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Current research status and trends in the bioactivity of postbiotics

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Postbiotics have similar bioactivity to probiotics and are safer and more stable. The results indicate that postbiotics exert their effects through direct (antibacterial, antioxidant, immune regulatory) and indirect (regulating microbial homeostasis and metabolic pathways) mechanisms. In the field of animals, postbiotics can enhance the disease resistance of aquatic animals, improve the intestinal health and meat quality of broiler chickens, and promote nutrient absorption in ruminant animals. In the field of humans, postbiotics exhibit potential for anti-allergy, prevention of respiratory or digestive tract infections, adjuvant therapy for anti-cancer, and improvement of liver cirrhosis. Meanwhile, postbiotics have been applied in the research and development of medical preparations and functional foods, but the mechanism of action still needs to be further explored. Compared to probiotics, postbiotics do not require live bacteria to produce health benefits and have advantages such as high safety, easy storage, and convenient production. In the future, postbiotics have broad prospects in the fields of functional foods with unique flavors and nutritional health benefits, and disease prevention and control.

KEYWORDS

postbiotics, probiotics, bioactivity, disease prevention, functional food

1 Introduction

The application of probiotics by humans can be traced back to ancient times. However, it was not until 1907 that people discovered the impact of these microorganisms on health. In 1953, the term "probiotics" was first used to refer to active substances essential for the healthy development of life (Gasbarrini et al., 2016). In 2001, a panel of experts from the Food and Agriculture Organization of the United Nations and the World Health Organization officially defined probiotics as live bacteria that, when ingested in appropriate amounts, can exert beneficial effects on the health of the consumer (Bognanni et al., 2024). In recent years, the field of probiotics has received increasing attention (Gibson et al., 2017). According to the current definition, probiotics must be live microorganisms, and it does not apply to dead bacterial cells or cellular components. However, studies have found that inactivated bacteria still retain their biological activity towards the host (Almada et al., 2016; Shafipour et al., 2020), thus giving rise to the concept of postbiotics, which refers to the components of probiotics that remain beneficial to the host even after losing their activity (Malagon-Rojas et al., 2020). Currently, postbiotics are collectively referred to as inactivated microorganisms and their related components that are

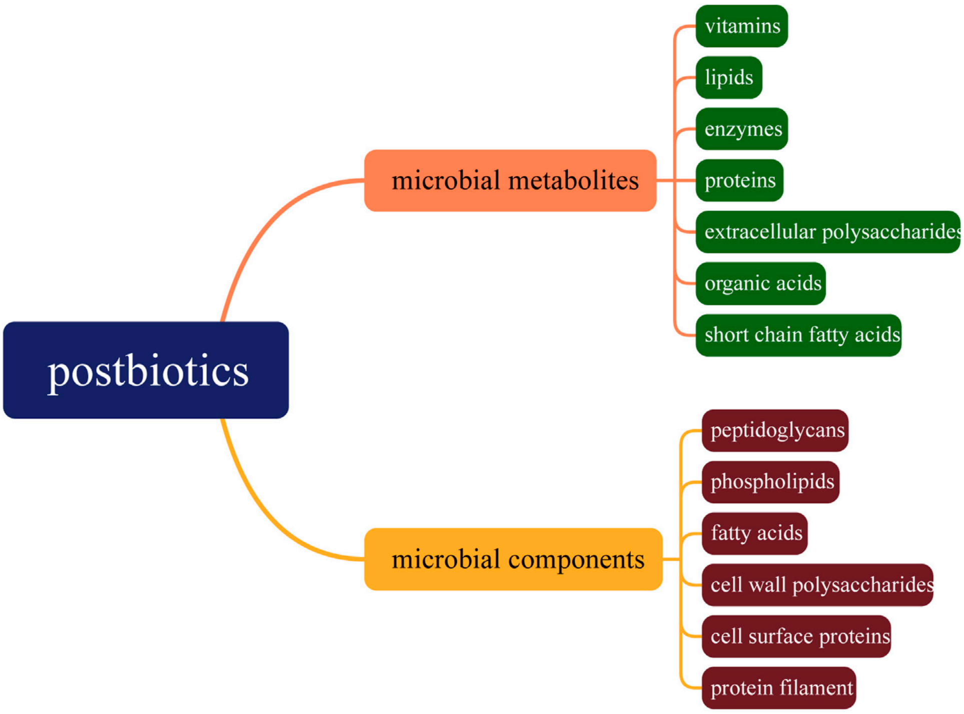

FIGURE 1
The components of postbiotics.

