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Review Article

Effects of Paraprobiotics and Postbiotics on Health and Their Application Areas

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Abstract

There are numerous studies regarding probiotic microorganisms, which are widely utilized in both food and pharmaceutical industries today and provide health benefits to the host when administered in adequate amounts. Conversely, more limited data exist in the literature concerning the metabolites produced by probiotics and the non-living forms of probiotic cells. Although the standard definition of probiotics emphasizes that a probiotic microorganism must be viable to confer a benefit to the host, data obtained from recent studies have revealed that microorganisms can provide health benefits even when they are non-viable. Consequently, the concepts of paraprobiotics and postbiotics have emerged. Postbiotics are defined as metabolic products secreted by probiotics—such as enzymes, proteins, short-chain fatty acids, vitamins, surfactants, amino acids, peptides, and organic acids—or as structural components released from non-living probiotic cells, including teichoic acids, peptidoglycan-derived muropeptides, exopolysaccharides, and cell surface proteins. On the other hand, paraprobiotics are defined as non-viable cells of probiotics that have been inactivated through various applications. Therefore, postbiotics indirectly represent a broader category that encompasses the concept of paraprobiotics; for this reason, some sources in the literature examine both postbiotics and paraprobiotics under the single category of "postbiotics." In this review, the definitions of paraprobiotics and postbiotics, their isolation methods, their effects on health, their utilization in the food and pharmaceutical industries, and their potential future roles are comprehensively discussed.

Keywords

Probiotic, Paraprobiotic, Postbiotic

1. Probiotic

The basis of the probiotic concept relies on Ilya Ilyich Mechnikov's observation of the dietary habits of elderly people living in a Bulgarian village in 1907. Mechnikov suggested that the healthy and long lifespans of the village population stemmed from their regular consumption of fermented milk products, such as yogurt, which contain lactic acid bacteria [1]. Extending from this observation to the present day, the concept of probiotics has been supported, developed, and updated through studies conducted by numerous scientists. The most recent valid definition of probiotics accepted by the scientific world is the one proposed by the expert panel convened by the International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2014. Here, probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [2]. Probiotic microorganisms are listed as Generally Recognized as

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Safe (GRAS) at the species level by the United States Food and Drug Administration (FDA) or as Qualified Presumption of Safety (QPS) at the species level by the European Food Safety Authority (EFSA). These lists, which contain a limited number of microorganisms, predominantly feature *Lactobacillus* and *Bifidobacterium* species [1].

Probiotics are reported to be effective in the treatment of diarrhea, lactose intolerance, colitis, irritable bowel syndrome, insulin resistance, obesity, certain cancers, and other diseases and symptoms, and these effects vary depending on the strain used and the type of disease [3]. According to the current definition of probiotics, although it is pointed out that a probiotic microorganism's ability to provide benefits to the host depends on the cell being in a live form, studies conducted after 2004 have revealed that probiotics can provide health benefits even when they are not in a live form [3-9]. This situation has brought a new perspective to the probiotic concept, leading to the emergence of new terms such as paraprobiotics and postbiotics. Although these concepts have emerged in recent years, they have been rapidly adopted in human health and nutrition as well as in the field of food science and technology, drawing particular interest for potential food, biotechnology, and pharmaceutical industry applications such as functional foods, nutraceuticals, and pharmaceuticals [10].

