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Synbiotics: a New Route of Self-production and Applications to Human and Animal Health

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Abstract
Synbiotics are preparations in which prebiotics are added to probiotics to achieve superior performance and benefits on the host. A new route of their formation is to induce the prebiotic biosynthesis within the probiotic for synbiotic self-production or autologous synbiotics. The aim of this review paper is first to overview the basic concept and (updated) definitions of synergistic synbiotics, and then to focus particularly on the prebiotic properties of probiotic wall components while describing the environmental factors/stresses that stimulate autologous synbiotics, that is, the biosynthesis of prebiotic-forming microcapsule by probiotic bacteria, and finally to present some of their applications to human and animal health.

Keywords Environmental stress · Exopolysaccharides · Probiotics · Prebiotics · Synbiotics

Introduction

Synbiotics are "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host." Two types of synbiotics have to be distinguished: (a) complementary synbiotics, which consist of a probiotic combining with an independently active prebiotic, and (b) synergistic synbiotics, in which the substrate is designed to be used selectively by the co-administered microorganism [1]. The synergistic effect of synbiotics is demonstrated by inhibiting the growth of pathogenic bacteria [2] and promoting the growth of beneficial organisms [3]. The term "probiotic" is designated for bacteria as well as some yeasts that can live until reaching the gut, and have beneficial effects on the host health. Among the microorganisms considered probiotics, lactic acid bacteria (LABs) are the most common probiotics known to have beneficial effects on the gastrointestinal tract [4]. Prebiotics are a group of non-digested substrates selectively utilized by host microorganisms conferring a health benefit [5]. Initially, it mainly consists of carbohydrate-based substances such as of fructans, galactans, beta-glucans, and exopolysaccharides (EPSs), leading to the formation and regulation of the host gut microbiota [6, 7]. However, substances such as polyphenols and polyunsaturated fatty acids converted to respective conjugated fatty acids may be considered prebiotics when there is an adequate evidence of their health benefits for the target host, according to the updated definition.

To improve host health through the beneficial activity of bacteria, it must be ensured that probiotic cell survival in any type of formulation should achieve a certain density depending on the expected dose–response effects for each strain [8, 9]. However, for ease of use, the probiotic ingredients are usually in a dried form. During the production, storage, and powder digestion, the bacteria may experience a variety of stresses, which can affect their survival and beneficial effects [10]. Importantly, ensuring the survival of probiotics needs to be considered when they were transported through the harsh acidic environment of the stomach to reach the target site, hence allowing adequate colonization and proliferation [11]. Protecting probiotics into macromolecular

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microcapsules successfully help them to survive from the harsh [12] and changing conditions of the gastrointestinal tract [13, 14]. The microencapsulation technique also stabilizes probiotics during storage at various temperatures and can significantly extend the cell shelf life [15–17].

It has been proven that probiotic strains such as Lactobacilli, propionibacteria, and bifidobacteria experience membrane injury under various stresses [18], such cell membrane acting as a barrier against adverse environmental conditions. In response to these challenges, bacteria are able to adopt various mechanisms. These include internal changes expressed by overexpression of molecular chaperones as well as the synthesis of stress-resistant proteins, and extrinsic changes through enhancing the synthesis of cell wall components such as membrane lipids, peptidoglycans (PGs), S-layer proteins, and EPSs [10]. Numerous studies have indicated that probiotic bacteria enhance the synthesis of EPSs, forming a protective envelope around the cells, so-called capsules, under environmental challenges [19, 20].

The current review outlines, on one hand, the basic concept of synbiotics and their various applications, and on the other hand, the prebiotic properties of probiotic wall components. A particular attention will be focused on the potential use of environmental stresses stimulating autologous synbiotics, that is, the biosynthesis of prebiotic-forming microcapsule by probiotic bacteria.

Synbiotic Composition and Definitions

Basically, synbiotics are composed by probiotics and prebiotics in the same preparation [2]. Probiotics are live microorganisms including bacteria and yeast that have been shown to have beneficial effects on the host health [21, 22] and gastrointestinal function [22], and may contain one or more selected strains. *Bacillus*, *Enterococcus*, *Lactobacillus*, *Pediococcus*, and *Streptococcus* as well as some fungi and yeast strains such as *Saccharomyces cerevisiae* and *Kluyveromyces* are various examples of microbial genera recognized as probiotics [22]. Prebiotics are a group of nutrients capable of stimulating the growth of probiotic bacteria [23]. Various compounds which have been functionally identified as prebiotics are fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), trans-galacto-oligosaccharides, short-chain fatty acids, peptidoglycans [23], and EPSs [24]. Previously, synbiotics were simply a combination of probiotics and prebiotics [3, 25] and required that each independently provides health benefits, which are dependent on the dose of each component. [5]. However, a more general definition has been given by the International Scientific Association for Probiotics and Prebiotics (ISAPP), which defines synbiotics as "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host." According to this formula, the microbial composition is not necessarily an independent probiotic, and the non-digestible substrate is not necessarily an independent prebiotic, but if they confer a health benefit, the mixture can be called a synergistic synbiotic [26].

