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REVIEW

Paraprobiotics: definition, manufacturing methods, and functionality

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Abstract

Probiotics are living microorganisms that are beneficial to the host, enhancing the immune response by promoting antibody production, regulating cytokine secretion, and stimulating T cells. However, probiotics have limitations in that they require viability control and have a short shelf life. Recently, the use of paraprobiotics has gained attention. These include dead bacterial cells, bacterial fractions, and cell lysate that have health benefits and are stable and safe for use. Paraprobiotics comprise molecules of bacterial cell wall compounds, such as peptidoglycans, teichoic acids, polysaccharides, and cell surface proteins. Paraprobiotics are manufactured by a diverse range of techniques, including thermal treatments, high pressure, ultraviolet rays, sonication, ionizing radiation, and pH modification. Their beneficial health effects include immunomodulatory, intestinal balancing, anticancer, and antimicrobial activities. Therefore, this review summarizes and discusses the manufacturing methods and bioavailability of paraprobiotics and suggests their potential health advantages.

Keywords Probiotics · Paraprobiotics · Production · Function · Safety

Introduction

Probiotics are defined as live microorganisms that when administered in adequate amounts confer health advantages on the host (FAO/WHO, 2001). Generally, their health benefits depend on the probiotic strain or administered dose. Probiotics influence the gastrointestinal flora and help maintain intestinal health by alleviating diarrhea or constipation (Mendoza et al., 2023; Pramanik et al., 2023). The probiotic strain can produce antimicrobial substances, such as bacteriocin and organic acid, and compete physicochemically with potential pathogens.

Bifidobacterium and *Lactobacillus* are the most studied probiotic species. Moreover, *Clostridium* and *Bacteroides* species are promising next-generation probiotics despite safety issues (O'Toole et al., 2017). In addition, a total of 32 *Lactobacillus* species were granted the Qualified Presumption of Safety status by the European Food Safety Authority for human application in a view of safety perspectives (Basavaprabhu et al., 2020).

Despite their health benefits, probiotics have several limitations, including toxin production, antibiotic resistance, strain-specific activities, short shelf life, unknown molecular characteristics, unclear beneficial effects, stability during manufacturing process, opportunistic infections, sepsis, and bacteremia for immunocompromised persons (Evivie et al., 2017). Probiotics are influenced by various host-specific factors in the gastrointestinal tract (GIT) that influence various metabolic pathways (Baugher and Klaenhammer, 2011). The metabolites and inactivated forms of probiotics can be used to promote human health (Sharma et al., 2023). Postbiotics are defined as metabolites or resolvable products produced by probiotics that have biological benefits to the host (Aguilar-Toalá et al., 2018). Simply, postbiotics include metabolic byproducts (secreted enzymes and proteins, short chain fatty

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acid, vitamins, and phenols) from probiotic strains (Nataraj et al., 2020). They are suggested as alternatives to probiotics for enhancing human health.

Paraprobiotics (parabiotics, dead probiotics, or inactivated probiotics) are defined as non-living forms of bacteria that can enhance human health (Aguilar-Toalá et al., 2018). These include bacterial cell structure components, cell lysates, and bacterial fraction. Generally, paraprobiotics are composed of peptidoglycan, teichoic acid, cell wall polysaccharide, and cell surface proteins (Aguilar-Toalá et al., 2018). Their functions include anti-inflammatory, antioxidant, anti-cancer, immunomodulation, hypocholesterolemic, anti-obesogenic, and anti-hypertensive effect (Nakamura et al., 2016; Shin et al., 2010).

Therefore, the aim of this review was to investigate the manufacturing methods and bioavailability of paraprobiotics and suggest their potential health benefits.

Definition and synthesis of paraprobiotics

Paraprobiotics have been described using various terms, such as inactivated probiotics, ghost probiotics, and non-viable (dead) probiotics (Siciliano et al., 2021). Paraprobiotics comprise cell fractions or inactivated microbial cells that confer health advantage to the host (Aguilar-Toalá et al., 2018).

As per the Food and Agriculture Organization/World Health Organization (FAO/WHO), paraprobiotics are defined as inactivated (non-viable) microbial cells, which, when managed in sufficient amounts, present benefits to consumers (Aguilar-Toalá et al., 2018).

Paraprobiotics are synthesized frominactivated microorganisms following chemical or mechanical stress; the cells suffer from mechanical/chemical damage to the cell envelope, leading to disruption of the cell membrane or degradation of DNA filaments (Siciliano et al., 2021). After inactivation, cell viability is confirmed by plating in acceptable culture media. In addition, checking the cell intensity of paraprobiotics is important as damaged cells can still maintain residual metabolic activity. Paraprobiotics have been used two forms: intact and ruptured. The structure of paraprobiotics is illustrated in Fig. 1.

