

POSTBIOTICS AS EMERGING FUNCTIONAL INGREDIENTS: MECHANISMS, HEALTH BENEFITS, AND APPLICATIONS IN GUT AND METABOLIC HEALTH

Revathy S¹, Revathy.K², Firdose Fathima G³, Latha G⁴

II MSc Food and Nutrition¹, II MSc Food and Nutrition²,

II MSc Food and Nutrition³, Assistant Professor⁴

Department of Food Science and Nutrition,

Dr. N.G.P. Arts and Science College, Coimbatore, India

Abstract: Growing evidence of the link between gut microbiota and human health has created interest in dietary modifications that can impact microbial activity and host responses. Although probiotics and prebiotics have been widely studied for this purpose, it can be complex to preserve the viability and effectiveness of living microorganisms throughout food processing and storage. This has drawn attention to postbiotics, a relatively new class of microbial-derived alternatives that contain inanimate microorganisms and/or their constituents that have been shown to promote the host's health. Postbiotic preparations may contain inactivated bacterial cells, and their components such as cell-wall structures, proteins, polysaccharides depending on the microbe and processing applications. Certain preparations may affect immunological responses, inflammatory signaling, intestinal barrier function, and interactions within the gut microbial population, according to experimental data. Recent studies on humans have also shown that certain postbiotic preparations are useful, especially for specific immunological outcomes and gastrointestinal problems. From a food technology perspective, postbiotics have potential due to their non-viable nature, which may provide more processing and storage flexibility than live probiotic cultures. However, there are still significant variations in microbial strains, production techniques, inactivation processes, composition, and dose in the field. Furthermore, while human clinical evidence is still very limited, a large portion of the current evidence is derived from laboratory and animal investigations. The current understanding of postbiotics, their main compositions, sources and manufacturing, methods of action, possible roles in human health and the gut, uses in functional foods, safety concerns, research constraints, and future possibilities are all included in this study. Overall, postbiotics represent a promising area at the intersection of food science, microbiology, and nutrition, although stronger clinical evidence and standardized approaches are needed to support their wider application.

Keywords: Postbiotics; Gut health; Functional foods; Gut microbiota; Human nutrition.

1. INTRODUCTION

The gastrointestinal tract is an interactive biological system where host tissues, the microbes, and food components constantly influence each other. The microbes that live in the gut help to control activity keep the intestinal barrier strong produce microbial metabolites and help to break down food. Because of this changes in the ecosystem inside the gastrointestinal tract have been linked to many aspects of human health. This has raised interest in ways to support a healthy connection, between the host and the microbes. (Valdes *et al.*, 2018).

Probiotics, prebiotics, and synbiotics have drawn a lot of attention as a result of this expanding knowledge. Prebiotics are substrates that host microorganisms specifically use to produce health benefits, whereas probiotics are living microbes that offer health benefits when ingested in sufficient doses. Live microorganisms and substrates that the host bacteria specifically use are combined in synbiotics. The longevity of probiotic bacteria can be impacted by manufacturing conditions, storage, and the food matrix, which presents a persistent issue for food makers even if these technologies have significant uses. (Marco *et al.*, 2021).

Certain biological effects linked to microbes may continue even after the cells have been rendered non-viable, according to a growing body of research. This observation contributed to interest in what are now described as postbiotics. Non-viable microbial preparations were referred to by a number of terms in earlier literature, such as paraprobiotics and heat-killed probiotics. In order to differentiate postbiotics from other microbial products and establish a research and product development a standard framework, it was deemed important to define a common terminology. (Salminen *et al.*, 2021).

According to the current consensus, a postbiotic is a preparation of inanimate microorganisms and/or their constituents that has been shown to promote the host's health. Crucially, this term doesn't pertain to any material derived from microorganisms; rather, it relates to a preparation. The health benefit of isolated microbial metabolites alone must be proven for the appropriate preparation and target host in order for them to be categorized as postbiotics. This distinction is particularly important when interpreting the rapidly expanding postbiotic literature. (Salminen *et al.*, 2021).

