Full Length Article

POSTBIOTICS OF LACTIC ACID BACTERIA: POTENTIAL APPLICATIONS IN FOOD AND DAIRY INDUSTRY

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ABSTRACT

The review explores the emerging significance of postbiotics, the non-viable microbial cells or their metabolitesas potential alternatives to probiotics indevelopment of functional foods, bio preservation, food packaging and enhancing gut health. It highlights the advantages of postbiotics, including superior stability, effective functionality without the need to survive in the digestive tract and a reduced risk of allergic reactions. The review also discusses the incorporation of postbiotics into foods and supplements, emphasizing their bioactive role in modulating the gut microbiome. Additionally, it addresses the influence of environmental factors such as temperature and pH on postbiotic efficacy, underscoring the importance of controlled conditions to maximize health benefits.

Keywords: Postbiotics, gut health, bio preservation, packaging, functional foods.

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INTRODUCTION

Hippocrates's well-known statement "all diseases begins in the gut" has gained new relevance as modern research delves into the complex interactions between gut microbiota and health. Recent studies reveal that disruptions in gut microbiomes can significantly affect immune responses and contribute to various diseases. In this context, postbiotics are emerging as promising alternatives to probiotics.

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The International Scientific Association for Probiotics and Prebiotics defines postbiotics as "preparations of inanimate microorganisms and/or their components that confer health benefits on the host" (Salminen *et al*., 2021). Unlike probiotics, which are live microorganisms, postbiotics consist of non-viable microbial cells or their metabolites. Postbiotics have been shown to positively impact health, including immune modulation, antibacterial properties, improvements in conditions like inflammation, oxidative stress and cardiovascular health (Gurunathan *et al*., 2020; Yelin *et al*., 2019, Ma *et al*., 2023).

Various foods, such as yoghurt, flaxseeds, garlic and fermented products can foster an environment conducive to postbiotic formation. Despite being relatively new compared to probiotics and prebiotics, postbiotics offer several advantages. Postbiotics are less sensitive to environmental factors such as heat and light, making them easier to store and use (Nataraj *et al*., 2020). Unlike probiotics, which may lose viability before reaching the gut, postbiotics need not survive the digestive tract's harsh environment to function effectively (Izuddin *et al*., 2020). Since they contain no live microorganisms, postbiotics are less likely to trigger allergic responses.

Major advantage of postbiotics is their ease of incorporation into food and supplements, as they do not require the stringent conditions necessary for probiotic formulations. The mechanism of action of postbiotics is mediated by bioactive compounds, such as peptides and organic acids produced during fermentation. These compounds can directly benefit health by modulating the gut microbiome (Rad *et al*., 2020). In contrast, probiotics work by colonizing the gut with live bacteria that compete with harmful microorganisms and support immune function (Humam *et al*., 2021).

Temperature and pH are critical factors that influence the effectiveness of postbiotics. Research indicates that postbiotics function optimally within a pH range of 4 to 9, with foods like pasteurized milk and ground beef providing ideal environments (Moradi *et al*., 2020). However, excessive heat can reduce their efficacy.Studies shown that temperatures above 121°C can significantly diminish their antibacterial activity (Mirnejad *et al*., 2013). Therefore, careful control of environmental conditions is essential to maximize the health benefits of postbiotics.

Preparation of Postbiotics

The preparation of postbiotic mixtures typically involves the development of a cell-free supernatant solution, either with or without cell lysis. This solution generally contains products of microbial metabolism, metabolites synthesized by the action of lactic acid bacteria (LAB) on culture media or food ingredients or structural substances produced by LAB that are incorporated into the postbiotic mixture. The composition and quantity of these components largely depend on factors such as the type of bacterial strain, culture medium used and bacterial treatments after propagation.

In most of the studies focusing on the application of postbiotics in food, no post-propagation treatments were applied. In such cases, postbiotics consisted solely of soluble factors, including products or metabolic by-products secreted into the medium during bacterial growth. However, in some instances, bacterial cells were subjected to post-propagation lysis using methods such as enzymatic treatments, thermal processes, sonication, high-pressure techniques or combinations of these approaches to release additional bioactive compounds (Fig.1).