Synbiotic formulation simply includes two main components of a living microorganism and a certain substrate (Fig. 1). The combination of these ingredients into a synbiotic will provide better health benefits than the individual ingredients. The next section treats the mechanism of action of such a combination.

Synergistic Synbiotics

The synergistic effect of probiotics and prebiotics in synbiotics confers host health benefits. For complementary synbiotics, the probiotic and prebiotic ingredients can act independently and must meet minimum dosage criteria to achieve one or more health benefits [26]. However, both prebiotics and probiotics function optimally when they are combined. These synergistic benefits enhance the therapeutic and nutritional value of products containing these components [27, 28]. Therefore, prebiotics should be comprehensively characterized to evaluate not only their fermentability, but also their influences on probiotic properties likes adherence, because enhanced adhesion can prolong the residence time of bacteria in the gastrointestinal tract [29]. In meanwhile, probiotics confer positive effects on health by impacting the resident microbiota, intestinal epithelium cells, and the host immune system [30]. In addition, probiotics can use prebiotics as a source of nutrients, helping them stay longer in the gut [31]. This probiotic higher viability facilitates the delivery of the expected health benefits [27, 32]. Thus, the combination of both probiotic and prebiotic ingredients in a product will ensure superior efficacy compared to using them independently [33].

For synergistic synbiotics, substrates are designed for selective use by co-administered microorganisms, whereas live microorganisms are selected based on their ability to provide health benefits and to support the growth as well as activity of selected microorganisms [27]. Although the substrate may also enrich other beneficial members of the gastrointestinal microbiota, its primary target is the ingested microorganisms [27]. However, designing and demonstrating the efficacy of a synergistic synbiotic is an experimental challenge. Therefore, many of the commercial synbiotics used in clinical trials and nearly all synbiotics used in commercially available clinical trials are mostly in complementary synbiotics [34]. The mechanism of action of synbiotics can be described in Fig. 2.

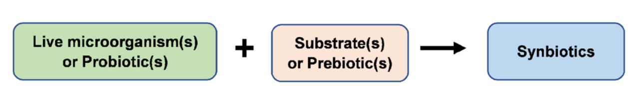

Fig. 1 The formulation of a synbiotic

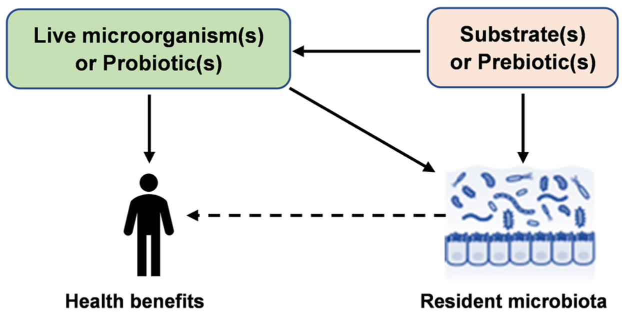

Fig. 2 Mechanism of action of synbiotics

When combining as a synbiotic, prebiotics play a role in improving the survival of probiotics [35]. It is not surprising that the components involved in the construction of the cell wall also have a similar function, contributing to the enhancement of the probiotic properties of beneficial bacteria. In addition, some ingredients such as EPSs have been proven to exhibit prebiotic activities [24].

Probiotic Bacterial Cell Wall Containing Prebiotic Components

LAB is the most common group of probiotic bacteria [4]. The cell wall of LAB is composed of a thick PG sacculus (multi-layered) that surrounds the cytoplasmic membrane and is embedded with teichoic acids, lipoteichoic acids, proteins, and polysaccharides [36] (Fig. 3). Each cell surface macromolecule impacts the probiotic activity of LAB because it is involved in the interaction between bacteria and the host [37]. The PG layer is an essential component which protects cell integrity and resists lysis [38, 39]. In addition, other cell wall components such as teichoic acids, lipoteichoic acids, S-layer proteins, and polysaccharides are non- or covalently bound to PGs which serve as a permanent framework for these components [38]. The chemical structure of PGs consists of glycan chains interspersed with N-acetylglucosamine and N-acetylmuramic acid linked via β-1.4 linkage. The peptide chain is covalently linked via the N-terminus to the lactyl group of N-acetylmuramic [36]. The negatively charged polymers covalently bonded to PGs were identified as teichoic acids, or directly attached to the cytoplasmic membrane were identified as lipoteichoic acids (LTAs) [36].