The non-viability of postbiotics has gained the interest of the food manufacturers because their functionality does not rely on the presence of living microbial cells. This could make it easier to include probiotics in foods that are subjected to processing conditions that may not be appropriate for them. However, non-viability does not mean that postbiotic preparations are completely unaffected

by processing. The stability or activity of their functional components can still be changed by temperature, moisture, oxygen, pH, and interactions with the food matrix. (Thorakkattu *et al.*, 2022).

Simultaneously, the growth of postbiotic research has accelerated the creation of standardized techniques for manufacturing and assessing these preparations. The microorganisms employed, culture conditions, inactivation methods, composition, dosage, and experimental paradigms vary among studies. As a result, conclusions drawn from a single preparation cannot always be applied to all postbiotics. One of the main problems that now prevents comparisons between studies is this heterogeneity. (Wei *et al.*, 2024). Clinical research is still far less extensive than the preclinical literature, despite the fact that a number of experimental investigations have documented impacts on intestinal barrier function, immunological responses, and inflammation. For instance, both live and heat-treated *Bifidobacterium longum* CECT 7347 significantly reduced symptoms when compared to a placebo in a randomised controlled trial including 200 persons with diarrhea-predominant irritable bowel syndrome. Although these results are positive, they are still unique to the population and preparation under study. (Srivastava *et al.*, 2024).

Therefore, postbiotics are better viewed as an emerging group of preparation-specific functional ingredients rather than a single uniform category with established effects. A critical review is needed to connect their microbiological characteristics with food applications and human health outcomes while also identifying the limitations that must to be addressed before wider nutritional use. This review therefore examines the concept, components, sources, production methods, mechanisms, health effects, functional food applications, safety issues, research gaps and future prospects of postbiotics.

2. CONCEPT AND DEFINITION OF POSTBIOTICS

The idea behind postbiotics is based on the observation that microbial cells contain structures capable of interacting with host tissues independently of their ability to grow. Once a microorganism has been deliberately inactivated, its cellular structures may remain biologically recognizable. Depending on the preparation, these structures can interact with epithelial cells, immune cells or other microorganisms and may contribute to measurable biological effects. (Salminen *et al.*, 2021).

Before the consensus definition was introduced, the literature contained considerable variation in terminology. Terms such as paraprobiotics, non-viable probiotics, tyndallized probiotics and bacterial lysates were used for related preparations. Although these terms sometimes referred to similar materials, their definitions were not necessarily equivalent. The adoption of the term postbiotic was intended to provide a clearer scientific framework for preparations that meet specific criteria. (Salminen *et al.*, 2021).

For a preparation to meet the current postbiotic concept, the source microorganism should be adequately characterized, the method used to inactivate it should be described, complete inactivation should be demonstrated, the composition of the final preparation should be documented, and a health benefit should be established in the relevant host. Safety for the intended use is also an essential consideration. These requirements distinguish a scientifically characterized postbiotic from an unspecified mixture of microbial products. (Salminen *et al.*, 2021).

The difference from probiotics is simple but significant. While postbiotics are purposefully made from inanimate microbial material, probiotics require live microbes. This distinction might provide technological benefits, but it shouldn't be taken as proof that postbiotics are inherently better. The microbial strain, preparation technique, content, and dosage all affect the biological response. (Thorakkattu *et al.*, 2022).

3. MAJOR COMPONENTS OF POSTBIOTICS

Postbiotic preparations can differ significantly in content. They may include intact, inactivated cells, cell wall structures, surface proteins, polysaccharides, enzymes, nucleic acid-associated material, and microbial metabolites that are still present in the preparation, depending on the microorganism and manufacturing technique. Different postbiotics may function through distinct biological pathways, which can be explained by this compositional diversity (Wei *et al.*, 2024).

3.1 Inactivated microbial cells

Whole inactivated cells are among the most recognizable forms of postbiotic preparations. Although they cannot reproduce, their surface structures remain available for interaction with host receptors. Evidence from both experimental and clinical studies demonstrates that certain heat-treated microorganisms can retain biological activity, suggesting that viability is not an absolute requirement for all beneficial microbial effects. (Srivastava *et al.*, 2024).