Dairy-based substrates like low-heat milk and milk permeate have been optimized as fermentation media, with ideal incubation time and temperature, to produce antifungal postbiotic solutions using Lactobacillus spp.

Postbiotic mixtures prepared with low-heat milk demonstrated remarkable antifungal activity, likely due to differences in protein and fat content. Both low-heat milk and milk permeate exhibited compatibility with dairy/food applications. Additionally, skim milk has been successfully utilized at an industrial scale for the production of postbiotics using an adjunct culture.

Alternative dairy substrates, including whey and buttermilk, have also been explored as suitable propagation media for various commercial probiotics. These substrates have shown promising results, indicating their potential in supporting the development of effective postbiotic mixtures for food applications (Moradi *et al.*, 2021).

Stages in the Preparation of Postbiotics

There are five major stages in the preparation of postbiotics as given below.

Inoculation of LAB, propagation of LAB, followed by heat treatment, then postbiotic harvesting and finally postbiotic concentration.

Postbiotics have notable applications in food safety, including bio-preservation, packaging, biofilm removal and the degradation of toxic contaminants. They offer a promising avenue for enhancing host health, with current research focusing on elucidating their mechanisms of action and developing innovative functional food and preventive medication formulations.

Moreover, the inherent stability of postbiotics across a broad range of temperatures and pH levels enables their incorporation into foods and ingredients prior to thermal processing, providing producers with technical and economic advantages. Postbiotics also serve as essential components in delivery systems for functional foods and pharmaceutical products, as their optimal dosage can be controlled during production and storage, where survival viability is not a primary concern (Rad *et al*., 2020; Thorakkattu *et al*., 2022).

Overall, the growing interest in postbiotics reflects a shift toward holistic approaches to health and wellness, with nutrition playing a central role. As research continues to uncover the full potential of postbiotics, more innovative products and solutions are expected to emerge, catering to the diverse needs and preferences of consumers worldwide (Aggarwal *et al.,* 2022). This review primarily focuses on the following applications of postbiotics:

1. Bio-preservation
2. Food packaging
3. Development of novel functional foods
4. Control of biofilms

1. Bio-preservation

Bio-preservation is a method used to enhance the shelf life of food by utilizing natural microbiota or antimicrobial agents. In this context, postbiotics play a crucial role by exhibiting antimicrobial properties against spoilage and pathogenic microorganisms. The mechanism of action includes creating pores in cell membranes, altering cell wall proteins and lowering cytoplasmic pH. When combined with other preservation methods, postbiotics help to reduce foodborne pathogens while maintaining the sensory qualities of food (Homayouni *et al*., 2021).

In the meat industry, postbiotics are applied to products such as fish fillets and ground meats to prevent spoilage and extend shelf life. Common spoilage bacteria, including *Clostridium perfringens* and members of the Enterobacteriaceae family, often cause undesirable organoleptic changes. Utilizing lactic acid bacteria (LAB) and their antimicrobial metabolites, such as bacteriocins, helps topreserve meat products without compromising their sensory quality (Bhattacharya *et al*., 2022). For instance, postbiotics derived from *L. paracasei* and *S. boulardii* have been shown to extend the shelf life of chicken sausages (Godoy *et al*., 2022). Similarly, *L. plantarum* postbiotics have demonstrated effectiveness against pathogens such as Salmonella and *E. coli* in chicken breast meat, extending its shelf life up to 14 days (Serter *et al*., 2024). Furthermore, postbiotics from *L. salivarius* have exhibited significant antibacterial activity against *L. monocytogenes* (Moradi *et al*., 2019).