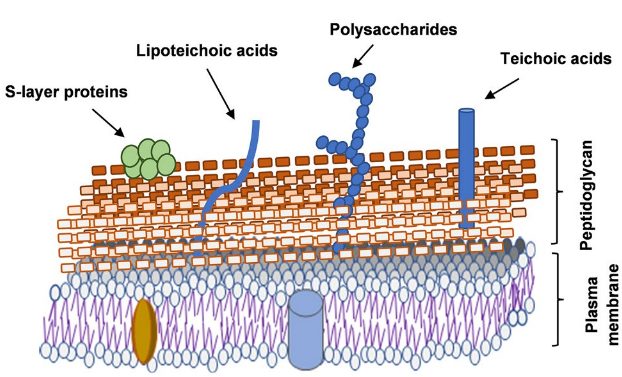

Fig. 3 Structure of the probiotic cell wall

The basic structure of teichoic acids (TAs) consists of repeating units of polyglycerol phosphate or polyribitol phosphate depending on various conditions such as species, stage or growth rate, pH of the medium, carbon source, and the presence of phosphate that the structure and abundance of this polymer can be different [40, 41]. Different roles are assigned to TAs, at least concerning their anionic properties or their distribution in the bacterial cell wall. TAs provide a reservoir of ions close to the cell wall that may be necessary for enzymes to function properly. Due to their anionic properties, TAs can bind both cations, such as Mg^{2+} and protons, thereby creating a pH gradient across the cell wall. TAs and their substitutes are crucial for the control of autolysis in certain species of Gram-positive bacteria [41]. LTAs were originally considered autolysin inhibitors. By determining the number of binding sites for autolysin cations, their D-alanylation level has also been proposed as a means of regulating autolysation [41]. LTAs appear to play a prominent role in host-Lactobacilli interactions [42]. LTAs have been reported to be essential for the adhesion of *Lactobacillus johnsonii* La1 to human intestinal epithelial cells (Caco-2), possibly through hydrophobic interactions [43].

Another important component of the LAB cell wall is surface proteins, which can be large or small, and consist of repeat domains or discrete domains [39]. One of the important surface proteins called the S-layer is tightly bound to PGs [40]. The surface proteins of probiotic or commensal bacteria are thought to facilitate the colonization and persistence of mucosa in the gastrointestinal tract. It has been suggested that the S-layer proteins may be involved in the adhesion properties of LAB to the intestinal epithelium and other extracellular complex components [44, 45].

Finally, the cell wall surface of probiotics contains polysaccharides [39]. These polysaccharides can covalently bind to PGs called capsule polysaccharides or secrete directly into the external environment called exopolysaccharides; they are sometimes collectively named EPSs [39]. Several roles have been assigned to EPSs in LAB such as in bacterial-host interactions. EPSs are required for normal cell morphology and play a role in cell division [46]. In addition, EPSs are also involved in a wide range of bacterial properties and functions, including adhesion to abiotic surfaces and biofilm formation [36]. EPSs have also been shown to protect *Lactococcus lactis* against macrophage phagocytosis [46]. A *Lacticaseibacillus casei* Shirota mutant synthesizing lower levels of high-molecular-weight EPSs produced higher levels of cytokines IL6, IL10, and IL12 after being co-incubated with mouse macrophages in vitro. These results highlight the immunosuppressive function of EPSs [47]. The monosaccharide composition in EPSs influences their protective efficacy. The galactose-rich EPSs of *Lacticaseibacillus rhamnosus* GG protect against host innate defense molecules, such as the antimicrobial peptide LL-37 [48].

Environmental Stress Factors Enhancing the Prebiotic Self-Producing Probiotics

In response to extreme environmental conditions, probiotics can strengthen their cell wall by enhancing the synthesis of S-layer proteins, peptidoglycans, and EPSs. As a result, the cell wall becomes thicker forming a protective microencapsulation. The following reviews will be more specific about the effects of environmental stresses on cellular mechanisms for improving survival.

