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Antibacterial, Antiviral, Antioxidant, and Anticancer Activities of Postbiotics: A review of Mechanisms and Therapeutic Perspectives

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Abstract: Several scientific evidence has shown the advantageous effects of probiotic-derived metabolites on human health. Postbiotics are a wide range of bioactive compounds derived from probiotics through a fermentation process and/or produced in pure forms in laboratory scales. These compounds have native biological activities that have been extensively studied in recent years. Immunomodulation, antimicrobial, anti-cancer, antioxidant, anti-diabetic, and reduction of food allergies compose the most important biological roles of postbiotics. In terms of safety, it has been confirmed that postbiotics, as potential substitute elements, might be superior to their parent live cells. Also, due to their appropriate economic, technological, and clinical features, they could be employed as favorable apparatuses in the food and drug industry to improve health benefits. This review comprehensively discusses the concept of postbiotics and their characteristics, emphasizing their potential antibacterial, antiviral, antioxidant, and anticancer activities.

Keywords: postbiotic; probiotic; antimicrobial; antiviral; antioxidant; anticancer.

1. Introduction

The use of beneficial microorganisms in the food and pharmaceutical industries has a long history. According to a definition, probiotics are a group of living microorganisms in the mammalian intestines, which, when taken in sufficient quantities, play a positive role in improving human health [1]. Beneficial intestinal bacteria mainly include *Bacteroides, Fusobacterium, Eubacteria, Peptococcus, Peptostreptococcus, Bifidobacterium, Escherichia*, and *Lactobacillus* [2]. Interestingly, their useful effects have been shown, which include reducing lactose intolerance, preventing cancers, lowering blood cholesterol and absorbing it from the intestine (by breaking down bile in the intestine), lowering blood pressure, strengthening the immune system and preventing infections, preventing and treating acute diarrhea, decreasing intestinal inflammation, reducing food allergies or eczema in children, enhancing vitamin and mineral absorption, improving symptoms of irritable bowel syndrome and colitis, preventing the growth and proliferation of invading bacteria, improving gastrointestinal function and nutrient uptake, and assisting the production of B and K vitamins [3,4]. The beneficial effects of probiotics are exerted in the body through food supplements and/or pharmaceutical products .Due to these favorable effects, probiotics have found special applications in recent years [5]. For example, probiotics' antimicrobial, antioxidant, and anticancer effects have been widely used in laboratory, animal, and clinical models. Moreover, the antimicrobial effect of probiotics has been widely taken advantage of in the food industry [6,7]. Probiotics inhibit the growth of spoilage bacteria and pathogens during food storage, thus being an important way to preserve food and prevent the transmission of pathogens through food. Probiotics also have very strong antioxidant properties that prevent food oxidation during food storage [8,9]. Probiotics have been used extensively as anticancer compounds in laboratory, animal, and clinical models in recent years. Many studies have been conducted on the anticancer effects of probiotics [10]. Despite the beneficial effects of probiotics, the use of these bacteria has recently been shown to have challenges. One of the challenges with using probiotics is their viability. Prescribing supplements containing live probiotic bacteria to people of different ages and physical conditions do not always show the same beneficial effects. These bacteria have adverse effects in people with weakened immune systems and can cause many clinical problems. These include people with Crohn's disease, pregnant women, the elderly, and infants [11]. Other important challenges of using probiotics are the emergence of antibiotic resistance and transmission potential. The genes that cause virulence and resistance to pathogens are located in the host intestine. There are also opportunistic pathogenic bacteria in the intestinal microbiome, the acquisition of antibiotic resistance in which could be associated with serious problems [12]. Researchers have come up with various solutions in recent years to solve these challenges, and have suggested that one of the most effective and practical approaches in this regard is the use of an inanimate form of bacteria (postbiotics) as substitutes for probiotics [13]. Postbiotics are called inanimate forms and metabolites of probiotic bacteria that have beneficial effects if taken in sufficient amounts. In this review, we comprehensively discuss the concept of postbiotics and their characteristics, with emphasis on their potential antibacterial, antiviral, antioxidant, and anticancer activities.