beneficial to host health, including microbial catabolic products, non-living microbial bodies, and components derived from cell lysis (Nataraj et al., 2020). As shown in Figure 1, postbiotics include microbial metabolites such as vitamins, lipids, enzymes, proteins, extracellular polysaccharides, organic acids, short chain fatty acids, as well as microbial components such as peptidoglycans, phospholipids, fatty acids, cell wall polysaccharides, cell surface proteins, and protein filament (Roy et al., 2024).

Probiotics generally refer to live, non-pathogenic microorganisms. However, clinical studies have shown that not only do live cells of probiotics exert health effects, but different non-viable parts of probiotic cells may also have certain beneficial effects (Sarkar, 2018). Depending on the process conditions for producing and storing probiotic products, the final product is a mixture containing both live and non-viable probiotic cells. By measuring the ratio of live cells (probiotics) to dead cells (postbiotics) in probiotic foods through *in vitro* experiments and assessing the properties of both fractions, it was found that the number of non-viable cells is much larger than that of live cells, and they have the same effects as live cells. This discovery indicates that a significant part of the health benefits of probiotic products is due to the presence of compounds such as postbiotics, which are naturally produced under special conditions (Fleming et al., 2019). Another technical issue with probiotic products is how to maintain the biological activity of probiotic cells. Compared to probiotics, postbiotics have the following advantages. The most significant advantage of postbiotics is their high stability. Compared to live probiotics that require storage under specific temperature and humidity conditions, postbiotics do not require live bacteria to produce health benefits, making them more stable and easier to store and transport (Zhong et al., 2024). Additionally, since postbiotics contain no live bacteria, they avoid secondary contamination and can be incorporated into products where adding live probiotics might cause discomfort, giving them broader application prospects in the food and beverage industry. The safety of postbiotics is another key advantage. They do not contain live bacteria that may cause infection or anaphylactic responses, making them more suitable for immunocompromised individuals or *homo sapiens* groups allergic to certain bacterial strains (Ma et al., 2023; Scott et al., 2022). This avoids potential issues such as the generation of antibiotic resistance genes and virulence factors in the body when using probiotics (Brial et al., 2018). The bioactivity of postbiotics also stands out as an advantage. The metabolites and cell wall components in postbiotics offer health benefits to the host, aiding in improving gut health, regulating the immune system, inhibiting pathogen growth, and reducing inflammatory responses. Therefore, it is necessary to obtain some safe and inexpensive alternatives to live probiotic cells. Evidently, postbiotics have research value as such alternatives.

2 Bioactivity of postbiotics

The biological mechanisms of most postbiotics are not fully understood. Scientific evidence indicates that postbiotics exert various biological effects through direct or indirect pathways, such as antibacterial, antioxidant, and immunomodulatory activities (Aguilar-Toala et al., 2018). Research suggests that the direct mechanism involves the interaction of postbiotics with different molecules or receptors, resulting in inhibitory effects (such as antioxidant, immunomodulatory, and antibacterial activities); the indirect mechanism involves the homeostasis of the microbiota, host metabolism, and signaling pathways, thereby affecting specific physiological responses (Singh et al., 2018).