Component of paraprobiotics

Peptidoglycan

The cell walls of most lactic acid bacteria (LAB) encompass a thick peptidoglycan layer, having a cross-linked glycan chain with alternating units of β-1,4-linked *N*-acetylmuramic disaccharide and *N*-acetylglucosamine (Teame et al., 2020). In addition, the fundamental composition of the oligopeptides and glycan strands is strain specific (Lebeer et al., 2010).

Teichoic acid

Teichoic acids (TAs) also form part of the cell walls of LAB. Owing to its anionic polymer nature, TA can be covalently linked to peptidoglycan or attached to the cytoplasmic membrane (Teame et al., 2020).

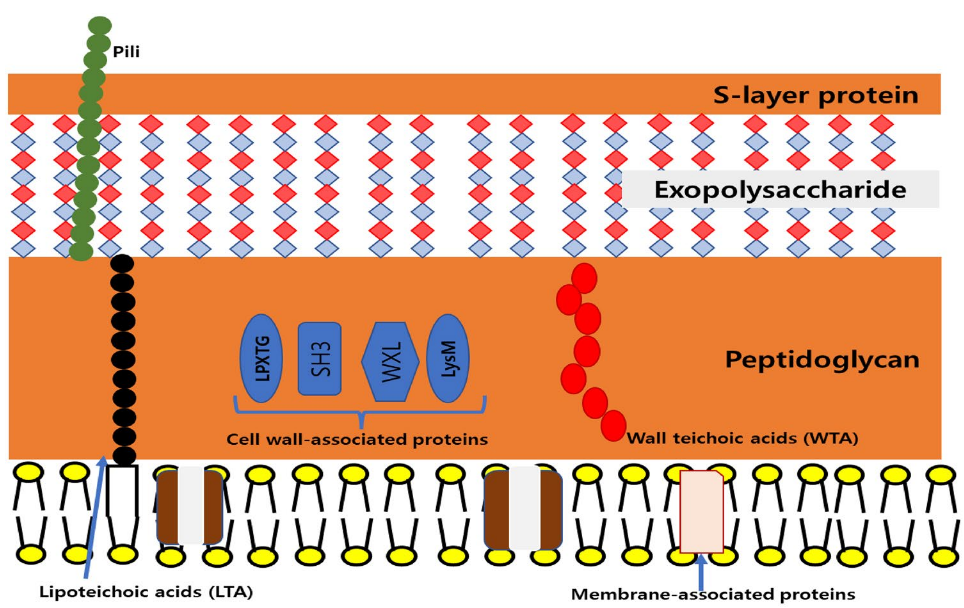

Fig. 1 Diagram showing the cell surface of *Lactobacilli*. The cell membrane (CM) with embedded proteins is covered by a multilayered peptidoglycan (PG) shell composed of lipoteichoic acids (LTA), wall teichoic acids (WTA), pili, proteins, and lipoproteins. Exopolysaccharides (EPS) form a thick covering closely associated with PG and are surrounded by an outer envelope of s-layer proteins

Cell wall polysaccharides

Exopolysaccharides (EPS) may assist the interactions between the environment and bacteria through their antipathogenic effects and adhesion properties (Chen et al., 2014). EPS derived from probiotic strains can modulate intestinal balance and mucosal immune responses (Bleau et al., 2010; Dinić et al., 2018).

Cell surface protein

Surface layer proteins are the valuable components of the LAB cell envelope. Cell surface proteins are classified based on whether they are covalently or non-covalently bound to the cell surface (Lebeer et al., 2010). A recent study reported that several types of surface proteins containing moonlight proteins, LPxTG proteins, pili proteins, and S-layer proteins are produced by LAB strains (Hynönen and Palva, 2013).

Moonlight proteins

Moonlighting proteins comprise several proteins, including ribosomal proteins, metabolic enzymes, translational elongation factors, and molecular chaperones (Kainulainen et al., 2012). They are located in many *Lactobacillus* sp., including *Limosilactobacillus reuteri*, *Lactobacillus crispatus*, *Lactobacillus jensenii*, and *Lactiplantibacillus plantarum* (Kainulainen et al., 2012; Saad et al., 2009).