The Synthesis of Internal Stress-Resisting Factors

Probiotics LAB can survive at high temperatures from 45 to 80 °C [49]. Grujović et al. reported that *Limosilactobacillus fermentum* (KGPMF28 and KGPMF2) was capable of growing at 45 °C for 24 h [50]. The viability of LAB at high temperatures is a very important criterion for the selection of LAB species as starter cultures and probiotics. At high temperatures, biomolecules such as proteins and nucleic acids can be degraded and lost their function, leading to the inhibition of metabolism [51]. High temperatures can also increase the fluidity of cell membranes, thereby disrupting cellular activities [52]. To avoid denaturation and degradation, LAB have multiple adaptive mechanisms including increased production of specific proteins [53]. These proteins include heat shock proteins, the chaperone protein DnaK prolyl-tRNA synthetase, chaperonins (GroEL), and cofactors (GroES) that play important roles in promoting the correct folding and subsequent translocation of newly synthesized polypeptides [54]. In addition, under heat stress conditions, LAB increase the synthesis of saturated and straight-chain fatty acids, providing the appropriate amount of fluidity required for membrane functions [55]. The expression of DNA-binding proteins is another way to protect biomolecules like DNA which is through the expression of DNA-binding proteins [56].

The ability of probiotics to maintain viability in cold is vital due to most commercial probiotic strains be supplied as lyophilized powders [57]. The viability of probiotic LAB during freeze-drying and storage before consumption is a determinant of their probiotic properties [58]. LAB cope with the effects of low temperatures by creating antifreeze and cold shock protein that ameliorate the harmful effects associated with cold environments [59]. LAB are known to be capable of synthesizing cold-adapted enzymes to remain active at freezing temperatures and support both transcription and translation [60]. Some LABs also produce anticoagulant proteins which bind to ice crystals to prevent them from penetrating cells [61].

Strengthening acid tolerance is crucial to promoting LAB survival and therefore ensures the quality and functionality of probiotics products. Acidity is one of the important barriers that LAB need to deal with to survive the passage from the stomach to the intestines. Probiotic LAB can experience extreme acid stress conditions in the stomach due to the presence of hydrochloric acid. However, some LAB are equipped with mechanisms that allow them to survive at low pH conditions [62]. Consequently, to qualify as a probiotic, LAB must have the ability to survive under the pH conditions of the gastrointestinal tract [63]. It is fortunate that LAB are equipped with molecules to protect against cell damage and improve tolerance to the harmful external environment [64, 65]. One such protective molecule secreted by LAB during fermentation is a proton-translocating ATPase [66], which stabilizes the pH inside the cell in response to a low external pH [67].

Under alkaline conditions, LABs regulate their intracellular pH by alkalizing the cytoplasm [68]. Zhang et al. proved that K^+ and Na^+ proton antagonists lower cytoplasmic pH undergrowth in alkaline conditions [69]. K^+ ions are required for LAB protection under alkaline pH because the expression of soluble shock proteins is activated by K^+ [70].

Probiotic LAB are often subject to osmotic pressure causing dehydration. To tolerate such changes, probiotics have developed systems to protect against osmotic stress. During growth in a highly osmotic medium, LABs regulate their intracellular osmolarity to maintain osmotic balance with the outside. Probiotic bacteria activate specific mechanisms such as K^+ or compatible solute uptake/synthesis to prevent cell death in media with high salt concentrations. Probiotic bacteria also produce protective molecules (mainly proteins), such as the operon proteins DnaK and HtrS, protecting cells from salt-induced damage [71].

S-Layer Proteins

Bacteria are surrounded by extracellular polymeric substances such as EPSs and proteins, which allow bacteria to exist with their different physicochemical states of modes of organization [72]. The surface properties of probiotic LABs are related to their ability to adhere to the gastrointestinal epithelium, a condition considered a prerequisite for the exclusion of enteric pathogenic bacteria [73, 74] and the regulation of host immunity [75]. Several species of *Lactobacillus* including mucosa-associated species such as *Lactobacillus crispatus, Lactobacillus acidophilus*, and *Lactobacillus gallinarum* as well as species related to milk fermentation such as *Lactobacillus kefiranofaciens* and *Lactobacillus helveticus* can form S-layer proteins which participate in the outermost structure of the cell envelope. These S-layer proteins are involved in critical cell functionalities such as maintaining cell shape, controlling the transfer of nutrients and metabolites, promoting cell adhesion, and acting as a protective barrier against adverse environments [76]. In some species of *Lactobacillus*, S-layer proteins mediate bacterial attachment to the extracellular matrix or the host cells [77]. There is evidence that bacteria can express alternative S-layer protein genes in response to different stresses, for example, the host immune response to pathogens dramatic changes in environmental conditions for non-pathogenic agents [78, 79].