2.1 Bioactivity of postbiotics in animals

Many researches on postbiotics in animals have shown that they possess certain health-promoting effects. It has been demonstrated that cell-free supernatant obtained from milk fermentation by controlling the pH of agar medium has the ability to protect mice from *Salmonella* infection (Dunand et al., 2019). At present, there is an urgent need to find environmentally friendly methods to replace antibiotics. Research indicated that the gut microbiota not only provided nutritional benefits, but also contributed to the development and differentiation of immune responses; probiotics can produce antibacterial compounds, compete for living space and nutrients, and inhibit virulence genes. However, the long-term addition of large amounts of live bacteria in aquaculture systems is often questioned, as these bacteria may also carry high levels of antibiotic resistance genes, making postbiotics a valuable alternative to live probiotics (Perez-Sanchez et al., 2018). Meanwhile, postbiotics have certain applications in the prevention and treatment of infectious diseases in aquaculture. Some of their soluble factors (such as short-chain fatty acids, organic acids, peptides, teichoic acid, peptidoglycan, extracellular polysaccharides, cell surface proteins, and vitamins) have effects on enhancing immune response, disease resistance, and antibacterial activity in certain aquatic animals (Yao et al., 2020), thereby reducing the risk of pathogenic infections in aquaculture animals. Adding four types of inactivated probiotics to rainbow trout feed can prevent furunculosis (Irianto and Austin, 2003). Furthermore, studies have shown that a new postbiotics extracted from lactic acid bacteria can modify the gut microbiota of rainbow trout and confer disease resistance (Mora-Sanchez et al., 2020). The beneficial bacterial cellular components and metabolites (postbiotics) of hybrid sturgeons have a positive effect on the growth of beneficial gut microbiota in hybrid sturgeons, but they do not affect the survival rate of sturgeons. They can promote the growth of beneficial gut microbiota in hybrid sturgeons, altering the composition and diversity of their gut microbiota (Wu et al., 2020).

Feeding broiler chickens with feed supplemented with postbiotics results in chicken meat with a 5% lower fat content compared to commercially available chicken meat, and a significant 11.13% increase in saturated fatty acid content. Broiler chickens fed with postbiotic supplemented feed not only have no residues of antibiotic substances, pesticides, and herbicides, but also exhibit better flavor (Kucheruk et al., 2019). Postbiotics have an immune-regulating effect on the jejunum tissue of broiler chickens; they modulated the activation of the innate immune response, and combined with pathogenic factors of gas-producing capsular protein, inhibited the activation of the standard gas-producing capsular protein immune response. Due to certain limitations on the addition of antibiotics with anti-inflammatory effects to feed, postbiotics were of great significance as alternatives to antibiotics for maintaining intestinal health in broiler chickens (Johnson et al., 2019). Researchers had shown that adding certain postbiotics to feed improved the growth performance of broiler chickens, reduced the number of *Escherichia coli*, increased acetic acid concentration, and accompanied related changes in cytokine expression in the ileum. Proportionally adding postbiotics could serve as an alternative to antibiotics in broiler chicken feed to improve growth and intestinal health (Kareem et al., 2016). Other research results indicated that under heat stress conditions, feeding postbiotics can enhance the antioxidant activity and meat quality of broiler chickens, and reduce acute-phase proteins, plasma cholesterol, and lipid peroxidation (Humam et al., 2020).

Postbiotics also exhibit certain biological activities in mammals. Wan Ibrahim et al. (Izuddin et al., 2020; Izuddin et al., 2019a) reported that supplementation of postbiotic *L. plantarum* RG14 in weaned lambs can promote rumen papilla growth, immune status, and gastrointestinal health. *Lactobacillus plantarum* RG14 also demonstrates high antioxidant capacity. Post-weaning supplementation with *L. plantarum* RG14 can increase antioxidant enzymes in serum and rumen fluid, and reduce lipid peroxidation in serum. After supplementation with *L. plantarum* RG14 in newly weaned lambs, rumen fermentation was improved, microbial parameters, blood metabolites, as well as post-weaning growth and nutrient intake genes were upregulated, indicating that it can enhance the growth performance, nutrient intake, and nutrient digestibility of weaned lambs (Izuddin et al., 2019b). Deoxynivalenol and aflatoxin in pig feed are harmful to newly weaned piglets, affecting their growth and nutrient digestibility. Supplementation with yeast cell wall mixture (postbiotics) can partially overcome the harmful effects of various mycotoxins in the diet on the growth and health of weaned piglets (Holanda et al., 2020). Based on research in various animals, postbiotics may be a suitable alternative to the use of probiotics, avoiding the potential risks associated with the use of live microorganisms. Postbiotics have broad application prospects in the field of animal feed.