LPxTG proteins

LPxTG proteins, characterized by a C-terminal located LPxTG signal, are covalently linked to the cell wall peptidoglycan via sortase A (SrtA) (Zong et al., 2023). LPxTG protein isolated from *L. reuteri* SH 23 showed anti-inflammatory effects by modulation via the MAPK-dependent NF-kB pathway in colitis mouse model (Zong et al., 2023).

Pili proteins

Pili are related to motility and pathogenicity. Pili promote the attachment of the bacteria to the intestinal mucosa and promote the persistence of *Lactobacillus* strains in the GIT (Reunanen et al., 2012; Troge et al., 2012). The SpaCBA pili of *Lacticaseibacillus rhamnosus* GG play a key role in the adherence of the bacteria to the host's intestinal mucosa (Lebeer et al., 2012).

S-layer proteins

S-layer proteins are commonly attached to the peptidoglycan through non-covalent bonds. *Lactobacillus* strains have an S-layer protein, accounting for 15% of the total cell wall protein (Malamud et al., 2018). Especially, S-layer protein are related to adhesion and immunity (Hynönen and Palva, 2013). The S-layer protein obtained from *Lentilactobacillus kefiri* CIDCA 8348 provides synergy effects on the action of macrophages to lipopolysaccharide (LPS) (Malamud et al., 2018). Notably, in some species of *Lactobacillus*, glycosylated S-layers are found in *L. kefiri* and *Lentilactobacillus buchneri* (Meng et al., 2014).

Manufacturing methods of paraprobiotics

Paraprobiotics can be synthesized from inactivated probiotics via numerous methods, including thermal treatment, high pressure, irradiation, ultra-violet rays, pulsed electric field, drying, sonication, and pH changes. These methods affect the cell membrane, nucleic acid, DNA, protein denaturation, chemical changes, inactivation of enzymes, and protein coagulation.

Thermal treatments

Thermal treatment is the most popular method to inactivate probiotics. The treatment is influenced by temperature, growth medium, pH value, mode of heating, water activity, and type of cells, such as vegetative cells or spores (Cebrián et al., 2017). The inactivation mechanisms using heat treatment are associated with the breakup of DNA filaments, ribosome aggregation, depletion of nutrients and ions, membrane damage, protein coagulation, and enzyme inactivation. The thermal treatment conditions vary widely (60–100 °C for 5–30 min) and are strain-dependent (Ou et al., 2011). The correlation of temperature with time varies based on the probiotic strain (Kawase et al., 2012).

High pressure

The high-pressure process can be used in combination with thermal treatment to inactivate the cells. At a pressure of 20–180 MPa, microbial growth is delayed, and protein synthesis is inhibited (Lado and Yousef, 2002). In addition, a pressure of 180 MPa renders all cells non-viable.

High pressure inactivates microorganisms owing to membrane damage, decrease in the intracellular pH, and protein denaturation, thereby leading to extensive loss of cell contents and coagulation of the nucleic acids and ribosomes (Mañas and Pagán, 2005).

Ultraviolet (UV) rays

UV rays are non-ionizing radiation having a germicidal effect (Lado and Yousef, 2002). The electromagnetic spectrum of UV rays lies in the range of 200–400 nm and is divided into three regions: short (200–280 nm), medium (280–320 nm), and long (320–400 nm) wavelengths (Lado and Yousef, 2002). Notably, the UV rays having the strongest germicidal action lie in the short-wave region.

Ionizing radiation

Ionizing radiation can be used for parabiotic production, and the sources are X-rays, gamma rays using Cs^{137} and Co^{60}, and high-energy electrons (Farkas and Mohácsi-Farkas, 2011; Ibrahim, 2013). Ionizing radiation renders the cellsinactive via damage to nucleic acids. Indirect damage leads to indirect actions resulting in the interaction of reactive species formed via the radiolysis of water, such as the hydroxyl radical with cell components (Mañas and Pagán, 2005). Direct damages are caused by the incorporation of radiation energy.

Pulsed electric field technology

Pulsed electric field (PEF) technology can be used for inactivation of probiotics. This technique functions by targeting and disrupting the cell membrane. The intensity of the electric field must be in the 2–87 kV/cm range (Devliegher et al., 2004).

Sonication

Sonication is a method using ultrasound to disrupt intermolecular interactions. This technology triggers chemical and physical changes in biological structures owing to intracellular cavitation. Cavitation results in microbial inactivation owing to DNA damage, thinning and perturbations of the cell membranes, and cell wall rupture through the formation of free radicals (Birmpa et al., 2013).