2. Paraprobiotics

The concept of paraprobiotics first emerged in 2011 when Taverniti and Guglielmetti published an article stating that inanimate forms of probiotics could positively affect the host's immune system [9]. The term paraprobiotic was created by adding the prefix "Para", which means "side by side" or "atypical" in Ancient Greek, to the word probiotic to indicate its close similarity to and simultaneous difference from the definition of a probiotic [11]. Paraprobiotics are defined as "inanimate microbial cells (either intact or ruptured) or cell extracts that, when administered in sufficient amounts orally or topically, confer a benefit to the human or animal host" [12-15]. In addition, designations such as inactivated probiotics, non-living probiotics, killed probiotics, ghost probiotics, modified probiotics, and sterilized probiotics are also used for paraprobiotics [8, 11, 14]. Paraprobiotics can be found in probiotic formulations at concentrations ranging from 1×10^5 to 1×10^{14} CFU/ml per unit dose (ml, gram, tablet, or capsule) [8]. Inactivated microorganisms are required to obtain paraprobiotics. Live probiotic cells can be inactivated by thermal treatments such as pasteurization and sterilization; non-thermal treatments such as ionizing radiation, ultraviolet (UV) rays, high pressure, sonication, dehydration, and altering the environmental pH; or alternative applications such as pulsed electric field (PEF), ohmic heating, and supercritical CO_2 technology. Among these applications, the most widely used methods are thermal treatments [3]. Information regarding the methods used to inactivate paraprobiotic microorganisms and their mechanisms of action is provided in Table 1. While physiological changes occur in the probiotic microorganism after inactivation processes, the microorganism partially or completely loses its viability. Nevertheless, the viability status, intracellular activity, and cell membrane integrity of probiotic microorganisms can be determined using fluorescent dyes such as carboxyfluorescein diacetate (cFDA), fluorescein diacetate (FDA), hydroethidine (HE), propidium iodide (PI), and SYTO-9 [16]. From a technological standpoint, the use of paraprobiotics offers certain advantages. For example, inactivated microorganisms can be added to food products at any desired stage of production, which is important for reducing the risk of contamination. Therefore, non-living materials of microbial origin, such as paraprobiotics, can provide more distinct benefits compared to probiotics for developing safer and more stable products [14, 17, 18]. Furthermore, compared to probiotics, paraprobiotics are reported to possess advantages such as interacting less with food components, being easier to process, having the feasibility of being added to the medium before heat treatment, being safer in conditions like infection risk, immune stimulation, and microbial translocation, as well as ease of storage and transport [19].

Table 1. *Methods used to inactivate a probiotic microorganism and their respective mechanisms of action.*

Inactivation Method	Mechanism of Action
Thermal applications	Cell membrane damage, loss of nutrients and ions, aggregation of ribosomes, breakage of RNA strands, inactivation of essential enzymes, coagulation of proteins
Ionizing radiation	Nucleic acid damage
High pressure	Cell membrane damage, protein denaturation, decrease in intracellular pH, loss of solutes, inactivation of enzymes, conformational changes in ribosomes and nucleoids
Sonication	Cell wall rupture, cell membrane damage, DNA damage
UV rays	Protein denaturation, formation of DNA photoproducts
Ohmic heating	Cell membrane damage, loss of nutrients and ions, aggregation of ribosomes, breakage of DNA strands, inactivation of essential enzymes, coagulation of proteins, electroporation

Inactivation Method	Mechanism of Action
Pulsed electric field (PEF)	Disruption of the cell membrane (Electroporation)
Supercritical CO_2	Decrease in intracellular pH, cell membrane damage, inactivation of enzymes, direct effect of HCO_3 and CO_2 on metabolism, disruption of the intracellular balance of electrolytes, removal of vital elements from the cell and cell membrane
Drying	Cell membrane damage, alterations in the structure of proteins, nucleic acids, and ribosomes
pH alteration	Cell membrane damage, chemical alteration in cell DNA

Paraprobiotics have aroused great interest in the scientific world due to their beneficial health effects, such as long shelf life, safety, and contributions to the modulation of immunity, modification of biological responses, reduction of cholesterol, and anti-inflammatory and anti-proliferative properties. Although not fully elucidated, it is stated that the beneficial effects of paraprobiotics on health are provided by improving certain physiological functions of the individual [14]. The health effects of paraprobiotics are summarized in Table 2 [8].

