

Postbiotics and their therapeutic effects: a review

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The microbiota is an ecosystem composed of microorganisms living in various parts of the body. The large majority of microorganisms live in the gastrointestinal system. The relationship between the microbial habitat and the host is complicated and dynamic. As a result, the microbiota plays a significant role in the development of numerous diseases. The use of probiotics to enhance the microbiota of the gastro-intestinal system attracts attention. However, it's reported in the literature that probiotics have side effects such as systemic infection, harmful metabolic activities, overstimulation of the immune system, gene transfer, and mild gastrointestinal symptoms. Consequently, the term "postbiotic" known as the structural components of probiotic bacteria and their metabolites has become increasingly important in recent years. Postbiotics can be grouped as lipid, protein, carbohydrate, vitamin, co-factor, organic acid, peptidoglycan according to their main components, or immunomodulator, antioxidant, anti-inflammatory, antihypertensive, hypo-cholesterolemic, antiobesogenic antiproliferative according to their physiological effects. Postbiotics have been reported to have positive impacts on some diseases by interacting with the microbial community and host. In this review, it was aimed to investigate the concept of postbiotics, the interaction of postbiotics with the gut microbiota and the host, and the effects of postbiotics on various diseases. As the number of studies on postbiotics is increasing day by day, it's aimed that this review will shed light on future studies. More research is required in this area to examine the effects of postbiotics on health and disease in a more comprehensive manner.

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Introduction

Microbiota is a microbial ecosystem consisting of bacteria, viruses and fungi living in different parts of the human body such as the gastrointestinal system, skin, lungs. More than 70% of the microbiota members have a mutualistic relationship with the host in the gastrointestinal system [1]. The microbial habitat in the human gastrointestinal tract has a complex and dynamic two-way relationship with the host. Microorganisms in the gut microbiota affect many physiological functions by producing a variety of compounds that can be used by both the host and other microorganisms. Such relationships are called host-microbial community interactions. Although more research is needed to determine the exact role of gut microbiota in specific aspects of health and disease, the microbiota composition has become an

important target for treatment [2]. When the host consumes probiotics at certain levels, they produce soluble postbiotic metabolites with molecular weights of less than 50, 50–100 and <100 kDa. These metabolites are short-chain fatty acids, organic acid, hydrogen peroxide, ethanol, acetaldehyde, diacetyl, acetoin, indole, peptides, bacteriocins, exopolysaccharides, reuterine, and they cause specific biological effects in the organism [3].

Many researchers who contributed to the literature have used terms like "biogenic" "cell-free supernatant", "abiotics", "metabiotics", "paraprobiotics", "ghost probiotics", "pseudoprobiotic", "postbiotics" for inanimate probiotics. Postbiotics are the most used of these terms; It includes inactivated probiotic bacteria (paraprobiotic), cell fractions (peptidoglycan-derived muropeptides, teichoic acid, endo and exopolysaccharides, cell surface

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proteins) or probiotic metabolites (short chain fatty acids, enzymes, bacteriocins, organic acids). The composition of the gut microbiota varies greatly between populations and even individuals, and its composition is dependent on the metabolic and functional phenotype. Thus, the degree of metabolism of microbial products varies from person to person and biotic treatment is different for each individual. This viewpoint has led to reassessment of food fermentation and gain importance of the concept of postbiotics [4]. According to the report of WHO and FAO, probiotics have side effects such as systemic infection, harmful metabolic activities, over-reacted immune system in an individual who is susceptible, gene transfer and mild gastrointestinal symptoms [5]. The risks of such probiotic side effects have induced postbiotics to attract greater attention.

Postbiotics may be distinguished by their main components, that is, lipids (butyrate, propionate, plasmalogen derived from dimethyl or acetyl), protein (lactocepelin, p40 molecule), carbohydrate (polysaccharides rich in galactose, teichoic acids), vitamins/co-factors, organic acids (propionic and 3-phenyllactic acid) and peptidoglycan-derived muropeptides, lipoteichoic acids, or physiological functions (immunomodulation, antioxidant, anti-inflammatory, antihypertensive, hypo-cholesterolemic, antiobesogenic, antiprolative) they provided to the host [6].

The effects of postbiotics on microbial habitat interactions

Fermentation products such as organic acids, especially acetic acid, and lactic acid, have a strong inhibitory effect against gram-negative bacteria and have been recognized as the main antimicrobial compounds responsible for the inhibitory activity of probiotics against pathogens [7].