Supercritical CO_2 technology

Supercritical CO_2 technology is a cheap, non-toxic, and environmentally friendly method that inactivates the microorganisms by modifying the cell membrane, decreasing the intracellular pH, inactivating key enzymes related to cell metabolism, eliminating components from the cell membrane, and disturbing the intracellular electrolytic balance (Efaq et al., 2015; Yuk and Geveke, 2011).

Dehydration methods

Dehydration methods mainly include freeze-drying (or lyophilization) and spray drying. Freeze-drying is a costly method, and this method is generally applied to biological materials. In this process, the water present in the materials is pre-frozen and subsequently dried under vacuum conditions (Prapa et al., 2023). Conversely, spray drying makes use of an atomizer for crushing the liquids, resulting in the formation of powders. Spray drying occurs owing to dehydration and heat shock, leading to alterations in nucleic acids, ribosomes, and protein structures and the destruction of cytoplasmic membranes (Puttarat et al., 2021).

pH modification

pH modification is a non-thermal method that can be used to inactivate probiotics (Sehrawat et al., 2021). However, the metabolic ability of microorganisms cannot be eliminated and, therefore, they can remain viable.

Functions of paraprobiotics

Paraprobiotics are effective in regulating the immune system by alleviating intestinal lesions, colitis, diarrhea, liver diseases induced by obesity or alcohol, visceral pain, and respiratory diseases (Table 1) (Aguilar-Toalá et al., 2018). Moreover, they inhibit cancer growth, prevent aging, alleviate lactose intolerance, and promote oral health. Paraprobiotics have been mainly used as heat-killed whole cells. However, the use of teichoic acid, exopolysaccharides, and S-layer protein as paraprobiotics has also been reported.

Regulation of immune system

The immune system can be classified into innate and adaptive systems. Innate immunity is related to components such as cytokines, monocytes, neutrophils, natural killer cells, dendritic cells, macrophages, complements, and host proteins. The adaptive immune response is caused by antigen-specific B and T lymphocytes.

IL-12, a proinflammatory cytokine, is the key product of inflammatory cells (dendritic cells, neutrophils, macrophages, and monocytes) (Llewellyn and Foey, 2017). IL-12 can act by inducing the innate and adaptive immune systems. The teichoic acid composition of IL-12P40 resembles that of heat shock proteins (Hsp) and was produced through thermal treatment. It enhances immunomodulatory activity. Probiotics exert direct or indirect immune modulatory effects via the JAK/STAT, NF-kB, MAPK, and P13K/Akt signaling pathways.

The paraprobiotic *Lactobacillus gasseri* TMC0356 and *L. gasseri* AI-88 exert immunomodulatory effects through an increase in the production of tumor necrosis factor (TNF)-α, IL-12, and interferon (IFN)-γ in macrophages (Miyazawa et al., 2011; Ou et al., 2011). Heat-inactivated *Levilactobacillus brevis* KU15159 increases IL-1β, IL-6, and TNF-α levels by activating the mitogen-activated protein kinase (MAPK) pathway (Hwang et al., 2022a). Paraprobiotic *L.*

Table 1 Bioavailability of some paraprobiotics

Bacteria	Components	Bioavailability	References
L. brevis KU15159	Heat-inactivated cells	Immune enhancing effects in RAW264.7 cells	Hwang et al. (2022a)
L. rhamnosus GG	Heat-inactivated cells	Anti-inflammatory effect in rat model	Li et al. (2009)
L. brevis SBC8803, *L. brevis*-8013, *B. longum*, and *S. faecalis*	Heat-inactivated cells	Anti-inflammatory and epithelial barrier permeability in Caco-2 cells and colitis mouse model	Ueno et al. (2011)
VSL#3 (*B. breve*, *B. longum*, *B. infantis*, *L. plantarum*, and *S. salivarius* susp. *thermophilus*	Heat-inactivated cells	Anti-inflammatory effect in colitis mouse model	Sang et al. (2014)
S. salivarius subsp. *thermophilus* ATCC 19258 and *L. delbrueckii* subsp. *bulgaricus* ATCC 11842	Intracellular contents	Antioxidant effect	Ou et al. (2006)
B. longum SPM1207	Sonicated cell suspension	Hypocholesterolemic effect in high cholesterol rat model	Shin et al. (2010)
L. amylovorus CP1563	Fragmented cells	Antiobesogenic effects in obese mouse model	Nakamura et al. (2016)
L. brevis KU15176	Heat-inactivated cells	Anticancer effects on AGS cell lines	Hwang et al. (2022b)
L. fermentum BGHV110	Cell lysate suspension	Hepatoprotective effect in human HepG2 cells	Dinić et al. (2017)
Lactobacillus spp.	Heat-inactivated cells	Anti-biofilm effect against oral pathogens in vitro	Ciandrini et al. (2017)
L. plantarum 200655 and *L. buchneri* 200793	Heat-inactivated cells	Neuroprotective effects against oxidative stressed SH-SY5Y cells	Choi et al. (2022); Cheon et al. (2021)
Leu. mesenteroides H40 and *Lc. lactis* KC24	Heat-inactivated cells	Neuroprotective effects against oxidative stressed SH-SY5Y cells	Lee et al. (2021); Lim et al. (2020)