Table 2. *Effects of paraprobiotics on health.*

Effects	Identified Health Benefits
Effect on the immune system	Immunomodulation, modulation of inflammation
Effect on the digestive system	Modulation of gut microbiota, healing of intestinal injuries, treatment of diarrhea, protection of the intestinal barrier, treatment of colitis, reduction of lactose intolerance
Effect on the respiratory system	Treatment of respiratory diseases, treatment of alcohol-related liver diseases
Other effects	Modulation of the response to visceral pain, suppression of certain age-related symptoms, prevention of dental caries, prevention of cancer development, reduction of cholesterol, treatment of atopic dermatitis, inhibition of pathogens, reduction of bacterial translocation

There are several studies examining the immunomodulatory effects of paraprobiotics on the immune system. It has been determined that the paraprobiotic *Lactobacillus casei* (*Lacticaseibacillus casei*) Zhang strain (1×10^6 CFU/ml), heat-inactivated and suspended in buffered peptone water, enhances the natural response of macrophages by improving the expression of pro-inflammatory cytokines and the transcription of Toll-like receptors (TLR2, TLR3, TLR4, and TLR9), thereby positively affecting the innate immunity system [20]. In a study investigating the ability of paraprobiotics to protect the host against infections, it was found that the heat-inactivated paraprobiotic *Lactobacillus plantarum* (*Lactiplantibacillus plantarum*) b240 strain, suspended in physiological saline, protected the host against systemic infection caused by *Salmonella enterica* serovar Typhimurium [21]. In another study, it was determined that the heat-inactivated paraprobiotic *Lactobacillus gasseri* TMC0356 strain, suspended in physiological saline (10^{11} CFU/ml), was effective against infection caused by the H1N1 influenza virus. Furthermore, it was determined that *L. gasseri* TMC0356 provides protection against infections by stimulating local and systemic immune responses, and its cell components provide protection against viral infections by increasing the immune responses of the gut and respiratory system [22]. Paraprobiotics are also known to improve the balance of the gut microbiota (dysbiosis). In a study conducted in this direction, the heat-inactivated, lyophilized paraprobiotic *Lactococcus lactis* ssp. *lactis* G50 strain was added to the diet of mice at 0.5 g/kg, and this application was observed to improve the gut microbiota by suppressing the development of intestinal pathogens in mice [23]. Beneficial and harmful microorganisms coexist in the intestine, and a balance is sought through host-microbiota interaction. In cases where the intestine is damaged, probiotics are used to observe healing and ensure the resolution of dysbiosis. In a conducted study, it was determined that the heat-inactivated paraprobiotic *Lactobacillus brevis* (*Levilactobacillus brevis*) SBC8803 strain, suspended in buffered peptone water, healed damaged areas by improving the intestinal barrier in mice with colitis induced by dextran sodium sulfate (DSS) [24].

2.1. Food Industry Applications of Paraprobiotics

In order for paraprobiotics to be widely used in food applications, the primary issue to be addressed is the selection of appropriate probiotic species and strains that offer an acceptable level of specific health benefits in obtaining paraprobiotics. The second step is the application of a series of inactivation conditions to probiotic species to obtain paraprobiotics. However, it is not known whether all probiotic species to be used will be suitable for food applications. Another issue to consider is that the beneficial effects of paraprobiotics remain stable throughout their shelf life and determining the effect of the inactivation method used in obtaining paraprobiotics on the shelf life. It is also extremely important to define the forms in which paraprobiotics can be commercialized and the health benefits they will provide when consumed with foods throughout their shelf life. Although the health benefits of paraprobiotics have been associated with data in the literature, paraprobiotics must be used in food applications for these benefits to be realized. Therefore, the use of paraprobiotics in foods brings certain opportunities as well as some challenges. In this case, the selection of probiotic species and strains to be used for paraprobiotic production, the use of appropriate methods for inactivation, the evaluation of their stability and activity in foods throughout the shelf life, and the use of appropriate methodologies to evaluate their biological efficacy are of paramount importance [8]. In some studies, the health effects of paraprobiotics directly added to the medium during food processing or produced within the food have been examined. In a study, it was determined that the consumption of yogurt containing heat-inactivated lactic acid bacteria such as *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus* prevented the disruption of intestinal epithelial barrier function in human intestinal cells (Caco-2) [25]. In another study, beverages prepared with the heat-inactivated paraprobiotic *L. gasseri* CP2305 strain were consumed by patients with a tendency toward constipation for 3 weeks, and at the end of the period, improvements were observed in the intestinal functions, defecation, and particularly feces color of the individuals [26]. Although the mentioned foods containing paraprobiotics are not yet commercially produced in the food industry, it is thought that they can take place in more product contents over time with the increase in data obtained from studies on this subject and the elucidation of the mechanisms of action of paraprobiotics.