Acetate, propionate, and butyrate that produced by bacteria in the colon, stimulate water and mineral absorption and colonocyte proliferation. Whereas propionate is used for gluconeogenesis in the liver, butyrate is used as the primary energy source for colonocytes, and they also improve barrier function and mucosal immunity [8]. Many lactic acid bacteria produce antimicrobial peptides such as bacteriocins. Bacteriocins are produced by gram-positive bacteria. A study has shown that bacteriocin production directly inhibits pathogen growth in the gastrointestinal system and increases bacteria that produce bacteriocins [9].

The effects of postbiotics on host-microbiota interactions

The most important factor for probiotics is that it reaches the gut alive, while in postbiotics it does not matter. Postbiotic activity is based on microbial metabolites,

proteins, lipids, carbohydrates, vitamins, organic acids, cell wall components or other complex molecules produced in a fermented matrix [6].

Microbial-based substances such as surface proteins of probiotic bacteria, capsule polysaccharide, lipoteichoic acid, lipopolysaccharide specifically bind to pattern recognition receptors (PRR) such as NOD-like receptor (NLR) and Toll-like receptor (TLR) in intestinal epithelial cells and regulates nuclear factor kappa B (NF- κ B), mitogen-activated protein kinase (MAPK), peroxisome proliferator-activated receptor gamma (PPAR γ) and other signaling pathways [10].

Heat-killed *Lb. casei* suspension has been shown to enhance pro-inflammatory cytokines and cause an increase in TLR-2, TLR-3, TLR-4, and TLR-9 expression [11]. Lactic acid bacteria species have received attention owing to their ability of produce exopolysaccharides [12]. Bacterial exopolysaccharides are involved in the adhesion of the probiotic surface, antibiotic resistance, and protection of the host's immune system. In the literature, it has been stated that exopolysaccharides provide cardioprotective, antioxidant, anti-inflammatory effect in the host [13–15]

Postbiotics with antioxidant properties can be illustrated by enzymes like glutathione peroxidase (GPx), superoxide dismutase (SOD), nicotinamide adenine dinucleotide oxidase and nicotinamide adenine dinucleotide peroxidase [16] Extracellular vesicles are lipid-layered products produced by both Gram-negative and Gram-positive bacteria. They are involved in a variety of potential biological functions, from roles in microbial community groups (transfer of message, genetic material, and proteins) to host-bacterial signaling. *Akkermansia muciniphila* and commensal *Escherichia coli* derived extracellular vesicles reduce gut permeability and activate signaling in the intestinal epithelial barrier *in vitro* [17].

Therapeutic effects of postbiotics

Inflammatory diseases

Heat killed microorganisms can still exert biological effects on the host's immune system, and thus heat-unaaffected structural components of bacteria can be a useful therapy for inflammatory diseases. Imaoka *et al.* have shown that heat-processed Bifidobacterium cells induced cellular immune and anti-inflammatory responses by inhibiting interleukin (IL)-8 in the intestinal epithelial cells of ulcerative colitis patients [18].

According to the results of preclinic studies, the effects of short-chain fatty acids on inflammatory diseases such as colitis, arthritis, asthma, gout, pneumonia are promising.

In addition, pathways that modulate anti-inflammatory effects are thought to be effects of cytokine release and cell aggregation at the inflammation site. However, these mechanisms vary according to the cell type and target site of the host [19].

Short-chain fatty acids may also regulate cellular responses through inhibition of histone deacetylase. For instance, short-chain fatty acids provide down-regulation of pro-inflammatory cytokines by enhancing T-regulatory cells and macrophages. These effects protect the balance between inflammation and immunity in the gut [20]. Linoleic acid and linolenic acid are taken into the body with diet are used for producing bioactive lipid mediators. These mediators modulate inflammation by reducing blood pressure and inflammatory cytokines. Species of *Lb. plantarum*, *Enterococcus faecalis*, *Bacteroides thetaiotaomicron* are known to produce various conjugated-linoleic acid, oxy-fatty acids, and hydroxy-fatty acids [21]. In a study, it has been shown that conjugated linoleic acid, along with fiber and vitamin supplements, may have a beneficial effect in the treatment of ulcerative colitis [22]. In addition, other metabolites such as taurine, histamine and spermin released by the microbiota affect inflammation-dependent signaling pathways in the host and the intestinal microbiota [23]. Taurine is an amino sulphonic acid modulated by commensal bacterial deconjugation of primary bile acids. In this way, commensal bacteria cause an increase in luminal taurine levels. Taurine has been found to stimulate the NLRP6 inflammation signal and therefore modulate inflammatory bowel diseases [24].