B., Bifidobacterium; *L., Lactobacillus*; *Leu., Leuconostoc*; *Lc., Lactococcus*; *S., Streptococcus*

casei Zhang (LcZ) improves the innate response of macrophages by increasing the transcription of Toll-like receptors (Wang et al., 2013).

Inflammation is a complicated response of the immune system involving the activation of plasma proteins and leukocytes. UV-inactivated *L. rhamnosus* GG reduced the inflammatory response induced by LPS in a rat model (Li et al., 2009). Paraprobiotic GG at 10^8 CFU/mL was effective in regulating the inflammation induced by LPS. Both probiotic and parabiotic GG lowered IL-8 production in the intestinal epithelium.

EPS obtained by *L. rhamnosus* RW-9595 M induced high levels of IL-10 and low or no levels of IL-6, IL-12, and TNF-α (Bleau et al., 2010). Furthermore, the EPS of *L. paraplantarum* BGCG11 showed anti-inflammatory properties (Dinić et al., 2018).

Alleviation of atopic dermatitis

Atopic dermatitis (AD) is a chronic and intractable skin disease, which normally reveals eczema, with recurring exacerbation and regression (Weidniger and Novak, 2016). At the beginning of AD, the skin barrier is destroyed or damaged owing to itching triggered by the outbreak. This increases the levels of proinflammatory cytokines, which activate several cells of the immune system (Itamura and Sawada, 2022). AD is characterized by responses mediated by Th2 cells. Th2 cells mediate the synthesis of immunoglobulin E (IgE) due to the production of chemical mediators and cytokines (Wollenberg et al., 2021).

Lactilactobacillus sakei probio-65 inhibited skin inflammation and lesions in a rat model. Two types of *L. sakei* probio-65 at 5×10^9 CFU/mL reduced the frequency of itching and skin lesions, and decreased the levels of IgE and Th2 cytokines (IL-4 and IL-6) (Kim et al., 2013). Furthermore, parabiotic *Lacticaseibacillus paracasei* K71 at 2×10^{11} cells inhibited the symptoms of AD in adult (Moroi et al., 2011). The severity of the scores for the skin lesions was reduced in comparison to the placebo group. Therefore, paraprobiotics can alleviate symptoms in patients with AD.

Regulation of the intestinal microbiota

Probiotics protect the hosts against invading pathogens. The antimicrobial mechanisms of probiotics include: (1) direct inactivation via the production of organic acid or antimicrobials, such as bacteriocins; (2) indirect inactivation via interfering with their adhesion to the intestinal cells and induction of IgA on the mucous surface (Bermudez-Brito et al., 2012). Moreover, paraprobiotics protect the host alongside infections by invading pathogens.

Parabiotic *Leuconostoc mesenteroides* 1RM3 of 1×10^9 CFU/mL prevented the enterogastric invasion and infection by *Listeria monocytogenes* both *in vitro* and *in vivo* (Nakamura et al., 2012). The S-layer protein of *L. casei*, *Lactobacillus paracasei* subsp. *paracasei*, and *L. rhamnosus* strains can inhibit the adhesion of *Shigella sonnei* to HT-29 cells (Zhang et al., 2010). A mixture of paraprobiotics using *Lactobacillus acidophilus* LAF1, LAH7, and LAP5 at1×10^{10} CFU/mL can inhibit the *S.* Typhimurium invasion through macrophage immunomodulation (Lin et al., 2007).

The intestinal barrier is the key defense system to keep intestinal integrity and defend the microflora from the environment. Dysfunction of the intestinal barrier can allow pathogens such as bacteria and toxins to enter (Teame et al., 2020). Paraprobiotics made of cell wall proteins protect the intestinal barrier (LPxTG, S-layer, moonlight proteins etc.).