It has been suggested that the surface properties of bacteria depend on the growth conditions and the composition of the culture medium [80]. A recent study showed that the probiotic strain *Lactiplantibacillus plantarum* 299v in the human intestine specifically regulates its metabolic capacity to acquire carbohydrates, synthesize EPSs, and express surface proteins [81]. Certain stressful conditions can also induce S-layer proteins by *L. acidophilus* IBB 801, presumably helping to increase the viability of this strain under adverse culture conditions. Proteomic studies have provided information on proteome changes when *L. acidophilus* IBB 801 is subjected to thermal stress [82]. The role of S-layer proteins in the adaptation of *L. acidophilus* ATCC 4356 to high salt-induced osmotic stress was also demonstrated. The pre-adaptation to high salt conditions favors the probiotic nature of *L. acidophilus* ATCC 4356 because the increased number and the release of S-layer proteins may be consistent with its antimicrobial potential [71].

Peptidoglycans

Peptidoglycans play an important role in the survival and growth of probiotics as well as in the regulation of host immune responses [83]. This represents a potential characterization as a prebiotic of PGs. PGs derived from *L. rhamnosus* MLGA are able to induce the antimicrobial peptide defensin while simultaneously avoiding the harmful risks of inflammatory reactions [84]. Under lethal pH, the MurC and GalE1 proteins involved in peptidoglycan synthesis are upregulated in response to acid stress [85]. In addition, previous transcriptome analysis revealed that inducing peptidoglycan synthesis is a strategy that enhances cell wall H^+ blocking in *Bifidobacterium* [86]. The production of PGs in the cells was significantly higher under low pH conditions. This suggested that the cell wall of the adapted cells has improved integrity and strength [87].

Exopolysaccharides

LAB's EPSs are important biopolymers, which are widely used in food and pharmaceuticals, and act as prebiotic. Among prebiotics, EPSs were examined for their prebiotic activities [24]. It has also been indicated that the EPSs produced by LAB are able to inhibit the formation of biofilms via certain pathogenic bacteria [88]. Glucan-type EPSs isolated from *Levilactobacillus brevis* ED25 have potential as a prebiotic which stimulates the growth of Lactobacillus GG [89]. A previous study reported that the EPSs produced by *L. plantarum*, *Weissella cibaria*, *Weissella confusa*, and *Pediococcus pentosaceus* can be utilized (as carbon source) by *Bifidobacterium bifidum* DSM 20456 [90]. The metabolic, physiological, and cell surface properties of probiotic bacteria can be altered under exposure to stressful gastrointestinal conditions, thereby affecting the production of colonization factors such as EPSs. As a result, their ability to adhere to the intestinal epithelium is significantly affected [91]. The production of EPSs in LAB can be stimulated by various environmental stresses [92]. Probiotic LAB enhance EPS synthesis making a physical barrier to protect cells from adverse environmental conditions [93]. There is evidence that sub-lethal thermal stress improves the survival of *B. bifidum* by enclosing the EPS layer around the cells [94]. A recent study also showed that there is an enhancement of EPS synthesis in *L. plantarum* VAL6 under stress conditions of pH and sodium chloride [20].

Synbiotics Applications

Synbiotics are currently considered one of the important approaches to better maintain human and animal health by preventing and lowering the risk of disease. There is evidence that synbiotics influence the microbial ecology of the intestinal tract and play a role in alleviating various diseases [3, 95]. These studies suggested that synbiotics can modulate the Firmicutes/Bacteroidetes ratio as well as inhibit harmful bacteria by direct antagonism, competitive exclusion, microbiota recovery healthy intestinal flora acceleration, e.g., maintaining the pH of the intestine, producing important metabolites, and promoting the restoration of the intestinal mucosal barrier. Furthermore, synbiotics have the potential to help fight multidrug-resistant microorganisms [96–98].

In humans, the effects of synbiotic supplementation were also studied in patients with chronic kidney disease [99], nonalcoholic fatty liver disease [100], autoimmune disease [101], diarrhea [102], and metabolic syndrome [103]. Although studies on the effects of synbiotics on livestock health and performance are still limited, it is worth mentioning that health impacts will likely depend on the combination