2.2 Bioactivity of postbiotics in humans

In recent years, due to the difficulty in determining the bioactivity of probiotics, some new research directions have gradually emerged. For instance, postbiotics—metabolites produced by probiotics that exhibit similar health benefits—hold considerable research promise (Taverniti and Guglielmetti, 2011). The following briefly describes the bioactivity of postbiotics in humans.

2.2.1 Prevention and treatment of allergies

Due to the different composition of gut microbiota in allergic patients, regulating gut microbiota is an effective method to control allergic reactions, and this research strategy has certain application prospects (Pascal et al., 2018). Some researchers have found that probiotics have a certain role in the prevention and treatment of food allergies (Santos et al., 2020). For example, some live microorganisms, when given in sufficient amounts, seem to bring health benefits to the host, such as balancing the microbiota, restoring intestinal permeability, improving barrier function, and regulating immune responses, but may produce some minor side effects (Sotoudegan et al., 2019). However, postbiotics do not pose such risks, postbiotics enhance the endogenous probiotics of each host, rather than adding unfamiliar strains of probiotics to the gut microbial ecosystem, which can restore missing allergen-protective microorganisms, improve immune tolerance, treat allergies, and have no adverse side effects. The positive effects of postbiotics largely depend on the selection of maternal microbial strains, different preparation methods, the presence of multiple bioactive molecules, dosage, and the appropriate choice of drug delivery system. Therefore, postbiotics can serve as a new strategy to improve immune tolerance and treat food allergies without adverse side effects, especially in infants and children. Despite some research progress in postbiotics in allergy treatment, further research is still needed to fully understand how postbiotics can be used as an adjunctive therapy to prevent or treat a wide range of chronic diseases.

2.2.2 Prevention of respiratory and gastrointestinal infections

Children under the age of 5 are particularly susceptible to respiratory and gastrointestinal infections (Frank et al., 2019). Since early 1980, probiotics have been used to mitigate infections from common infectious diseases in children and infants (Amaral et al., 2017). However, it is worth noting that due to rare cases of infections associated with probiotics, such as necrotizing enterocolitis and pneumonia, the scientific community does not endorse the use of probiotic interventions in young children (Kothari et al., 2018). Given these challenges, supplementation with postbiotics has been proposed as an alternative strategy to reduce the incidence of infectious diseases in children. There have been reports on the use of heat-killed *L. acidophilus* for the treatment of acute diarrhea and heat-killed *Lactobacillus acidophilus* CBA L74 for the prevention of gastrointestinal and respiratory infections (Malagon-Rojas et al., 2020). Postbiotics offer the same beneficial therapeutic effects as similar probiotics, while avoiding the risks associated with live microorganisms, especially in high-risk groups such as children under the age of 5.

2.2.3 Adjuvant therapy for anti-cancer

It has been reported that the postbiotics produced by *L. plantarum* exhibit selective cytotoxic effects on various cancer cells under different combinations of dosage and time (Chuah et al., 2019), without causing toxicity or hemolysis to normal cells. Fluorescence microscopy and flow cytometry observations indicated that these postbiotics inhibited the proliferation and induced apoptosis of human breast cancer cells, reducing the survival rate of human breast cancer cells. The postbiotics produced by *L. plantarum* were selective towards various tumorigenic cells, suggesting that they possess certain anti-cancer properties. Therefore, they have great potential as functional supplements and adjuvant therapies for anti-cancer treatment, which can be further explored.