Parabiotic *L. plantarum* b240 can defend the host from the invasion of *S.* Typhimurium via the reduction of its translocation in different organs and tissues [mesenteric lymph nodes (MLN), Peyer's plate (PP), spleen, liver, blood, and feces] (Ishikawa et al., 2010).

Yogurt containing paraprobiotics of *L. bulgaricus*, *S. thermophilus*, and *L. acidophilus* at 1×10^9 cells/mL is significantly effective at preventing the disruption of the gut epithelial barrier function in human Caco-2 cells (Zeng et al., 2016). The potential mechanism is related to the inhibition of iNOS and induction of proinflammatory cytokines.

Alleviation of colitis

Inflammatory intestinal disease is a chronic inflammatory condition that affects the GIT and comprises two main forms such as ulcerative colitis and Crohn's disease (Zhang et al., 2014). Some paraprobiotics have shown beneficial effects in the prevention of colitis. Paraprobiotics, *B. bifidum* and *B. breve* showed an anti-inflammatory effect on peripheral blood mononuclear cells (PBMC) in patients with ulcerative colitis. The paraprobiotics induced the production of IL-10 in the PBMC and reduced the secretion of IL-8 in epithelial cells. In addition, parabiotic *L. brevis* SBC8803 alleviated intestinal injuries in colitis mice models induced by dextran sodium sulfite (DSS) by enhancing the intestinal barrier (Ueno et al., 2011).

Reduction of lactose intolerance

Lactose intolerance refers to the lack of ability to digest lactose. It is caused by lactase deficiency. Symptoms of lactose intolerance include swelling, flatulence, vomiting, nausea, abdominal distension, diarrhea, and abdominal pain after ingesting lactose (Di Stefano et al., 2007). Lactose intolerance can be diagnosed using the positive hydrogen breath test (BHT). Rampengan et al. (2010) showed that paraprobiotics (Dialac) administration in children having lactose intolerance reduced its symptoms. A typical BHT test score of > 20 ppm indicates lactose intolerance. The use of dialac decreased the BHT score, and the results was similar to that obtained with the probiotic Lacidofil. However, these characteristics depend on β-galactosidase production in intestinal microbiota.

Reduction of cholesterol levels

High cholesterol levels in the blood increase the risk of coronary heart disease. Fibers, plant steroids, and probiotics can lower cholesterol levels (Bosch et al., 2014). Parabiotic *B. longum* at 10^8–10^9 CFU/mL demonstrated a hypocholesterolemic effect in a rat model (Shin et al., 2010). Parabiotic *B. longum* reduced total and low-density lipoprotein (LDL) cholesterol levels in serum and also reduced both the liver weight and atherogenic index. These hypocholesterolemic effects were observed in the following components: (1) the cell membrane, (2) the cytoplasm, (3) metabolic products related to inhibition of the cholesterol synthesizing enzyme, (4) the adsorption of cholesterol, and (5) interference that facilitated its elimination.

Alleviating the symptoms of respiratory disease

Paraprobiotics can alleviate symptoms of respiratory diseases, such as asthma, colds, pneumonia, colds, and allergic rhinitis, leading to the improved life quality of patients. Parabiotic *L. paracasei* 33 administration at 5×10^9 CFU/capsule showed the alleviation of disease symptoms in patients with perennial allergic rhinitis (Peng and Hsu, 2005). Paraprobiotic *E. faecalis* FK-23 (LFK) can combat nasal allergies and modulate the immune response in hamsters with allergic rhinitis (Zhu et al., 2012). LFK treatment reduced the frequency of sneezing and nose scratching. In addition, LFK treatment CD25 + and CD4 + T regulators in the spleen. The regulatory T cells can act by attenuating Th1 and Th2 responses and suppressing IgE secretion and the activity of effector cells, such as eosinophils.

Parabiotic *L. plantarum* KCTC 3104 and *L. curvatus* KCTC 3767 at 5×10^7 CFU/200 µL suppressed allergy and modulated intestinal immunity as a defense mechanism in the host (Hong et al., 2010). These paraprobiotics upregulated IL-10 and Foxp3 expression levels in intestinal cells in cases of hyper-responsiveness of the airways.

Improvement of liver health

The interaction between the liver and intestinal microbiota has been reported as the gut-liver axis. Alcohol can induce alterations in intestinal conditions, such as intestinal permeability and microbiota, and accelerate the progression of liver diseases (Wang et al., 2012). Alcohol can cause fatty liver disease by promoting the accumulation of triglycerides and cholesterol in the liver (Segawa et al., 2008).