2. Characteristics of Postbiotics

There are various definitions of postbiotics. Postbiotics are well-known as compounds generated throughout the process of fermentation in probiotic bacteria. They could also be generated by feeding particular fiber molecules to probiotics. They leave waste that is generally termed as postbiotic [14]. The most important example of postbiotics is SCFA, which is produced by the fermentation of carbohydrates [15]. Many terms have been used to name nonliving forms of probiotics in recent years, including postbiotic, pseudoprobiotic, ghost probiotics, paraprobiotic, metabiotic, abiotic, cell-free supernatant, and Biogenic [16]. Among these, "postbiotic" is more commonly used. However, 'paraprobiotic' and 'pseudoprobiotic' have also been used in a number of studies [15]. Various components are obtained with the lysis of probiotic bacteria, which could include short-chain fatty acids, cell wall fragments, enzymes, exopolysaccharides, and cell-free supernatants. There are two methods for producing and obtaining postbiotic compounds. The first is the natural method (fermentation process), and the second is the laboratory method [17]. In general, postbiotics have shown all the beneficial effects of probiotics, including strengthening the immune system, hypersensitivity, and antibacterial, antiviral, antioxidant, antiobesity, anti-diabetes, antihypertensive, antiproliferation, anti mutation, and anticancer effects, all of which have been proven both *in vitro* and *in vivo* [18]. In experimental and preclinical studies, no adverse effects (such as inflammation) of postbiotics have been observed. Therefore, postbiotics can be a safe alternative to probiotics, owing to their known chemical structures, safe dose, and longer shelf life [19]. Therefore, the safety of postbiotics is a good reason to apply them in the food and pharmaceutical industries. Several experiments have been conducted on the advantageous effects of postbiotics [20]. Importantly, the beneficial effects of postbiotics depend on the type of probiotic used to extract the postbiotics and the type of postbiotics themselves. Here, we examine the antibacterial, antiviral, antioxidant, and anti-cancer effects of postbiotics, focusing on their mechanism of function.

3. Antibacterial Effects of Postbiotics

Inhibition of pathogenic bacteria and food spoilage is one of the beneficial effects of postbiotics. Today, due to the advantages of postbiotics over antibiotics and chemical preservatives, the use of these compounds against pathogenic bacteria and food spoilage is common. Antibacterial effects of postbiotics depend on (a) the type of probiotic from which the postbiotic is prepared, (b) the type of target bacterium (Gram-positives are more resistant to gram- negatives against postbiotic compounds), and (c) the concentration of postbiotics [21]. Several postbiotics could exert direct antimicrobial effects as they seal the intestinal barrier and bind competitively to receptors that are needed by pathogens. They thus change host gene expression or modulate the local environment [22]. In this regard, in an in vitro study, the effects of postbiotics derived from *Lactobacillus plantarum* were investigated. Results indicated positive effects of postbiotics as inhibitory factors capable of reducing the adhesion and invasive ability of *Listeria monocytogenes* [23]. Postbiotic administration of probiotic bacteria *L. casei*, *L. acidophilus*, and *L. delbruckii* in mice enhanced gut IgA levels in small and large intestine lamina propria. This reduced the severity of enteric infections induced via pathogens, including *Escherichia coli* or *Salmonella enteritidis* serovar *Typhimurium* [24]. Antibacterial activity has also been shown in the supernatants of cultures of *Bifidobacterium* and *Lactobacillus* recently. This could be observed *in vitro* by preventing the invasion of enteroinvasive *E. coli* strains into enterocytes. The cell supernatants could also exert local effects on the expression of protective genes, cell barrier, and intestinal environment, even though such antibacterial features might be caused by inhibiting the adhesion of pathogenic bacteria (as they compete for receptor sites) [25]. The short-chain fatty acids (acetate) produced by lactobacilli prevented the infectivity of *E. coli O157: H7* in a mouse model. Lactic acid produced by six strains of Lactobacillus (*L. acidophilus* IBB 801, *L. amylovorus* DCE 471, *L. casei Shirota*, *L. johnsonii* La1, *L. plantarum* ACA-DC 287, and *L. rhamnosus* GG) from *Salmonella enterica serovar Typhimurium SL1344* blocks Caco 2/TC7 cells, including human enterocytes [26]. According to studies, it could be concluded that each of the postbiotic compounds with its mechanism can kill pathogenic bacteria.

