnosus* GD-11 *Lactobacillus delbrueckii* ssp. *bulgaricus* B-3	–	Exhibiting anti-biofilm activities against tested pathogens	Sarikaya et al. (2017)
Staphylococcus aureus ATCC 25923, *Listeria monocytogenes* ATCC 19115, *Pseudomonas aeruginosa* ATCC 33787, *Salmonella typhimurium* ATCC 14028	*Lactobacillus plantarum*	–	Exhibiting anti-biofilm activities against tested pathogens	Mahdhi et al. (2017)
Escherichia coli O157, *Shigella flexneri* CMCC (B), *Staphylococcus aureus* AC1, *Salmonella typhimurium* S50333	*Lactobacillus plantarum* YW32	Mannose, fructose, galactose and glucose	Exhibiting anti-biofilm activities against tested pathogens	Wang et al. (2015)
Bacillus cereus, Escherichia coli O157:H7, *Salmonella typhimurium*, *Staphylococcus aureus*	*Lactobacillus casei* NA-2	–	Exhibiting anti-biofilm activities against tested pathogens	Xu (2021)
Pseudomonas aeruginosa CMCC10104, *Escherichia coli* O157:H7, *Salmonella Typhimurium* ATCC13311, *Staphylococcus aureus* CMCC26003	*Lactobacillus plantarum* WLPL04	Xylose, glucose and galactose	Exhibiting anti-biofilm activities against tested pathogens	Liu et al. (2017)
Mice	*Lactobacillus plantarum* JLK0142	Glucose and galactose	Increasing the intestinal IgA content and promoting secretion of the cytokines TNF-α and IL-2.	Wang et al. (2018a)
Mice	*Lactobacillus rhamnosus* KL37	–	Inhibiting T-cell proliferation and reducing the secretion of IFN-γ	Nowak et al. (2020)

(*continued on next page*)

Table 3 (*continued*)

In vitro model	Microorganism strain	Main sugar composition	Highlights	Reference
Mice	*Lactobacillus casei* ATCC 393	Galactose, glucose, mannose, xylose, arabinose, rhamnose, and ribose	Promoting the differentiation of $CD4^+$ T cells into Th17 cells	Ren et al. (2020)
Mice	*Bifidobacterium breve* UCC2003 *Bifidobacterium breve* JCM7017	–	Preventing maturation of DCs, inhibiting the antigen-specific activation of $CD4^+$T cells by DCs	Hickey et al. (2021)
Rats	*Lactobacillus paraplantarum* BGCG11	–	Reducing the expression of pro-inflammatory cytokines IL-1β and iNOS and increasing the expression of anti-inflammatory cytokines IL-6 and IL-10 in rats' paw tissue	Dinić et al. (2018)
Mice	*Streptococcus thermophilus* MN-BM-A01	Rhamnose, glucose, galactose, and mannose	Reducing the pro-inflammatory cytokine secretion (IFN-γ, IL-6, and TNF-α)	Chen et al. (2019)
Mice	*Lactobacillus helveticus* KLDS1.8701	–	Decreasing the levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and increasing the levels of anti-inflammatory cytokines (IL-10)	Liu et al. (2021)
Fish	*Lactococcus lactis* Q-9	–	Increasing the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), anti-inflammatory cytokines (IL-10, TGF-β) and nitic oxide	Feng et al. (2020a)
Fish	*Lactococcus lactis* Z-2	Rhamnose, xylose, mannose, glucose, and galactose	Increasing the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), anti-inflammatory cytokines (IL-10, TGF-β) and nitic oxide	Feng et al. (2020b)

in porcine intestinal epithelial cells. The expression of IFN-α and IFN-β in porcine intestinal epithelial cells and the expression of the antiviral factors MxA and RNase L were upregulated by treatment with *L. delbrueckii* EPS (Kanmani et al., 2018). Feng et al. (2020a) and Feng et al. (2020b) reported that the EPS from *L. lactis* Z-2 and *L. lactis* Q-9 had immunomodulatory effects on common carp. These *L. lactis* EPS could not only promote proliferation and phagocytosis in head kidney cells, but also increase the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), anti-inflammatory cytokines IL-10 and transforming growth factor β (TGF-β) and nitic oxide.

In brief, several in vitro and in vivo studies clearly demonstrated immunomodulatory and beneficial health effects of LAB-EPS. LAB-EPS can regulate the immune system by stimulating lymphocyte proliferation, enhancing macrophage phagocytosis and modulating gene expression of nitric oxide and different cytokines including TNF-α, IL-1β, IL-6 and IL-10. The existing literature suggests that LAB-EPS can be used as an immunomodulatory and anti-inflammatory agent and may be added to animal diets as supplements to improve animal health.

6. Anti-biofilm property of LAB-EPS

Biofilms are complex bacterial communities embedded in an extracellular matrix, which consist of polysaccharides, proteins and nucleic acids. Bacterial biofilms cause chronic and recurrent microbial infections because they protect bacteria against the host immune response and environmental stresses, and increase tolerance to antibiotics and antimicrobial agents (Hooshdar et al., 2020). Biofilms play a crucial role in pathogenesis as approximately 80% of microbial infections are associated with biofilm. Microorganisms growing in a biofilm have 10 to 1,000 times more antibiotic resistance compared with their planktonic counterparts (Sharma et al., 2019). Most bacterial pathogens in the livestock body can form biofilms. However, the use of antibiotics, such as colistin and imipenem, can only reduce the biofilms but is unable to eliminate the biofilm entirely (Roy et al., 2018). Therefore, it is highly important to find suitable alternatives to antibiotics for treating biofilm-related infections in livestock and thus improve animal health and production efficiency. Many studies have reported that LAB-EPS can reduce or inhibit biofilm formation by a broad range of Gram-positive and Gram-negative pathogens, suggesting the potential application of LAB-EPS in the treatments of biofilm-associated infections (Angelin and Kavitha, 2020; Li et al., 2014b; Werning et al., 2022).