In dairy products also, postbiotics play a vital role in bio-protection and preservation. They inhibit the growth of foodborne pathogens, destroy microbial biofilms and degrade harmful chemicals. A novel approach involves the use of postbiotics derived from ultra-filtered cheese whey to preserve mozzarella cheese, demonstrating significant microbial inhibition (Sharafi *et al*., 2022). Likewise, lyophilized postbiotics from *L. acidophilus*, *Bifidobacterium bifidum* and *Lactobacillus plantarum* have effectively controlled pathogens in soft feta cheese (Hamad *et al*., 2017). Additionally, postbiotics from *L. acidophilus* and *B. animalis* have shown strong antibacterial activity against spoilage organisms, making them indispensable for dairy preservation.

In summary, postbiotics derived from LAB and other beneficial microorganisms are valuable for the bio-preservation of meat and dairy products. They extend shelf life, enhance safety and maintain product quality (Ozma *et al*., 2023; Incili *et al*., 2023). With their potent antimicrobial properties, postbiotics are a promising tool in modern food preservation strategies.

2. Food Packaging

Food packaging consists of products manufactured from various materials designed to protect, pack, manipulate, distribute, transport and identify food throughout the supply chain from farms to consumers. Meat, with its high water-binding capacity and abundance of nutrients, serves as a suitable substrate for microbial growth (Bekhit *et al*., 2021). Packaging plays a crucial role in maintaining the nutritional, sensory, physicochemical and microbiological qualities of food. In this context, the most notable innovation in the dairy and meat packaging industries is smart packaging. Currently, two primary types of packaging-intelligent and active-are used for various purposes in meat and dairy products (Kuswandi and Moradi, 2018). Among active packaging approaches, antimicrobial packaging (AMP) is widely recognized. AMP typically consists of three components: film-forming polymers, solvents and antimicrobial agents.

Research highlights the application of postbiotics in antimicrobial packaging. The primary antibacterial mechanism of postbiotics involves acidifying the mobile cytoplasm, halting energy production and inhibiting the growth of pathogenic microorganisms. This is achieved through the formation of pores in cellular membranes, structural and functional modifications of sensitive components such as proteins and peptides, increasing acidity within bacterial cell membranes and inducing bacterial cell oxidation (Hosseini *et al*., 2021).

Rasouli *et al*., (2021) prepared postbiotics from *Lactobacillus sakei*, a well-known probiotic bacterium, to develop anti-Listeria paper based bacterial nanocellulose (BNC) for meat applications. They found that BNC supplemented with postbiotics exhibited significantly higher antibacterial activity (a reduction of over 2 log cycles) compared to control. Similarly, Yordshahi *et al*., (2020) utilized lyophilized postbiotics from *Lactobacillus plantarum* to impregnate BNC using an ex-situ method, creating an antimicrobial nano-paper for wrapping ground meat. The optimized P-BNC film (21.21% concentration and 28-minute impregnation time) demonstrated a substantial reduction (~5 log cycles) in *Listeria monocytogenes* counts after nine days of storage at 4°C. Additionally, the film significantly reduced total mesophilic and psychrophilic counts and TBA values compared to control.

BNC has proven to be an effective carrier for developing antimicrobial films using postbiotics from lactic acid bacteria (LAB) for food applications. However, Mohammadi *et al*., (2022) observed that wet-form postbiotic-BNC exhibited greater antimicrobial activity than the lyophilized form due to its high adsorption capacity and open 3D structure. The wet-form BNC demonstrated zones of inhibition exceeding 20 mm for all bacterial pathogens, classifying them as "extremely sensitive microorganisms" to postbiotics at a 50% concentration. In contrast, fungal strains showed lower zones of inhibition (<17 mm). The antimicrobial susceptibility order was: *Listeria monocytogenes* > *Staphylococcus aureus* > *Escherichia coli* > *Salmonella typhimurium* > *Aspergillus flavus* > *Penicillium citrinum*.

Sharaf *et al*. (2019) developed chitosan nanoparticles containing postbiotics produced by seven LAB strains (*L. plantarum, L. helveticus, L. rhamnosus, L. reuteri, Streptococcus thermophilus, Enterococcus faecium* and *L. lactis*). These nanoparticles demonstrated both antibacterial and antifungal activity in Egyptian cheese. In another study, Salvucci *et al*., (2019) produced postbiotics from *Enterococcus faecium* and used their semi-purified bacteriocin extract as an antimicrobial agent in the formulation of triticale flour films.