Table 1 Studies applying synbiotics

Subjects	Synbiotics	Dose*		Duration of administration	Effects	Ref.
		Probiotics	Prebiotics			
Humans						
Soccer players	*Bifidobacterium lactis* CBP-001010, *L. rhamnosus* CNCM I-4036, *Bifidobacterium longum* ES1, and FOS	10^9 CFU/day	200 mg/day	02 weeks	Improving anxiety, stress, and sleep quality, particularly in sportspeople, is linked to an improved immuno-neuroendocrine response	[25]
Patients with nonalcoholic fatty liver disease	*L. casei, L. rhamnosus, Streptococcus thermophilus, Bifidobacterium breve, L. acidophilus, B. longum, Lactobacillus bulgaricus,* and FOS	2×10^8 CFU/day	-	28 weeks	Inhibiting NF-κB (nuclear factor κB) and reducing the production of TNF-*α* (tumor necrosis factor α)	[106]
Middle-aged adults	*Bifidobacterium animalis* subsp. *lactis* plus FOS	5×10^9 CFU/day	4.95 g/day	30 days	Reducing abdominal discomfort and pro-inflammatory conditions associated with aging naturally	[107]
Infants aged from 6 to 19 weeks	*B. breve* M-16 V plus GOS/FOS (9:1)	10^6 CFU/day	0.8 g/day	06 weeks	Reducing potential pathogens and the infant's intestinal physiology	[108]
Adults	Unspecified (a systematic review and meta-analysis)	-	-	-	Improving cardiometabolic and oxidative stress parameters in patients with chronic kidney disease	[99]
Patients with nonalcoholic fatty liver disease	*B. animalis* subspecies *lactis* BB-12 plus FOS	2×10^{10} CFU/day	8 g/day	10–14 months	Altering the fecal microbiome but did not reduce liver fat content or markers of liver fibrosis	[100]
The age > 12 years old	Unspecified (a systematic review and meta-analysis)	-	-	-	Effects on several markers of inflammatory and oxidative stress	[101]
Poultry						
Pullet chickens (1-day old)	*L. acidophilus, L. casei, Streptococcus faecium, Bacillus subtilis, S. cerevisiae* plus yeast-derived carbohydrates	-	-	06 weeks	Improving humoral immunity by increasing IgG concentration in serum; modulating the adaptive antibody-mediated immune response against infectious bronchitis virus	[109]
Broiler chickens (1-day old)	*B. longum* PCB133 plus xylooligosaccharides	10^{10} CFU/kg feed	2 g/kg feed	30 days	Reducing *Campylobacter jejuni* and *Campylobacter* sp. in the caecum	[110]
White Leghorn chicks (1-day old)	*Limosilactobacillus reuteri, Enterococcus faecium, B. animalis, Pediococcus acidilactici,* and FOS	-	1 g/kg feed	28 weeks	Increasing body weight, enhanced performance; protecting against the infection of *Salmonella enterica* serotype Enteritidis	[111]

Table 1 (continued)

Subjects	Synbiotics	Dose*		Duration of administration	Effects	Ref.
		Probiotics	Prebiotics			
Broiler chicks	*S. cerevisiae, E. faecium, B. subtilis, B. licheniformis* plus *β*-glucans, mannan oligosaccharides (MOS), and FOS	-	1 g/kg feed	42 days	Increasing body weight; decreasing mortality; adjusting feed conversion ratio, and necrotic enteritis-associated mortality with no decrease in the severity of intestinal lesion points in broiler chickens challenged with *Clostridium perfringens*	[112]
Male broiler chicks (1-day old)	*E. faecium, P. acidilactici, B. animalis,* and *L. reuteri* plus FOS	2×10^6 CFU/kg feed	1 g/kg feed	42 days	Promoting growth to reduce fear responses and stress states of heat-stressed broilers	[113]
Female chicks (1-day old)	*L. lactis, Carnobacterium divergens, L. casei, L. plantarum,* and *S. cerevisiae* plus RFO (extracted from lupin seeds)	-	8 g/kg feed	42 days	Increasing the body weight; affecting the morphometric parameters of the small intestine of chickens such as jejunum, ileum, and crypts	[114]
Livestock						
Piglets (28-day old)	*L. plantarum*-Biocenol LP96 (CCM 7512), and *L. fermentum*-Biocenol LF99 (CCM 7514) plus flaxseed	4×10^9 CFU/day	100 g/kg feed	14 days	Positive effects on the blood serum levels of total lipids, the ratio of n-3 polyunsaturated fatty acids (PUFAs)/n-6 PUFAs, and gut health and adaptation process after weaning	[115]
Piglets (6-week old)	*E. faecium* NCIMB 11,181 and lactulose	10^9 CFU/kg feed	5 g/kg feed	2 weeks	Decreasing proteobacteria abundances; increasing the average population of *Lactobacillaceae*; decreasing sharply in the proportions of *Enterobacteriaceae* in feces	[116]
Sows (256.7 ± 16.4 kg)	*L. plantarum ŁOCK 0860, S. cerevisiae ŁOCK 0118, L. reuteri,* and *S. cerevisiae* plus inulin	-	5 g/kg feed	24 weeks	Improving the immune status of healthy sows and their offspring	[117]
Weaned pigs (21-day old)	*Bacillus* sp. and xylanase	2×10^8 CFU/kg feed	13,370 XU/kg feed	20 days	Benefits on growth performance; reducing diarrhea, immune response, and the oxidative stress status in the small intestine	[118]
Growing-finishing pigs (25.29 ± 1.33 kg)	*Clostridium butyricum* endospores, *B. subtilis* endospores, and *Rhodopseudomonas capsulata* plus FOS	3×10^6 CFU/kg feed	1 g/kg feed	77 days	No effects on growth performance, nutrient digestibility, and fecal microbial shedding after supplementation with or without antibiotics in growing phase	[119]
Holstein heifer calves (34 ± 7 kg)	*L. acidophilus, E. faecium, B subtilis, S. cerevisiae*, and MOS	-	-	85 days	Improving diet digestibility and animal health	[120]