Compare *et al.* reported that the postbiotic produced by the *Lb. casei* DG strain reduced the inflammatory mucosal response in infected irritable bowel syndrome in an ex-vivo organ culture model study [25]. Heat killed *Lb. plantarum* L-137 (HK L-137) has been shown to have an anti-inflammatory effect on heart and adipose tissue in rats with metabolic syndrome [26]. In addition, it has been reported that heat-killed *Lb. plantarum* L-137 (HK L-137) was determined to improve liver enzymes in overweight individuals [27].

Immune system diseases

Paraprobiotics are known to modulate the immune system by cell wall compounds as well as inhibition of pathogens. Additionally, the metabolites released from paraprobiotics may provide beneficial effects in the host. Despite these findings, the mechanism of action of paraprobiotics in gastrointestinal and respiratory system diseases has not been fully explained [28]. Ryan *et al.* (2008), compared live and UV-killed *Lb. salivarius* against *Helicobacter pylori* infection and determined that there was no difference between IL-8 production inhibition [29]. Furthermore, Piqué *et al.* found that nonviable bacteria could pass through mucus and stimulate epithelial cells more effectively than living bacteria [30].

Short-chain fatty acids can affect immune cells not only through direct inhibitory effects on cell division, but also through modulation of cell signals, epigenetic regulation, metabolism pathway, and molecular mechanisms [31]. Matsuguchi *et al.* found that inactivated *Lb. casei* Shirota cells caused a decrease in Immunoglobulin-E production. This data suggests that paraprobiotics may have a positive effect therapeutically or prophylactically for food allergies [32]. Furthermore, according to a published study, paraprobiotics stimulate the production of type 1 interferon by activating dendritic cells, thereby eliminating viruses [33].

Jeong *et al.* compared the immunomodulatory effects of heat killed *Lb. plantarum* KCTC 13314BP and live *Lb. rhamnosus* GG bacteria in macrophage cell culture and, interestingly, found that inactivated *Lb. plantarum* provided stronger immunomodulation [34]. Rocha-Ramírez *et al.* reported that heat-killed *Lb. casei* may be effective in regulating the immune functions of macrophages in children with severe malnutrition and infection [35]. In a preclinical study, the antigen-specific immunoglobulin A (IgA) in the small intestine was found to be significantly higher in the small intestine with the oral administration of heat-killed *Lb. paracasei* MCC1849 for 5 weeks [36].

Cancer

Cancer is a global epidemic, which affects individuals of all ages and socioeconomic levels. Although there are many methods for cancer treatment, it continues to be the leading cause of death. The biggest challenge in cancer treatment is cytotoxicity, which is nonspecific. Many studies have shown the importance of the gut microbiome in the effectiveness of cancer treatment [37,38]. Besides, microbiota dysbiosis causes cancer cachexia through systemic inflammation, barrier dysfunction, and muscle loss [39]. In recent years, the use of postbiotics in cancer treatment has been introduced to the literature as “bacteriotherapy”. This therapeutic approach provides a unique anticancer effect by causing selective toxicity in cancer cells, creating hypoxic or necrotic conditions for the tumor, and genetic modifications. The administration of postbiotics as adjunctive therapy in cancer prevention and treatment has been strongly associated with the function and response of the host's immune system [3].

In a study conducted with mice, it was stated that combined immunotherapy with heat-killed *Lb. casei* significantly increased nitric oxide production and cytotoxicity of natural killer cells in spleen cell culture in breast cancer mice [40]. Chuah *et al.* have shown that postbiotic metabolites of *Lb. plantarum* are selectively protect normal cells while have a cytotoxic effect in various cancer cells (colon, breast). One of the most important factors in the occurrence of colon cancer is inflammation [41]. Chung *et al.* have reported that heat-killed *Enterococcus faecalis* plays a role in the alleviation of

colitis and inflammation-associated colon cancer by modulating the Nod-like receptor protein-3 (NLRP3). In another study, "ferrichrome", a metabolite of *Lb. casei* ATCC334 strain, was found to suppress colon cancer cells [42]. Tukenmez *et al.* determined that exopolysaccharides of *Lb. plantarum* GD2, *Lb. rhamnosus* E9, *Lb. brevis* LB63 and *Lb. delbrueckii* ssp. *bulgaricus* B3 bacteria induced apoptosis in colon cancer cells *in vitro* [43]. Riaz-Rajoka *et al.* have found that the cell-free supernatant of *Lb. rhamnosus* SHA111, SHA112, and SHA113 strains isolated from breast milk has a very strong antioxidant activity and has an anticancer effect on cervical cancer cells.