3.Development of Novel Functional Food

Food is a fundamental need that meets the nutritional requirements of individuals. Essential nutrients like fat, carbohydrate and protein provide energy for growth and maintenance, while non-nutrient components (e.g., fiber, phytochemicals, antioxidants, probiotics and prebiotics) enhance human health by positively modulating host physiology. Functional meat and dairy products can be developed by incorporating naturally occurring ingredients such as prebiotics, probiotics, vitamins, minerals, flavouring agents and extracts from plants, fruits and other sources. Among these, probiotics and their metabolites are gaining prominence in the food industry as valuable functional ingredients.

In the dairy industry, probiotics are described as "live microbial food ingredients that are beneficial to health" (Clancy, 2003). However, a major challenge in probiotic product formulations whether pharmaceutical or commercial food-basedis maintaining bacterial viability during manufacturing and storage. The effectiveness of probiotic delivery systems can be affected by various factors. In contrast, postbiotics, which are stable metabolites of probiotics, offer advantages in terms of stability and functionality (Aguilar-Toala *et al*., 2018).

Recent studies have highlighted the benefits of postbiotics as functional food ingredients. Postbiotics the health-promoting metabolites derived from probioticsare emerging as a significant advancement in functional biotics. They offer several advantages over traditional probiotics, including known molecular structures, availability in purified forms, specific mechanisms of action and better accessibility (Nataraj *et al*., 2020). In functional food applications, postbiotics can be incorporated into a wide range of products, including dairy and non-dairy alternatives, beverages, snacks and supplements. This versatility allows consumers to seamlessly integrate postbiotics into their diets, promoting gut health and overall well-being.

Ramos *et al*., (2022) reported the first study on postbiotic compound production by Leuconostoc strains in sheep milk. Leuconostoc strains, isolated from artisanal Manchego cheese and craft beer, were analyzed for safety traits, postbiotic potential, α-glucosidase inhibitory activity and their ability to withstand technological stress conditions. These strains exhibited promising properties from both technological and health perspectives.

Warda *et al*., (2019) investigated the effect of postbiotics derived from *Limosilactobacillus fermentum* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in a mouse model. The postbiotics, produced through high-temperature fermentate treatments, influenced behaviour of mouse by increasing sociability, lowering baseline corticosterone levels and subtly altering gut microbiota composition. While postbiotics did not prevent Citrobacter infection, postbiotic-fed mice demonstrated physiological benefits, such as reduced colon crypt depth compared to control (Warda *et al*., 2020). Rad *et al*., (2020) reviewed the role of postbiotics in colorectal cancer prevention and treatment, noting their ability to mimic the health benefits and biological responses mediated by probiotics.

Postbiotics were found to have prophylactic effects through various mechanisms, potentially aiding in the prevention and management of colorectal cancer.

Sadighbathi *et al*., (2023) investigated the effects of adding postbiotics derived from *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB) and *Streptococcus thermophilus* (ST) to cheese whey (CW) and skim milk (SM) during yoghurt production. Over a 22-day storage period, the LB-CW yoghurt sample exhibited the strongest antioxidant activity, with 18.71% inhibition ($p>0.05$), compared to ST-SM yoghurt. Postbiotics have also improved yoghurt's nutritional value and enhanced its mouthfeel, making it more appealing as a healthy food option.

4. Control of Biofilms

Biofilm formation is a resilient attachment process where a collection of microorganisms adheres to a specific surface, significantly contributing to microbial contamination in food and dairy processing plants. Biofilms form on abiotic surfaces that come into contact with food. These microbial communities are embedded in a self-produced exopolymer matrix, which reduces the efficacy of detergents. Due to the challenges posed by biofilms in food safety and quality, a consistent and stringent sanitation program is essential in food processing environments (Khani *et al*., 2023).