Table 1 (continued)

Subjects	Synbiotics	Dose*		Duration of administration	Effects	Ref.
		Probiotics	Prebiotics			
Aquatic animals						
Nile tilapia, *Oreochromis niloticus* (8.84 ± 1.29 g)	*L. acidophilus*, *E. faecium,* and *Bifidobacterium* sp. plus MOS or chitosan	3×10^9 CFU/kg feed	1 g/kg feed	63 days	Improving the protection against *Aeromonas hydrophila* infection without growth reduction	[121]
Pacific white shrimp, *Litopenaeus vannamei* (1.5 ± 0.12 g)	*B. subtilis* and *S. cerevisiae* plus *β*-glucan and MOS, etc.)	*B. subtilis:* 1.3×10^8 CFU/kg feed *S. cerevisiae:* 2.8×10^8 CFU/kg feed	3 g/kg feed	56 days	Improving the growth, feed utilization, intestine health and non-specific immunity, spraying synbiotics on the diet presented better performance than adding synbiotics in diet for pelleting	[122]
Pacific white shrimp, *L. vannamei* (0.5 ± 0.1 g)	*L. plantarum* and GOS	10^8 CFU/kg feed	4 g/kg feed	60 days	Enhancing immunity and disease resistance against *Vibrio alginolyticus* infection	[123]
Rainbow trout, *Oncorhynchus mykiss* (2.06 ± 0.07 g)	*P. acidilactici* plus citrus flavonoids, or yeast paraprobiotics	-	1.5 g/kg feed	63 days	Improving lipid utilization; contributing to minor increases in disease resistance	[124]
Pacific white shrimp *(L. vannamei)* larvae	*Pfiesteria piscicida* 1UB and MOS, through the bio-encapsulation of *Artemia* sp.	10^6 CFU/mL	12 mg/L	13 days	Stimulating total hemocyte count, phenoloxidase activity, respiratory burst activity, expression of immune-related genes; increasing disease resistance	[125]
White shrimp *(L. vannamei)*	*L. plantarum* and GOS	10^8 CFU/kg feed	4 g/kg feed	60 days	Improving colonization of *L. plantarum*; reducing the prevalence of *Vibrio harveyi* and *Photobacterium damselae* in the intestines	[126]
Pacific white shrimp, *L. vannamei (156 ± 39 mg)*	*B. subtilis* or *P. acidilactici* and *β*-glucan	5×10^7 CFU/kg feed	0.5 g/kg feed	90 days	Increasing phenoloxidase activity, and superoxide dismutase activity	[127]
White shrimp *(L. vannamei)*	*L. plantarum* and GOS	10^8 CFU/kg feed	4 g/kg feed	60 days	Improving weight gain, LAB, protease, leu-aminopeptidase, and *β*-galactosidase activity; reducing *Vibrio* counts in the intestine	[128]

* (-) Not given

of synbiotics and seem to be promising for the regulation of gut microbiota composition [104]. The beneficial effects of synbiotics have also been extensively studied in poultry and aquatic animals [2, 105]. The results of the in vivo trials performed are promising. Furthermore, recent developments in the application of synbiotics have significantly focused on evaluating their beneficial effects on animal health and performance (Table 1).

Recent studies have shown that the use of synbiotics is a promising approach to strengthen the immune system of chickens. The combination of probiotics and prebiotics can improve the survival and persistence of health-promoting organisms in the poultry gut because the substrate for fermentation is readily available [129]. Bodyweight gain and feed efficiency were significantly improved by the synbiotic treatment, and it is therefore recommended that synbiotics can be used as non-antibiotic growth promoters to improve the growth index in poultry [130].