2.2.4 Therapy for cirrhosis

Cirrhosis is the terminal stage of chronic hepatitis development, dysbiosis of gut microbiota, loss of intestinal barrier function, and bacterial translocation are important causes of complications in cirrhosis. Although probiotics are well tolerated in patients with cirrhosis and can protect intestinal barrier function at multiple levels, studies have shown that in critically ill patients or those with extremely low immunity, probiotics may lead to opportunistic infections and even increase mortality. Therefore, their efficacy in treating intestinal microbiota dysbiosis in cirrhosis is unclear. In recent years, postbiotics have been proven to great potential therapeutic value in patients with cirrhosis. Postbiotics have various effects such as maintaining gut microbiota, protecting intestinal barrier function, and immune regulation, which are expected to improve the current situation of intestinal microbiota dysbiosis in cirrhosis, delay disease progression, and reduce the occurrence of subsequent complications. Postbiotics have a certain preventive and therapeutic effect on intestinal barrier damage in cirrhosis, and there is currently some research on the treatment of diseases related to intestinal microbiota dysbiosis with postbiotics; some researchers have found that postbiotics produced by *E. coli* 1917 can significantly inhibit the abnormal proliferation of pathogenic Enterobacteriaceae under intestinal inflammation conditions, alleviate intestinal microbiota dysbiosis, and ultimately treat enteritis (Sassone-Corsi et al., 2016). Postbiotics produced by *Clostridium butyricum* had good therapeutic effects on mice with non-alcoholic hepatitis induced by high-fat diet, improving intestinal microbiota dysbiosis in non-alcoholic hepatitis mice and enhancing intestinal barrier function, thus achieving good therapeutic effects on non-alcoholic hepatitis (Zhou et al., 2017). It can be seen that postbiotics have broad application prospects in regulating intestinal microbiota dysbiosis in cirrhosis, thereby improving disease progression and complications related to intestinal microbiota dysbiosis. However, most of the research is based on animals, and in order to evaluate the beneficial effects of postbiotics on humans, it is hoped that large-scale researches can be conducted on humans in the future.

In addition to focusing on the biological activity and other advantages of postbiotics, the safety of their application must also be considered. The negative effects of postbiotics were evaluated in a randomized controlled trial of infants using inactivated *L. acidophilus*, which showed an indirect correlation with infant gastrointestinal tympanites, severe dehydration, and vomiting responses (Florez et al., 2020). Although postbiotics exhibit promising biological activity, most of these effects are limited to *in vitro* or animal studies and do not account for host pharmacokinetics and pharmacodynamics. Therefore, the health benefits of postbiotics must be clinically validated (Salminen et al., 2021). In the preparation of postbiotics, exogenous microbial metabolites or lysates can be directly added, or microorganisms can be inactivated through methods such as pyrolysis, ultrasonication, high-pressure treatment, or enzymatic treatment, allowing for the study of whole inactivated microorganisms. Thus, the selection of inactivation methods must be aligned with existing production processes and cost considerations. In addition, sensory and nutritional factors need to be further considered. The extensive autolysis of microorganisms may release flavor-active metabolites such as peptides, nucleotides, and amino acids, which can enhance the taste and flavor of the product. However, inactivation treatment methods may also lead to the loss of antioxidants, polyphenols