3.2 Cell-wall structures

The host immune system can recognize several molecules found in microbial cell walls. Lipoteichoic acid-associated structures, peptidoglycan, and other surface elements can interact with pattern-recognition receptors to affect cellular signaling. Although their effects vary depending on the microbial source and preparation, experimental investigations suggest that these interactions may impact inflammatory responses and epithelial integrity (Salminen *et al.*, 2021).

3.3 Proteins and polysaccharides

Surface proteins and microbial polysaccharides are also being investigated as functional constituents. These molecules may interact directly with intestinal epithelial cells or alter the surrounding microbial environment. Experimental work using components derived from *Lactocaseibacillus paracasei* has demonstrated changes in epithelial barrier-related responses and microbial fermentation, highlighting the possibility that individual cellular components may contribute to postbiotic activity. (Xiao *et al.*, 2024).

3.4 Microbial metabolites

Microorganisms produce a wide range of metabolites including organic acids and short-chain fatty acids during growth and fermentation. These compounds can have important effects on host physiology. However, an important distinction must be maintained: a purified microbial metabolite by itself does not meet the current consensus definition of a postbiotic. It may instead be a component of a postbiotic preparation. (Salminen *et al.*, 2021).

4. SOURCES AND PRODUCTION OF POSTBIOTICS

The development of a postbiotic begins with the selection of a suitable microorganism and the establishment of conditions that support reproducible growth. Factors such as strain characteristics, nutrient composition, temperature, pH and incubation time can

influence the cellular composition and metabolites generated during cultivation. Therefore, production conditions can become an important determinant of the properties of the final preparation. (Zhong *et al.*, 2024).

Fermentation is particularly relevant to food applications because it can be performed using food-compatible microorganisms and substrates. Depending on the substrate and microbial strain, fermentation may modify the nutritional and chemical characteristics of the food while simultaneously generating microbial biomass and bioactive compounds. This creates an opportunity to combine conventional food fermentation with postbiotic production. (Viswanathan and Muthusamy, 2022).

The microbial cells must be made non-viable after cultivation. Due to its ease of use and compatibility with numerous industrial processes, heat treatment is one of the most often researched techniques. However, since excessive heat can change proteins and other elements that could support biological activity, the temperature and heating time must be carefully regulated. (Thorakkattu *et al.*, 2022).

Alternative physical methods, including high-pressure processing and other non-thermal technologies, have attracted interest because they may reduce damage to heat-sensitive compounds. The most appropriate method will depend on the characteristics of the microorganism and the desired composition of the final preparation. Thus, the inactivation step should be treated as part of product design rather than merely as a means of eliminating microbial viability. (Zhong *et al.*, 2024).

Subsequent processing may include separation, concentration, drying and stabilization. These steps influence moisture content, storage stability and the recovery of functional components. For a postbiotic to be reproducible, researchers therefore need to describe not only the microbial strain but also the matrix, inactivation method and downstream processing conditions. (Salminen *et al.*, 2021).

5. MECHANISMS OF ACTION

Postbiotics are unlikely to function through a single, universal mechanism. Their effects depend on the biological target as well as the chemicals and structures found in the preparation. Interactions with intestinal epithelial cells, immunological signaling regulation, microbial community manipulation, and modifications to oxidative or inflammatory pathways are some of the potential processes (Wei *et al.*, 2024).

5.1 Intestinal barrier function

The intestinal barrier is crucial in regulating permeability and divides the internal environment from the contents of the gut. Increased permeability and inflammatory reactions can result from the disruption of tight-junction proteins, which aid in preserving the integrity of the epithelial layer. The capacity of several microbially generated preparations to maintain or restore epithelial barrier function has been studied. A preparation made from *Lactiplantibacillus plantarum* was shown to enhance barrier integrity in an experimental intestinal epithelial model after exposure to inflammatory stimuli. The researchers connected this effect to modifications in tight-junction regulation-related signals. Although cell-based research cannot be regarded as equal to clinical evidence in humans, such findings offer a potential molecular explanation for postbiotic effects on the intestinal barrier. (Zhang *et al.*, 2022).

5.2 Interaction with the gut microbial environment

The intestinal microbiota may potentially be impacted by postbiotics. Changes in the generation of short-chain fatty acids and variations in the abundance of specific bacterial groups have been observed in *in vitro* fermentation experiments employing microbially derived components. These results imply that even though postbiotic preparations don't include live microorganisms, they could indirectly affect the microbial ecosystem (Xiao *et al.*, 2024).