Foodborne pathogens can form biofilms on various abiotic surfaces commonly used in the food industry, including silicone rubber, plastic, glass, stainless steel and rubber gloves. This biofilm formation poses a severe threat to food safety by causing to contamination, food spoilage and equipment erosion, which negatively impacts the financial health of food industry (Hossain *et al*., 2017). Ensuring food safety involves eliminating or eradicating microbial and chemical contaminants during the production, transportation and storage of food.

Although postbiotics have received less scientific attention than probiotics, they represent an emerging concept in the food industry with promising applications, particularly in controlling biofilm formation. Hosseini *et al*., (2021) reported that postbiotics significantly inhibit the swimming motility and expression of key target genes associated with the pathogenicity and biofilm formation of *Listeria monocytogenes*. These multifunctional effects suggest that postbiotics could serve as alternative biological agents to prevent *L. monocytogenes* biofilm formation in the food industry.

Similarly, Moradi *et al*., (2019) found that *Lactobacillus spp*. cell-free supernatant (CFS) effectively controlled bacterial pathogens in meat and milk. CFS derived from *L. salivarius* exhibited strong biofilm-reducing properties, likely due to the presence of exopolysaccharides, organic acids and biosurfactants. Additionally, postbiotics derived from *L. acidophilus* demonstrated a strong antibiofilm effect against both gram-positive and gram-negative bacteria. Postbiotics derived from *L. acidophilus* LA5, *L. casei* 431 and *L.*

salivarius were shown to damage biofilms formed by *L. monocytogenes* on polystyrene surfaces, primarily due to their bacteriocin- and organic acid-based mechanisms (Moradi *et al*., 2019).

Clinical Applications of Postbiotics

Beyond the food industry, postbiotics are gaining attention for advanced clinical treatments. Kim *et al*., (2023) demonstrated the potent antibacterial activity of postbiotics produced by *Lentilactobacillus kefiri* LK1, isolated from kefir, against pathogens causing bovine mastitis. These postbiotics significantly suppressed auto-aggregation and exopolysaccharide production in *Enterococcus faecalis* while down regulating wspA and pelA gene expression in *Pseudomonas aeruginosa*. Compared to postbiotics from *Enterococcus faecium* EFM2, the postbiotics of *L. kefiri* LK1 showed greater efficacy.

Sevin *et al*., (2021) also highlighted the potential of postbiotics derived from *Lactobacillus sakei* EIR/BG-1, originating from milk microbiota in preventing mastitis. These postbiotics exhibited antibacterial and antibiofilm activity against key mastitis-causing pathogens, offering sustainable alternatives for livestock farming and novel strategies for mastitis management in bovines.

According to Khani *et al*. (2023) postbiotics stimulated persistent antibody generation and NK-cell activity, which improved immunization effectiveness in the elderly. Older adults who consumed heat-killed *Lactobacillus paracasei* jelly had better antibody responses to H1N1 A and B antigens. Additionally, immunomodulatory and antibacterial properties of postbiotics make them promising for treating infections in children under five, much like their probiotic counterparts. Additionally, because of their special qualities, postbiotics may be a new way to treat foodallergies in children.

Cell-free supernatant (CFS) of Lactobacilli, have demonstrated promise in the treatment of dental disorders in humans. In a study conducted by Ishikawa *et al*., (2020) the viable counts and biofilm biomass of *Aggregatibacter actinomycetemcomitans* (Aa), a major pathogen in periodontitis, were reduced by CFS of *Lactobacillus acidophilus* LA5. Notably, Aa colonization has been decreased and virulence factors that aid the bacteria in avoiding human defences have been modulated.

The use of postbiotics as adjuvant for the prevention and treatment of COVID-19 was studied. Research has demonstrated that postbiotics, in addition to probiotics and prebiotics, can lessen the intensity of symptoms and slow down the causes of the disease's course. They can also stop SARS-CoV-2 from replicating by focusing on single-stranded RNA. Postbiotics target the respiratory tract's epithelial cells, where SARS-CoV-2 infections occur. They also encourage the immune system and gut microorganisms to communicate, which is essential for fighting infections. Products that include postbiotics like bacteriocins have been shown to lessen the symptoms of COVID-19 (Isaac-Bamgboye *et al*., 2024 and Catalano *et al*., 2022).