2.2. Pharmaceutical Industry Applications of Paraprobiotics

Paraprobiotics find application areas in pharmacological tablets due to their health-improving properties. Today, commercially produced paraprobiotic products, which have proven effects in preventing or treating certain diseases, are used as supplements. It is stated that Pylopass™ (Novozymes Berlin GmbH, Berlin, Germany), which contains the paraprobiotic product *Lactobacillus reuteri* (*Limosilactobacillus reuteri*) DSMZ 17648 strain, is effective in controlling *Helicobacter pylori* infection. Another product available on the market is the Lacteol® Fort capsule (Carnot Laboratories, Houdan, France), which contains the heat-treated *L. acidophilus* LB strain effective in the treatment of diarrhea [10].

3. Postbiotics

Microbial metabolites secreted by probiotics, such as enzymes, short-chain fatty acids, bacteriocins, vitamins, organic acids, amino acids, and peptides, or structures released into the medium as a result of the inactivation of a probiotic cell via bacterial lysis, such as teichoic acids, cell surface-associated proteins, cell wall-bound biosurfactants, peptidoglycan-derived muropeptides, and pili, fimbriae, and flagella located on the microorganism surface, are defined as postbiotics [3, 10, 27-32]. Postbiotics are also known as metabiotics, biogenics, and cell-free supernatants [8-10, 29, 31]. However, postbiotics have not yet had a definition recommended by any international regulatory body or scientific association [32]. Postbiotics exist in two different forms: extracellular and intracellular (Table 3) [33]. In recent years, *in vitro* studies on cell lines and *in vivo* studies on obese/hypertensive rat models have been applied to evaluate the potential bioactivity and/or health effects of various postbiotics, including intracellular metabolites and cell wall components [34]. While postbiotics are obtained from *Lactobacillus* and *Bifidobacterium* strains in most of the studies, it is reported that *Streptococcus* and *Faecalibacterium* species can also be sources of postbiotics [29]. When evaluated from technological, biological, and safety perspectives, the facts that postbiotics possess high stability, can be easily standardized, are transportable, exhibit appropriate absorption, provide transition into the blood or tissues without the risk of translocation from the intestinal lumen, do not lead to risks of virulence and antibiotic transfer, and do not have any effect on the colonization of the beneficial microbiota present in the intestine are important points that distinguish postbiotics from probiotics [31].

In the production of postbiotics, cell disruption methods including ionizing radiation, solvent extraction, thermal treatments, ultraviolet rays, high pressure, enzymatic processes, and sonication are mostly used. Additional extraction and purification processes such as centrifugation, column purification, and freeze-drying are also utilized in the production of postbiotics [31, 33]. Furthermore, postbiotics are naturally produced frequently during the fermentation process of fermented products like kefir, kombucha, yogurt, pickled vegetables, etc. Additionally, postbiotics can also be added later to various fermented or non-fermented foods [35].

Table 3. Types of postbiotics.

Microbial Metabolites (Intracellular)	Microbial Cell Wall Components (Extracellular)
Short-chain fatty acids, Organic acids, Surfactant components, Vitamins, Peptides, Gamma-aminobutyric acid (GABA), Bacteriocins, Enzymes, Antioxidants	DNA, RNA, Proteins, Peptides, S-layer proteins, Teichoic acids, Peptidoglycan-derived muropeptides, Polysaccharides

It has been proven that postbiotics possess immunomodulatory, anti-inflammatory, anti-carcinogenic, anti-proliferative, and antibacterial properties, show positive local effects on certain tissues of the intestinal epithelium, and provide systematic effects by influencing multiple organs or tissues. Moreover, it is stated that postbiotics can also be used in the prevention of celiac disease [36].