The extent to which these consequences occur in humans remains unknown. It is challenging to predict how a certain postbiotic will function in every individual because the intestinal microbiota is regulated by diet, age, medicine, lifestyle, and individual microbial composition. Therefore, it would be crucial to conduct human microbiome research to ascertain whether the microbial alterations observed in laboratory settings may be replicated *in vivo*.

5.3 Immune modulation

Microbial structures can be recognized by receptors present on immune and epithelial cells. This provides a plausible route through which inactivated microorganisms may influence immune responses without colonizing the intestine. In a randomized human trial, daily consumption of heat-killed *Lactocaseibacillus paracasei* MCC1849 was associated with changes in selected immune-related outcomes, supporting the biological activity of the preparation. (Kato *et al.*, 2024). However, biological activity should not be confused with a proven clinical benefit. Changes in immune biomarkers may indicate that a preparation interacts with the immune system, but they do not necessarily demonstrate improved resistance to infection or prevention of disease. Clinical outcomes therefore remain essential when evaluating the nutritional significance of postbiotics.

5.4 Inflammatory and oxidative pathways

Inflammatory signalling and oxidative stress frequently occur together in intestinal disorders. Several experimental postbiotic preparations have been reported to influence inflammatory mediators and antioxidant responses. Such findings provide a mechanistic basis for further investigation, but the majority of evidence for these effects remains preclinical and should therefore be interpreted cautiously. (Zhou *et al.*, 2023).

6. POSTBIOTICS AND GUT HEALTH

Because microbial-derived components directly interact with intestinal epithelial and immunological systems, the gut is a particularly significant area for postbiotic research. Supporting intestinal microbial activity, controlling local immunological responses, and maintaining epithelial integrity are some possible advantages. However, there are significant differences in the strength of the evidence between different preparations (Wei *et al.*, 2024). Heat-treated *Bifidobacterium longum* CECT 7347 was tested in individuals with diarrhea-predominant IBS in one of the more instructive human trials. Over the course of 12 weeks, the heat-treated product significantly reduced the severity of IBS symptoms compared to a placebo in a randomised, double-blind, placebo-controlled trial with 200 individuals. This offers clear evidence that a particular non-viable microbial preparation can have a gastrointestinal impact that can be measured clinically (Srivastava *et al.*, 2024).

The importance of this finding should nevertheless be interpreted within its limitations. The observed effect was demonstrated using one defined strain, one preparation method and a specific patient population. It therefore cannot be used to claim that postbiotics in general are effective treatments for IBS or other gastrointestinal disorders. Replication with independent studies and different preparations will be necessary to determine the wider applicability of the finding. Food-based approaches have also been explored. A fermented oat preparation was investigated in people with IBS, with findings suggesting an influence on markers associated with intestinal barrier function. Such work is particularly relevant to nutrition because it demonstrates how postbiotic activity could potentially be incorporated into a familiar food rather than delivered exclusively as a capsule or concentrated supplement. (Bednarska *et al.*, 2022).

7. POSTBIOTICS AND HUMAN HEALTH

7.1 Gastrointestinal health

Current human evidence is strongest for selected gastrointestinal outcomes. The trial involving heat-treated *Bifidobacterium longum* CECT 7347 demonstrated a significant improvement in IBS symptom severity compared with placebo. The finding is important because it shows that non-viable microbial material can retain measurable biological activity after processing. (Srivastava *et al.*, 2024).

At the same time, clinical evidence should be interpreted on a preparation-by-preparation basis. A positive result with one microorganism cannot be assumed to apply to another strain, even when both belong to the same bacterial genus. This strain-specific nature is a central consideration when translating postbiotic research into nutritional recommendations.

7.2 Immune health

Immune modulation is another potential field. Studies using heat-killed *Lactobacillus paracasei* MCC1849 in healthy individuals have shown that supplementation alters several immunological markers. These findings suggest that non-viable microorganisms can influence host immunity without necessitating bacterial life in the gastrointestinal tract (Kato *et al.*, 2024). However, it is still unclear how significant these immunological alterations are clinically. Beyond laboratory biomarkers, future research should examine outcomes such as infection incidence, disease interval, or other validated clinical metrics as needed.