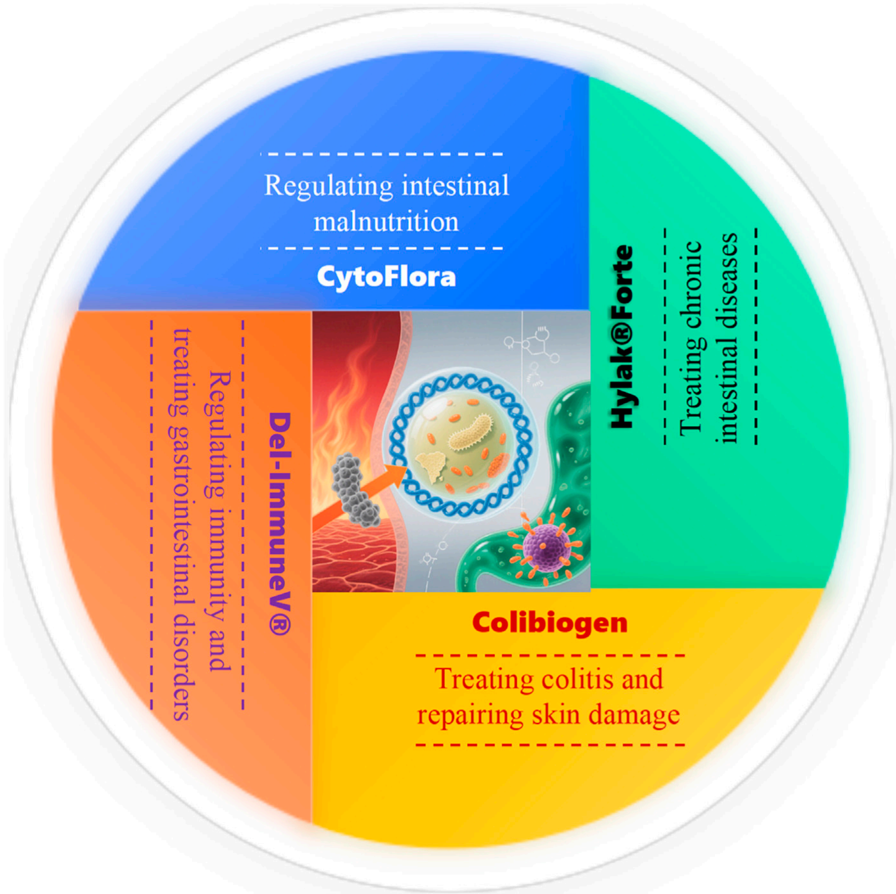

FIGURE 2
The main functions of the four types of postbiotics.

and vitamins, necessitating precise regulation during the process to ensure the product's nutritional value (Marson et al., 2020).

3 Application of postbiotics

In recent years, postbiotics has been introduced into the fields of medicine, veterinary medicine, and food, playing a unique role in preventing and treating certain diseases, improving animal health, and developing functional foods (Rad et al., 2020; Rinaldi et al., 2020; Riwes and Reddy, 2020).

3.1 Applied to the prevention and treatment of diseases

At present, free cell preparations obtained from different beneficial bacteria have been introduced into the prevention or treatment of diseases, with potential drug application value (Klein et al., 2013). Researchers are attempting to apply it to the matrix of pharmaceuticals and food, which may be a new strategy for preventing and assisting in the treatment of certain diseases. As shown in Figure 2, Colibiogen is a protein free commercial filtrate extracted from *E. coli*, containing amino acids, peptides, polysaccharides, and fatty acids. It can effectively resist antibiotic resistant and *Salmonella*, improve colitis in mice, and significantly reduce skin damage in patients with polymorphic solar rash (Aguilar-Toala et al., 2018). There is a product of postbiotics under the trademark CytoFlora, which is a mixture of cell wall isolates of *Lactobacillus casei*, *L. plantarum*, *L. acidophilus*, *L.* rhamnosus, *L.* salivarius, *L.* bulgaricus, *L.* acidophilus, *Bifidobacterium bifidum*, and *Streptococcus thermophilus*. It is used to regulate intestinal malnutrition, promote immune balance, and improve symptoms in children with autism (Ray et al., 2010; Roda et al., 2007). Hylak®Forte is a sterile liquid containing metabolites from *E. coli* DSM 4087, *S. faecalis* DSM 4086, *L. acidophilus* DSM 414, and *Lactobacillus helveticus* DS 4183, while, the main health benefits of Hylak®Forte include (Marov et al., 2014; Patil et al., 2019): promoting the growth of beneficial gut microbiota; adjusting the pH value of the intestinal environment and promoting the normal function of the digestive tract; energy supply of intestinal epithelial cells; regulating the balance of vitamin K. Another type of postbiotics is Del ImmuneV®, which is a registered and approved formulation by the US Food and Drug Administration (FDA), which contains rodentiyl peptides, amino acids, and DNA fragments of *Lactobacillus rhamnosus*. It is usually recommended to use it together with probiotics for treatment, and research results had shown that Del-ImmuneV® could effectively reduce the degree of gastrointestinal disorders in patients with autism spectrum disorders.