3.1. Food Industry Applications of Postbiotics

In recent years, along with the increasing awareness of consumers regarding healthy nutrition, the demand for probiotic products is also increasing day by day. In order to call a product "probiotic", there must be at least 10^6 CFU/ml of live probiotic microorganisms in that food [37]. Therefore, adding live probiotic cells to the matrix of various foods, the survival of probiotic cells in this environment during production, and the continuation of their viability during storage are of great importance [31]. Postbiotics, on the other hand, provide an advantage in functional food production in terms of not being obliged to preserve viability and thus being controllable under production and storage conditions [15]. Because postbiotics are safe, have a long shelf life, do not show toxic effects, can be standardized, and are easily transportable, they can be evaluated as a safe and affordable alternative as a new strategy for therapeutic purposes in the pharmaceutical and food industries within pharmaceutical formulations and/or food supplements [29, 31]. Postbiotics exhibit properties as a potential biopreservative, detoxification agent, and improver of physicochemical and sensory factors in food applications. It was determined that the supernatant of the *L. plantarum* YML007 strain isolated from Kimchi had a positive effect on soybeans and extended the shelf life up to 2 months, thus the postbiotic showed a potential biopreservative property in soybeans [38]. It is stated that the exopolysaccharide postbiotic (EPS) produced by lactic acid bacteria such as *Lactobacillus rhamnosus* (*Lacticaseibacillus rhamnosus*) can improve the physicochemical and sensory properties of foods [29]. Another biological role of postbiotics is their detoxification functions toward toxic metabolites, which has attracted great interest from scientists in recent years. In most studies, the main mechanisms of action of various postbiotics are termed as surface adsorption of toxic substances by cell wall components and/or degradation by various metabolites such as enzymes. In a conducted study, it was determined that yeasts are mycotoxin-adsorbing agents and that functional carbohydrates present in their cell walls are responsible for this adsorption. Furthermore, it was determined that adding 0.2% yeast cell wall extract to feeds effectively prevented the toxicity caused by 0.4 mg/l zearalenone in piglets [39]. Other studies indicating the food applications and benefits of postbiotics are summarized in Table 4 [40].

Table 4. Food application and benefits of postbiotics.

Postbiotic	Food	Benefit	Reference
Lactarius volemus Fr. polysaccharide extracts	Yogurt	Significantly improved water holding capacity and decrease in pH value	[41]
L. rhamnosus exopolysaccharide	Cheddar cheese	8.2% increase in yield	[42]
L. rhamnosus S93 enzyme	Cheddar cheese	Higher levels of soluble nitrogen in phosphotungstic acid and free amino acids	[43]
Lactobacillus sakei (*Latilactobacillus sakei*) NRRL B-1917 supernatant	Grilled steak	Decrease in *Escherichia coli* and *Listeria monocytogenes* counts without sensory alteration	[44]
Gassericin A bacteriocin of *L. gasseri* LA39 strain	Cheese whey	Inhibition of *Bacillus cereus*, *Lactococcus lactis* subsp. *lactis*, *Achromobacter denitrificans*, and *Pseudomonas fluorescens*	[45]
L. plantarum ST16Pa bacteriocin-like inhibitory substance	Chicken breast	Bioconservative effect against *Enterococcus faecium* for 7 days	[46]

Postbiotic	Food	Benefit	Reference
Pyrrolo (1, 2-a) and pyrazine-1, 4-dione derived from Lactobacillus salivarius (Ligilactobacillus salivarius)	Ground beef	Elimination of L. monocytogenes biofilm	[47]