7.3 Metabolic health

The possible significance of postbiotics in metabolic health is receiving increased attention. Microbial components and metabolites can alter intestinal permeability, inflammatory signaling, and metabolic pathways, implying multiple potential links to glucose and lipid metabolism. However, evidence for many of these effects is still based solely on laboratory models, and clinical trials are required before precise metabolic advantages can be determined (Wei *et al.*, 2024).

7.4 Gut-brain interactions

An expanding area of study in postbiotics is the gut-brain axis. Molecules generated from microbes can interact with the host via immunological, endocrine, metabolic, and neurological pathways. Although some psychological outcomes have changed in early inactivated microorganism intervention studies, there is currently insufficient data to support generalisations about mental health. Carefully monitored clinical research with verified psychological and physiological results is needed in this field.

8. POSTBIOTICS AS FUNCTIONAL FOOD INGREDIENTS

The food industry has a particular interest in postbiotics because their functionality is not dependent on microbial survival. This may provide greater flexibility when developing products that undergo heat treatment, acidic processing or extended storage. However, the practical advantage depends on whether the relevant bioactive components remain stable and functional under the actual conditions used for the food product. (Viswanathan and Muthusamy, 2022).

8.1 Dairy products

Dairy foods are among the most logical matrices for postbiotic development because lactic acid bacteria are already widely used in dairy fermentation. Fermentation can generate microbial-derived compounds while modifying the nutritional and sensory characteristics of the milk matrix. This provides an opportunity to develop foods in which the fermentation process itself contributes to the functional properties of the final product. (Oglio *et al.*, 2023).

8.2 Cereal-based foods

Cereal-based products are particularly interesting for nutrition research because they can combine the nutritional value of grains with microbial fermentation. Fermentation may influence starch digestibility, phenolic compounds, organic acids and other components while simultaneously generating microbial-derived material. Research using fermented oat preparations illustrates the potential of cereals as carriers of postbiotic functionality. (Bednarska *et al.*, 2022).

For future product development, however, the biological activity of a postbiotic should not be evaluated independently from the food itself. Processing, drying, storage and interactions with cereal components may affect the availability of the functional constituents. Sensory characteristics and consumer acceptance are also important because a product with demonstrated laboratory activity will have limited practical value if it is unacceptable to consumers.

8.3 Functional beverages

Postbiotic preparations can also be administered through beverages. Because they can incorporate nutrients and phytochemicals from plants with microbial fermentation, fermented plant-based drinks are very appealing. However, the stability of functional components can be affected by pH, oxygen exposure, temperature, and storage time. Therefore, before practical emphasizes can be made, stability tests relevant to the product are required. (Zhong *et al.*, 2024).

8.4 Food preservation

Microorganisms derived preparations have potential beyond health-conscious diets. Certain microbial products may be used in food preservation because they possess antibacterial activity against foodborne pathogens. However, as antimicrobial efficacy can be influenced by proteins, lipids, carbohydrates, pH, and water activity, results obtained in culture mediums cannot be immediately translated into real food systems (Mishra *et al.*, 2024).

9. SAFETY AND REGULATORY CONSIDERATIONS

The non-viability of postbiotics may alleviate some of the issues associated with delivering live microbes, but it does not eliminate the necessity for a safety evaluation. When evaluating a postbiotic preparation, it is necessary to take into account the source microorganism, production conditions, inactivation efficiency, purification procedure, composition, and planned dose (Salminen *et al.*, 2021). Another challenge is the absence of complete uniformity among postbiotic preparations. Two products may both be described as postbiotics while differing substantially in microbial source, processing conditions and active components. Comparing safety and efficacy data between products becomes challenging without sufficient characterisation. (Zhong *et al.*, 2024). Additionally, regulatory factors are changing. The scientific definition provides an important foundation, but regulatory treatment may vary according to the product composition, intended use and health claims. Clear characterization and human evidence will therefore be increasingly important as postbiotics move from experimental research toward commercial functional foods. (Salminen *et al.*, 2021).