3.2 Applied to functional foods

Compared with probiotics, postbiotics are more stable and safe in industry, therefore, the application of postbiotics in the research and development of functional foods has certain technological advantages (Barros et al., 2020). Currently, there are various food products with bioactive ingredients, such as dairy and non-dairy products with added probiotics, to meet the nutritional needs of consumers with different dietary styles (milk protein allergy sufferers, lactose intolerance sufferers, vegetarians). Researchers have shown that the secondary metabolites such as chlortetracycline and bacteriocins extracted from *Bacillus* sp. CS93 have stable and antibacterial properties, which make them widely used in food (Phister et al., 2004). On the one hand, some milk products and other products (such as Kombucha and pickled Chinese cabbage) may produce postbiotics during fermentation. On the other hand, postbiotics can also be purposefully added to various foods (Wasilewski et al., 2015). Researches have shown that the postbiotics from the supernatant of *L. plantarum* YML 07 can serve as a potential biological preservative, which can increase the shelf life of soybeans to 2 months (Rather et al., 2013). Nisin is an antibiotic produced by specific *Streptococcus lactis* and the only bacteriocin approved for use as a food preservative (Fusieger et al., 2020). At present, foods containing nisin include canned soups, baked goods, mayonnaise, and dairy products. Furthermore, extracellular polymeric substances (EPS) containing rare sugars have found new applications in the food industry due to their physicochemical properties (thickening, stability, or water binding ability) and palatability in food. However, except for dextran, due to the low EPS production of lactic acid bacteria, they have not yet been commercially developed as food additives (Ispirli et al., 2020). Another biological function of postbiotics is its detoxification of toxic metabolites, which has attracted widespread attention from scientists in recent years (Tomasik and Tomasik, 2020; Wang et al., 2019).

3.3 Applied to food packaging

Due to the harmful environmental impacts of synthetic packaging, the food manufacturing industry has developed organic packaging materials such as edible coating materials, which exhibit excellent gas barrier properties, extend food shelf life, and potentially contain antioxidant and antimicrobial compounds. MOHAMMADI R et al. utilized postbiotics to enhance the antibacterial properties of bacterial nanocellulose, developing antimicrobial films based on bacterial nanocellulose packaging materials (Mohammadi et al., 2022). The postbiotics demonstrated concentration-dependent antimicrobial activity against all studied bacterial strains. Research has shown that edible coatings developed using fermentation products of *Saccharomyces boulardii* ATCC MYA-796 and polysaccharides exhibit preservative effects on lamb meat, effectively reducing microbial growth, extending meat shelf life, and maintaining favorable sensory characteristics of lamb (Sivananthan and Petersen, 2018). ABBASI A et al. found that yeast composite films incorporating bacterial cellulose produced by yeast fermentation, along with carboxymethyl cellulose and glycerol, displayed high water solubility (42.86%), extended the shelf life of food packaging materials (Abbasi et al., 2023).

According to data from Future Market Insights, the global postbiotic supplements market is projected to reach $28.3 million by 2032, with the compound annual growth rate expected to increase from 7.6% during 2016–2021 to 11.5% during 2022–2032. Additionally, patent database searches revealed that from 2001 to 2020, there were a total of 2,215 global patents related to prebiotics and postbiotics. Compared to 2001–2010, the number of patents grew by 1.45% during 2011–2020 (Asif et al., 2023; Zang et al., 2024). Therefore, more research and clinical trials are needed in the future to enhance the control over the safety issues of postbiotics. It is necessary to establish industry, national, or international standards for postbiotics and improve the evaluation systems for their safety and functionality. In terms of technology and product development, it is also essential to explore more plant-based raw materials and microbial strains suitable for fermentation, optimize fermentation processes, and increase the yield of functional components in postbiotics. This will enable their broader application in products and facilitate the development of novel postbiotics with unique flavors and nutritional health benefits.

4 Conclusion

Compared with probiotics, postbiotics not only has the same beneficial effects, but also has some other advantages, such as known molecular structure, use after purification, specific mechanism of action, easier industrial scale production, easier production and storage, etc. Therefore, postbiotics has great application prospects in medical treatment and food. However, we lack the understanding of the specific mechanism of postbiotics *in vitro* and *in vivo*, and further research is needed to understand their beneficial effects on humans and animals.

Author contributions

XC: Writing – original draft, Methodology, Conceptualization. CY: Investigation, Writing – original draft, Visualization. JH: Writing – review and editing, Investigation. WL: Writing – original draft, Methodology. CL: Conceptualization, Writing – review and editing.

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Conflict of interest

Author WL was employed by Yibin Branch of Sichuan Tobacco Corporation.

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

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