Alcohol-induced liver diseases were controlled by the probiotic *L. rhamnosus* GG (Bull-Otterson et al., 2013). Parabiotic *L. brevis* SBC8803 administration in alcohol-treated mice could alleviate alcohol-induced liver disease by inhibiting the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum and decreasing total cholesterol and triglycerides levels in the liver. Parabiotic *L. brevis* SBC8803 suppressed the over-expression of TNF-α, sterol regulatory element binding protein (SREBP)-1, and SREBP-2 mRNA levels in the liver and increased the expression of heat shock protein (Hsp) 25 in the small intestine. TNF-α has a role in liver pathogenesis and is an inflammatory cytokine. A decrease in TNF-α expression level is probably due to an inhibition of its production in the liver. SREBPs caused a reduction in triglycerides and cholesterol in the liver. Hsps confer protection to the cells against oxidative, thermal, and inflammatory stresses. An increase in the Hsp25 level in the epithelial cells boosts the epithelial barrier against damage, preserving its function. *L. brevis* improves injuries induced in the liver and fatty liver disease by decreasing the expression levels of TNF-α and SREBPs (Segawa et al., 2008).

Anti-proliferative effects against cancer

Probiotics have been used as adjuvants against cancer (Rafter, 2003). Parabiotic *L. paracasei* IMPC2.1 and *L. rhamnosus* GG (10^8 CFU/mL) showed anti-proliferative activity and pro-apoptotic effect on HGC-27 (gastric) and DLD-1 (colorectal) cancer cell lines. Parabiotic *Lactobacillus* strains of various types, such as whole cells, cell wall, peptidoglycan, and cytoplasmatic materials, can show an anti-proliferative effect against human cancer cell lines. Heat-inactivated *Levilactobacillus brevis* KU15176 induced the apoptosis of AGS cell lines (Hwang et al., 2022b). EPS produced by *L. acidophilus* 20079 can control apoptotic, nuclear factor kappa B (NF-κB), IkBα, P53, and TGF genes in human colon cancer (Deepak et al., 2021).

Attenuation of age-associated conditions

Age-associated chronic inflammation is caused by age-linked disorders, such as chronic activation of the inflammasome, autophagy dysfunction, cell senescence, DNA damage, and gut dysbiosis (Shin et al., 2021). Parabiotic *L. lactis* H61 affects bone density, skin ulcers, and hair loss (Kimoto-Nira et al., 2012). The osteoclast has a principal role in bone reabsorption. Consequently, the consumption of these paraprobiotics affects reabsorption instead of bone formation.

Rheumatoid arthritis is an autoimmune disorder characterized by swollen joints with chronic pain and reduced functions. This disease is related to a proinflammatory state; therefore, probiotics having anti-inflammatory properties reduce the severity of rheumatoid arthritis (Paul et al., 2021). Heat-inactivated *L. sakei* suppresses collagen-induced arthritis by modulating T helper 17 cells and regulatory B cells (Jhun et al., 2020).

Inhibition of oral health

Oral health is important from infancy to old age in view of being linked with heart disease, diabetes, respiratory disease, digestion, and brain disease. The oral microbiome is linked to gut microbiota (Bowland and Weyrich, 2022). Dental caries is characterized by the initial dissolution of the enamel and dentine. *Streptococcus mutans* is the main microorganism causing active dental caries. Heat-inactivated *Lactobacillus* spp. inhibited the biofilm formation by *S. mutans* (Ciandrini et al., 2017).

Protection of neurohealth

The gut-brain axis (GBA) is a communication system whereby gut microbes can interact with the brain. Gut bacteria influence depression and behavior. Additionally, anxiety symptoms are directly related to microbiota composition. Psychobiotics are defined as probiotics that influence mental health, and gut microbes affect the production of neurohormones (serotonin, dopamine, and epinephrine), neurotransmitters, proteins, and short-chain fatty acids (SCFA) (Del Toro-Barbosa et al., 2020).

Some paraprobiotics including heat-inactivated *Lactococcus lactis* KC24, *L. buchneri* 200793, and *Leuconosotc mesenteroides* H40 showed neuroprotective effects as psychobiotics through exerting antioxidant effects and modifying intestinal conditions (Cheon et al., 2021; Lee et al., 2021; Lim et al., 2020).

Benefits and commercial use of paraprobiotics

The use of probiotics has increased over time and more functions are being discovered. The probable side effects of probiotics in sick people have not been reported; however, the guidelines on the safety of probiotics have been formulated by the Food and Drug Administration (FDA) in the Unites states and the European Directorate for Drug Quality and Health (EDQM) (Yunes et al., 2022).