CONCLUSION

The exploration of postbiotics as a dynamic and integral component of the dairy and food industry represents a promising paradigm shift towards functional and safer food alternatives. Unlike probiotics, postbiotics are less susceptible to environmental stressors, offering a more reliable and consistent functional ingredient for food applications. With their stability, versatility and health benefits postbiotics are emerging as a cornerstone of modern food technology and nutraceutical development. The multifunctional applications of postbiotics in food, including bio preservation, packaging and biofilm control underscore their capacity to revolutionize food quality and safety. As research into their potential continues to expand, postbiotics promise to usher in a new era where food serves not only as sustenance but also as a proactive tool for health management and disease prevention. The vision of incorporating postbiotics into daily diets globally aligns with the growing emphasis on health and wellness, emphasizing naturally derived and clean-label food ingredients. This approach caters to the needs of health-conscious consumers while meeting the rigorous demands of scientific validation. As we move forward, it is vital to sustain this journey of discovery, ensuring that the benefits of postbiotics are fully harnessed and made universally accessible. By doing so, we can leave a lasting mark on the landscape of food science and human health, paving the way for a future where food plays a central role in holistic well-being.

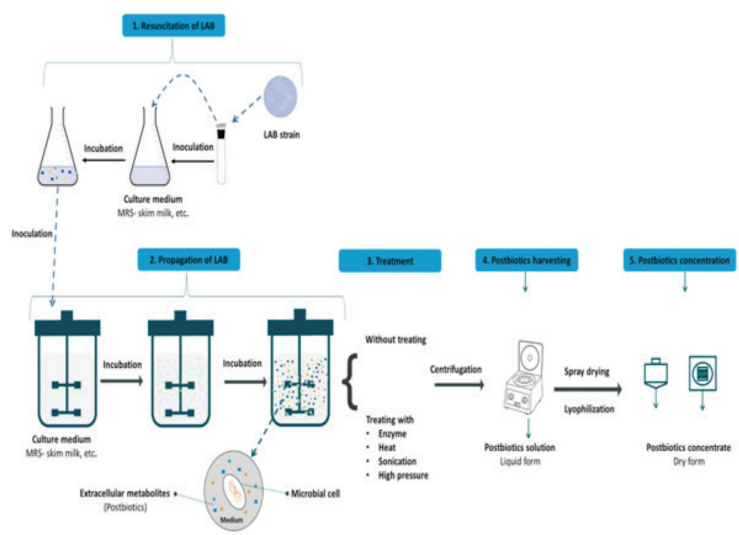

Fig.1. Stagesof preparing postbiotics (cell free supernatant) mixture from lactic acid bacteria

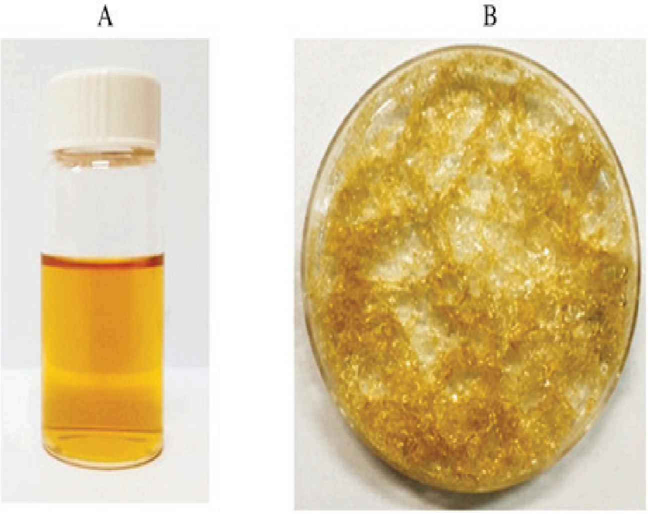

Fig.2. Appearance of Postbiotics (A- Solution and B-Lyophilized)

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