Dietary and water-based probiotics and prebiotics together with synbiotics supplements are most beneficial for the control or treatment of bacterial, viral, and parasitic diseases in aquaculture. The effectiveness of these supplements has been determined by enhancing immune responses, stimulating the production of antimicrobial agents, altering the gut microbiota, competing for nutrients and binding sites, and conducting enzyme-related activities [131].

It is evident that most of the synbiotics used are mixtures of one or more strains of live microorganisms with one or more prebiotics, mainly FOS, GOS, and MOS (Table 1). Prebiotics appear to be used in this combination to help probiotics survive during the passage through the upper digestive tract while also impacting the intestinal microflora positively [132]. However, it has been reported that excessive intake of prebiotics, especially oligosaccharides such as FOS and GOS, could cause bloating owning to their fermentation in the colon [133]. In addition, prebiotics, in this case, also failed to protect during the production of probiotic powder before being incorporated into synbiotics. Therefore, the prebiotic biosynthesis within the probiotic for synbiotic self-production is a promising alternative.

Future Outlook

It should be noted that the positive health effects of probiotics and prebiotics are highly dependent on their appropriate combinations, which is necessary to consider the protective potential of prebiotics to probiotics. To further improve the efficiency of synbiotic utilization and to ensure their stability and viability, different strategies have been applied such as microencapsulation [134]. In addition, environmentally adaptive treatment is also a potential strategy to enhance the survival rate of probiotics and promote their functional properties in synbiotics [135]. Approaches using environmental adaptation to enhance the synthesis of prebiotic characterized components on the cell wall that improve bacterial viability have been discussed. According to the study results, it is possible to propose a model for enhancing synbiotics by applying environmental stresses (Fig. 4). In particular, exposure of probiotic strains to environmental challenges can trigger the reprogramming of cellular mechanisms for cell wall biosynthetic pathways, leading to microencapsulation with ingredients featured in prebiotics. Probiotics change the properties of the cell wall by producing more surrounding polysaccharides, S-layer proteins, peptidoglycans, and lipoteichoic acids in response to environmental challenges such as temperature and pH. As a result, living microbial cells contain both components characteristic of synbiotics.

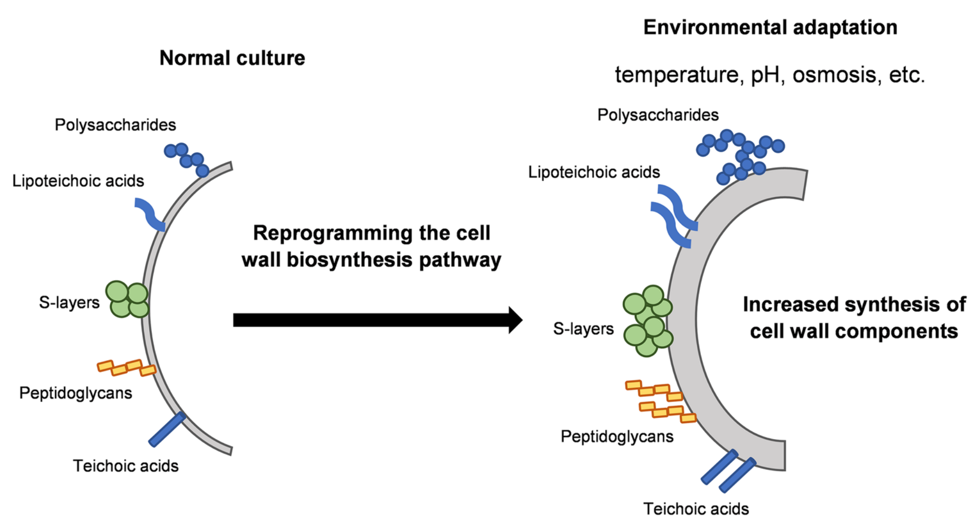

Fig. 4 Proposed model for the enhancement of cell wall components in probiotic bacteria. Environmental stresses trigger the reprogramming of the cellular mechanism for cell wall biosynthesis pathway, resulting in increased synthesis of prebiotic characterized components such as EPSs, S-layer, and peptidoglycan

Conclusions

Synbiotics have been shown to provide positive health benefits through the synergistic effect of prebiotics and probiotics. For maximum effectiveness, there is one aspect to consider that is the proper combination of these two ingredients and the viability of the product to achieve its goals. Using environmental stress adaptation may be a promising strategy to positively alter the biosynthesis of cell wall components to enhance survival. As a result, the probiotic strain fully exhibits the characteristics of a synbiotic with high viability by the protection of its microencapsulation which contains the prebiotic characterized components.

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Declarations

Conflict of Interest The authors declare no competing interests.

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