Mental illnesses

Gut bacteria interact bidirectional with the brain, and this interaction is called the gut-brain axis. Potential mechanisms affecting this relationship include changes in microbial content, immune stimulation, vagus nerve, tryptophan metabolism, intestinal hormonal response, and bacterial metabolites. Hormonal, neuronal, and bacterial changes in the gut are transmitted to the brain via the vagus nerve [44]. Vagal afferents are not in direct contact with the gut microbiota or lumen content but can detect lumen signals indirectly through the diffusion pathway through the gastrointestinal barrier of bacterial compounds or metabolites [45].

Wei *et al.* have demonstrated that heat-inactivated *Lb. paracasei* PS23 reverses the reduced protein levels of brain-derived neurotrophic factor, mineralocorticoid, and glucocorticoid receptors in the hippocampus, thereby exerting an antidepressant effect in depressed mice [46]. Another study reported that depressive and anxiety-like behaviors improved in mice when heat-killed *Lb. helveticus* MCC1848 was given to mice with sub-chronic and mild social defeat stress [47]. Liu *et al.* have showed that short-chain fatty acids produced by gut bacteria provided behavioral improvements in mice with postpartum depression [22].

Metabolic diseases

Short-chain fatty acids produced from nondigestible carbohydrates by the gut microbiota, inhibit insulin-dependent lipid accumulation via free fatty acid receptor 2-FFAR 2 signaling and reduce inflammation in adipose tissue. In addition, acetate increases leptin secretion in adipocytes [48]. Short-chain fatty acids are used by colonocytes as an energy source, they have beneficial effects on lipid and glucose metabolism. When short-chain fatty acids enter the bloodstream, they influence epigenetic factors such as G protein receptors (GPR41, GPR43, GPR109A, and olfactory receptor 78) and histone deacetylase inhibitor [49]. GPR41 plays a role in the regulation of intestinal hormones, epithelial immunity, T-regulator cell differentiation, GPR43 plays a role in releasing glucagon like peptide-1, which responsible for adipocyte improvement, adipogenesis, antilipolysis,

regulation of appetite, GPR109A plays a role in antilipolysis, HDL-cholesterol metabolism, and immune regulation [50]. Ge *et al.* applied acetate or propionate to the adipocytes of mice and reported that the activation of GPR increased adipogenesis, inhibited lipolysis and thus lowered plasma free fatty acid levels [51].

In a randomized double-blind placebo-controlled study, it was reported that there was a significant decrease in waist circumference and waist/height ratio when heat-killed *Bifidobacterium animalis* subsp. *lactis* CECT 8145 was administered to individuals with abdominal obesity [52]. Seo *et al.* found that there was a significant decrease in body weight and adipose tissue weight in mice that gave prebiotic grape seed flour together with heat killed kefir bacteria [53]. It has been reported that heat-killed *Lb. reuteri* GMNL-263 reduces insulin resistance and hepatic steatosis in mice with high-fat diet-induced obesity, resulting in a decrease in glucose, total triglyceride, total cholesterol, and LDL/HDL ratio [54]. In another study, the same postbiotic has been shown to slightly lower cholesterol levels and reduce myocardial cell death in 8 weeks [55].

In a study conducted with prediabetic individuals, participants given the heat-killed beverage containing *Lb. plantarum* OLL2712 for 12 weeks reported significant decreases from baseline in fasting blood glucose, serum glucoalbumin, insulin resistance index, and serum monocyte chemotactic protein-1 and IL-6, which play a role in insulin resistance and hyperglycemia [56]. In a placebo-controlled study conducted by Roshanravan *et al.*, with 60 individuals with Type 2 diabetes, it was reported that butyrate supplementation decreased the expression of genes that affect planned cell death due to inflammation, namely pyroptosis, and increased total antioxidant capacity [57].

Conclusion and suggestions

In recent years, modulation of intestinal microbiota has become a target in the prevention and treatment of various diseases, and the use of probiotics has become widespread in this context. However, the concept of postbiotics has gained importance due to factors such as the different intestinal microbiota phenotype of each individual, the limited shelf life of probiotics, the reported side effects of probiotics, and the number of probiotic bacteria reaching the intestines varying from individual to individual. Postbiotic metabolites produced by probiotic bacteria affect health through various metabolic pathways. In addition, structural components of inactivated bacteria have positive effects on various diseases. Although preclinical studies show that postbiotics have positive effects on many diseases, there are few studies conducted with humans. Therefore, further research is necessary in this regard.

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Conflicts of interest

All authors declare that they have no conflicts of interest.

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