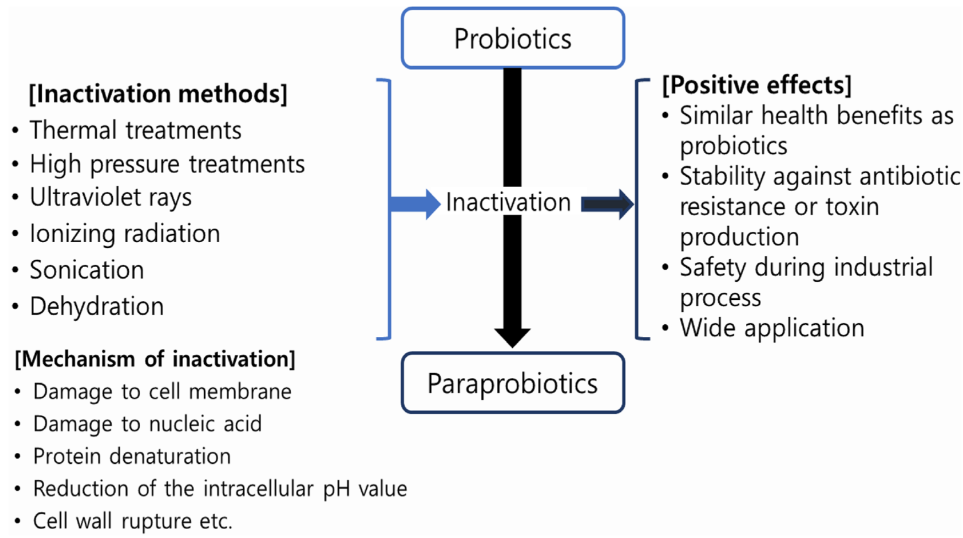

Fig. 2 Manufacturing methods and positive effects of paraprobiotics

As shown in Fig. 2, paraprobiotics have various advantages (Nataraj et al., 2020): no risk of translocation from the gut lumen to blood; stability against the transfer of antibiotic resistance genes, and limited interference with normal colonization of gut microbiota. In addition, physiological characteristics of inactivated forms could release active molecules from the inactivated cells that would pass through the mucosal layers, and stimulate epithelial cells directly. Therefore, loss of cell viability and cell lysis products can have more beneficial effects than viable cells. Furthermore, they are easier to standardize, transport, and store (Piqué et al., 2019).

Heat-inactivated *Lacticaseibacillus paracasei* MCC1849 is used in various food products such as miso soup, confectioneries, tofu, and seasonings (Maehata et al., 2021). Its commercial name is LAC-Shiled™ (Morinaga Milk Industry Co., Ltd, Japan), and is inactivated by thermal treatment. This paraprobiotics has influenced common cold infections and mood states (Murata et al., 2018). In addition, Staimune® (Blossom Water LLC., USA) is composed of inactivated *Bacillus coagulans* GBI-30 and is applied in beverages. Its main functions are to exert antioxidant and anti-inflammatory effects (Jensen et al., 2010). Colimil® Baby (Humana Spain S.L., Spain) is composed of chamomile, lemon balm, and heat-inactivated *L. acidophilus* HA122 to reduce infant colic (Martinelli et al., 2017). Lacteol™ (Reig Jofre, S.A., Spain), a parabiotic product using heat-inactivated *L. acidophilus* LB is effective in alleviating diarrhea related to intestinal infectious diseases (Liévin-Le Moal, 2016).

In conclusion, paraprobiotics are non-viable microbial cells or cell constituents having a similar function as probiotics. Paraprobiotics have various health benefits and are easy to synthesize and have the potential for wide application. However, some limitations have been reported including: (1) selection of probiotic species to make the paraprobiotics, (2) adequate inactivation methods, (3) demonstration of stability and shelf life, and (4) evaluation of health benefits. The function and required amounts of paraprobiotics have to be determined for clinical applications as per the guideline of the Korean Food and Drug Administration (KFDA) in Korea, Food and Drug Administration (FDA) in USA, and European Medicines Agency (EMA) in Europe. Although many inactivation methods have been used to synthesize paraprobiotics, these methods are not standardized owing to the different strains available. Moreover, the impact of food treatments through their shelf life needs to be investigated. If these issues are solved, the development of paraprobiotics as ingredients in food industries may gurantee their safety and shelf lives. Moreover, they may be an effective funtional food material for immunocompromised people.

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Declarations

Conflict of interest All authors confirmed that they do not have conflicts of interest to disclose in this manuscript.

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