10. RESEARCH GAPS AND CHALLENGES

The absence of standardised production and characterization is a significant obstacle in the field. Preparations with drastically varied compositions can be produced by variations in microbial strains, fermentation settings, inactivation techniques, and downstream processing. It is challenging to replicate biological effects or compare results across research due to this diversity (Wei *et al.*, 2024). The evidence hierarchy also requires greater attention. Cell culture and animal experiments are useful for understanding mechanisms, but they cannot establish human efficacy by themselves. Although clinical trials are emerging, the number of studies remains relatively small compared with the rapidly expanding experimental literature. More randomized controlled trials are therefore required before postbiotics can be associated confidently with specific health claims. (Srivastava *et al.*, 2024).

Postbiotic preparations can contain several potentially active components, measuring the dose simply as total preparation weight may not adequately describe biological exposure. Future research should report the composition of preparations more precisely and investigate dose-response relationships. (Thorakkattu *et al.*, 2022). Food matrix effects also deserve greater attention. A preparation that shows activity in a laboratory assay may behave differently after incorporation into a complex food containing proteins, carbohydrates, lipids and minerals. Processing and storage may further alter its composition. Therefore, future studies should evaluate on postbiotic activity under food processing and storage conditions. (Viswanathan and Muthusamy, 2022).

11. FUTURE PERSPECTIVES

The future development of postbiotics will depend strongly on improving standardization. Researchers should provide detailed information about microbial identity, cultivation conditions, inactivation procedures, chemical composition, dosage and demonstrated biological activity. Such reporting would make it easier to compare studies and establish reproducible preparation methods. (Salminen *et al.*, 2021). Clinical research should become a greater priority as the field develops. To identify whether postbiotic preparations are truly helpful and for which health outcomes, well-designed randomized controlled studies involving a variety of groups are required. Instead of depending solely on biochemical or immunological biomarkers, future trials should preferably incorporate established clinical objectives. (Kato *et al.*, 2024).

Potential uses for postbiotics include fermented dairy products, cereal goods, drinks, nutritional powders, and other functional foods. However, for development to be effective, biological activity, processing stability, shelf life, sensory quality, consumer acceptance, and safety must all be considered simultaneously (Muthusamy and Viswanathan, 2022). It is essential to explore the potential of combining postbiotics with probiotics, prebiotics, or dietary fiber. Such combinations may provide complementary mechanisms, but possible synergistic effects should be proven experimentally rather than assumed. Similarly, depending on their gut microbial and metabolic profiles, individuals who respond differently to particular postbiotic preparations may potentially be identified with the aid of the emerging concept of personalized nutrition (Wei and others, 2024).

12. CONCLUSION

Postbiotics have emerged as an interesting area of research linking microbiology, nutrition and food technology. Using inanimate microbes and/or their components that have been shown to have health advantages instead of depending on living microbial cells is what distinguishes them. According to recent studies, certain postbiotic preparations can affect inflammatory pathways, microbial interactions, immunological responses, and intestinal barrier function. (Salminen *et al.*, 2021). Crucially, some of the effects shown in experimental studies are starting to be supported by human investigations. While heat-killed *Lactobacillus paracasei* MCC1849 has shown impacts on specific immunological parameters in healthy individuals, heat-treated *Bifidobacterium longum* CECT 7347 has demonstrated positive effects on the intensity of symptoms in adults with IBS-D. However, these results are unique to the preparations under investigation and should not be generalised to postbiotics in general. (Kato *et al.*, 2024; Srivastava *et al.*, 2024).

Postbiotics may be an appealing pathway for generating functional foods in food science because their activity is independent of microbial survival. Potential delivery matrices include dairy products, fermented cereals, and drinks; chemicals generated from microbes may potentially be used in food preservation. However, stability, safety, sensory acceptability, standardised characterisation, and compelling human evidence are necessary for effective translation into commercial goods. (Bednarska *et al.*, 2022; Mishra *et al.*, 2024). All things considered, postbiotics are a promising but still-developing family of functional substances. The most crucial next step is to determine which preparations are effective, at what doses, by which mechanisms, and in which human populations, rather than just finding additional possible advantages. If these gaps are addressed, postbiotics could become valuable functional ingredients within evidence-based food and nutrition strategies.

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