The study of Sarikaya et al. (2017) demonstrated the anti-biofilm effects of LAB-EPS against four pathogenic bacteria including *B. cereus* RSKK 863, *L. monocytogenes* ATCC 7644, *Enterococcus fecalis* ATCC 25175, and *P. aeruginosa* ATCC 72853. The EPS of *L. fermentum* LB-69, *L. rhamnosus* GD-11 and *L. delbrueckii* ssp. *bulgaricus* B-3 isolated from children's feces and milk products displayed significant biofilm inhibition with increasing concentration of EPS (Sarikaya et al., 2017). These results are similar to the findings of Mahdhi et al. (2017) and Wang et al. (2015) on EPS from *L. plantarum*, which indicated the anti-biofilm effects of the EPS were dose dependent. The development of biofilms of *S. aureus* ATCC 25923, *S. aureus* AC1, *E. coli* O157, *Shigella flexneri* CMCC, and *S. typhimurium* S50333 was dramatically inhibited by the EPS. In addition, the *L. plantarum* EPS was effective on the biofilm produced by *P. aeruginosa* ATCC 33787, *L. monocytogenes* ATCC 19115 and *S. typhimurium* ATCC 14028 where more than 50% of their biofilms were eliminated (Mahdhi et al., 2017). When Xu (2021) investigated the anti-biofilm effects of the EPS from *L. casei* NA-2 by using fluorescence spectrophotometry, the results suggested that the *L. casei* EPS exhibited strong inhibitory activities against biofilm formation by *B. cereus*, *E. coli* O157:H7, *S. typhimurium* and *S. aureus*. These findings agree with a previous study from Liu et al. (2017) on the EPS produced by *L. plantarum* WLPL04 in that the EPS could largely reduce the bacterial biofilm formation by *E. coli* O157:H7, *S. aureus* CMCC26003, *S. typhimurium* ATCC13311 and *P. aeruginosa* CMCC10104. LAB-EPS reduced the initial attachment and auto-aggregation of pathogenic bacteria cells by weakening cell surface modifications and/or inhibiting cell-to-cell surface interactions. Furthermore, they could act as signalling molecules to

inhibit gene expression related to biofilm formation, and therefore prevent or eradicate biofilm-related infections (Werning et al., 2022).

Existing literature has demonstrated the biofilm inhibition and eradication activity of LAB-EPS against bacterial pathogens and supported the potential of LAB-EPS as an antimicrobial natural ingredient for livestock feed. However, no LAB-EPS have displayed a broad-spectrum biofilm inhibition thus far. Further research should be conducted to discover LAB-EPS with broad-spectrum biofilm inhibition.

7. General discussion

After antibiotics are banned as feed additives, it will be important to find alternatives to antibiotics for protecting animal health and enhancing livestock production efficiency. LAB-EPS are biodegradable, nontoxic and bio-compatible and have the potential to be natural and safe alternatives to antibiotics. LAB-EPS can improve intestinal health by adhering to intestinal epithelial cells, regulating intestinal microbiota and restoring impaired intestinal barrier function. In addition, LAB-EPS have exhibited multiple biological properties including anti-inflammatory activity, anti-biofilm properties and antimicrobial activity. They are able to regulate the immune system and enhance immunomodulatory activity. Also, LAB-EPS can reduce or inhibit biofilm formation by bacterial pathogens and hence prevent or eradicate biofilm-related infections. However, the EPS secreted by different LAB strains are varied in their structural composition, molecular weight, chain length, spatial arrangements and thus show significant differences in their biological activities and physiological functions. The relationship between the function and structure of LAB-EPS has not been completely understood due to their complex structure and diverse biological activities. The investigations on the mechanisms of antibacterial activity and immunomodulatory activity of LAB-EPS are also at a preliminary stage. So far, the research on the application of LAB-EPS is mainly focused on the food industry. There are only a few studies on the application of LAB-EPS in livestock and most of them are in vitro or in vivo studies in mice.

8. Conclusions

LAB-EPS are abundantly present in natural sources as metabolic by-products of many LAB strains. There is potential for LAB-EPS to replace conventional antibiotics in the feed industry due to their biological properties and physiological functions. Future research should be conducted to investigate factors influencing LAB-EPS absorption and metabolism in different livestock species. In addition, a simple and efficient procedure for commercial scale production of LAB-EPS is needed to improve yield and reduce production costs of LAB-EPS.

Author contributions

Shuo Yang: drafting the original manuscript, writing, reviewing, and editing. **Xiaoqing Xu, Qing Peng** and **Lan Ma:** software, resources, reviewing and editing. **Yu Qiao** and **Bo Shi:** reviewing, editing, providing critical feedback, approving the final version of the manuscript to be published.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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