3.2. Pharmaceutical Industry Applications of Postbiotics

Postbiotics are used in various products in the pharmaceutical industry and are sold commercially. CytoFlora® (BioRay Inc., Laguna Hills, CA, USA), the most well-known postbiotic product in the pharmaceutical industry, contains a mixture of cell wall isolates of *L. casei*, *L. plantarum*, *L. acidophilus* DDS-1, *L. reuteri*, *L. salivarius*, *L. rhamnosus*, *L. sporogenes*, *L. bulgaricus*, *L. acidophilus*, *S. thermophilus*, *Bifidobacterium bifidum*, *B. infantis*, and *Bifidobacterium bulgaricus* in its content. CytoFlora® has some important beneficial effects on maintaining intestinal homeostasis, improving intestinal dysbiosis, and improving immune response and symptoms in autistic patients [48]. Another postbiotic pharmaceutical product is Del-Immune V® (Pure Research Products, LLC, Boulder, CO, USA), which is generally recommended to be consumed together with probiotics for therapeutic purposes, and contains a mixture of *L. rhamnosus* V amino acids, muramyl peptides, and DNA fragments in its content. It has been determined that Del-Immune V significantly reduces the intensity of gastrointestinal disorders in patients with autism spectrum disorder [49]. Zakofalk® is another commercial product consisting of a mixture of sodium butyrate and inulin, and is recommended for the treatment of active intestinal inflammatory disorders [50]. Bactistatin® (Kraft, St. Petersburg, Russia) has a complex structure containing the metabolites of the *Bacillus subtilis* VKPM V-2335 strain, and possesses properties that normalize the microbial ecology of the digestive system, ensure the restoration of the intestinal barrier function to its former form, stimulate the development of symbiotic lactobacilli and bifidobacteria, and prevent the spread of pathogens, opportunistic bacteria, fungi, and some viruses [51]. Pro-Symbioflor® (SymbioPharm GmbH, Herborn, Germany) is an oral suspension containing the cell lysate of *Enterococcus faecalis* DSM 16440 and *E. coli* DSM 17252, which alleviates gastrointestinal discomforts and assists the immune system [52]. This postbiotic preparation has also been reported to be effective against the development of atopic dermatitis [53, 54]. Hylak® Forte (Ratiopharm / Merckle GmbH, Germany) consists of a mixture of soluble metabolites of *E. coli* DSM 4087, *Streptococcus faecalis* DSM 4086, *L. acidophilus* DSM 414, and *Lactobacillus helveticus* DS 4183 used to alleviate bacterial symptoms, and is used in the treatment of imbalances in the gastrointestinal tract including flatulence, diarrhea, and constipation [55]. Furthermore, it has been reported to be effective in the treatment of salmonellosis in infants, chronic gastritis, and reducing the incidence and severity of radiation-induced diarrhea in radio-oncology patients [56-58].

4. Future Role of Paraprobiotics and Postbiotics

In order to add postbiotics and paraprobiotics to food and pharmacological products, it is vital to understand the impact on host-microorganism interactions and evaluate their potential benefits and risks to support their health-related claims. It is reported that future *in vivo* studies should be carried out in animal models and human clinical trials to help determine the feasibility of postbiotics that improve the health of the gastrointestinal tract. At the same time, it is thought that conducting research on the production methods of paraprobiotics and postbiotics will lead to the development of new pharmaceutical products and functional foods.

5. Conclusion

Paraprobiotics and postbiotics comprise a group of non-living microbial cells, cell components, and metabolic by-products that can contribute to improving host health. The fact that a clear distinction and definition has not yet been made for the concepts of paraprobiotics and postbiotics by a scientific institution or association causes confusion in the literature. Thanks to data to be obtained from further studies, it will be possible to make a clearer distinction between these concepts. With scientists turning their research toward the topics of paraprobiotics and postbiotics in recent years, it is anticipated that these structures, which provide health benefits to the host, will find a place of use and become widespread in different food and pharmacological products today. Furthermore, current data are not sufficient to determine the level of efficacy of paraprobiotics and postbiotics. Consequently, more *in vitro* and *in vivo* studies need to be performed to support the clinical and protective effects of paraprobiotics and postbiotics.

Abbreviations

ISAPP	International Scientific Association for Probiotics and Prebiotics
GRAS	Generally Recognized as Safe
FDA	United States Food and Drug Administration

QPS	Qualified Presumption of Safety
EFSA	European Food Safety Authority

Author Contributions

Melike Kasap: Conceptualization, Data curation, Writing – original draft, Writing – review & editing

Conflicts of Interest

The author declares no conflicts of interest.

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