3.1. Antibacterial mechanism of postbiotics.

As mentioned, the effect of postbiotics on bacteria depends on factors such as the type of postbiotic and the bacteria. Postbiotics are not generally effective or are less effective, against Gram-positives bacteria since the antibacterial postbiotics cannot permeate the outer membrane of Gram-negative bacteria. It was shown by Enan *et al.* that *L. plantarum* UG1 had a more potent antibacterial activity against Gram-positive bacteria in comparison with Gram-positive microorganisms. Therefore, due to the resistance of gram-negative bacteria, pesticides should be extracted from probiotic bacteria with high antimicrobial properties [27]. Postbiotic compounds derived from probiotics each have a specific antibacterial mechanism. In the following, we examine the bacterial mechanism of each postbiotic compound.

3.1.1. Organic acid-based postbiotics.

Organic acid-based postbiotics are compounds appropriate as antimicrobial agents [28]. Organic acids are known as one of the key postbiotics. Citric acid, acetic acid, and tartaric acid are the most important acids produced by probiotic bacteria and have strong antibacterial effects. Lactic acid, acetic acid, tartaric acid, malic acid, and citric acid inhibit pathogens by significantly reducing pH value [29]. The inhibitory effect of lactic acids is related to their effect on bacterial cell membranes. The antibacterial mechanism of organic acids is mainly observed through lowering intracellular pH and reducing membrane integrity. [30]. The antimicrobial activity of organic acids can be linked in two ways [29].

3.1.2. Bacteriocin-based postbiotics.

Bacteriocins are peptides or proteins with antibacterial activity produced by various bacteria, such as *Lactubacillus* and *bifidobacters* [30]. Bacteriocins have a high antibacterial activity that has been used for thousands of years by humans in producing fermented foods. Bacteriocins are classified based on size, mechanism of action, and inhibitory spectrum. Bacteriocins have many positive effects, such as inhibiting the growth and development of pathogenic bacteria, and are also heat-resistant and pH-resistant [31]. The antibacterial mechanism of bacteriocins is mainly observed through their effect on bacterial cytoplasmic membranes. Bacteriocins are linked by affecting sensitive components, such as bacterial peptides, and inhibiting spore growth and pore formation in the cell membrane of pathogenic bacteria [32].

3.1.3. Fatty acid-based postbiotics.

Fatty acid-based postbiotics can be used as suitable alternatives to antibiotics due to their high antibacterial activity. In recent years, using short-chain fatty acids (FACs) produced by probiotic bacteria has become very common. FACs are formed from a saturated and an unsaturated carbon chain attached to a carboxylic (hydrophilic) group. Among fatty acids, lauric and meristic acids are highly active against bacterial growth and development [33]. Antibacterial mechanisms of FACs on bacteria include increasing membrane permeability (and leading to cell lysis), disrupting the electron transport chain, disrupting the structure and activity of enzymes, and morphological/functional changes on sensitive components such as proteins [34].

3.1.4. Peptide-based postbiotics.

Microorganisms produce antibacterial peptides. Peptide-based postbiotics destroy microbes through pleiotropic (multiple actions) mechanisms, such as microbial membrane degradation and inhibition of macromolecule synthesis [35]. Antibacterial peptides are divided into ribosomal and non-ribosomal types. Peptides produced by the bacteria are ribosomal [36], which show strong antibacterial activity *in vitro* by disrupting microbial membranes [37]. Peptides are commonly present in all bacteria. As mentioned earlier, the main target of some peptides is the cell membrane, while that of other peptides is the cytoplasm and disruption of metabolic accumulation of the nucleus [38]. The antibacterial mechanisms of peptides include creating physical holes that leak cellular content, damaging sensitive intracellular components of the microbes, activating lethal processes such as inducing hydrolases that have detrimental effects on the cell wall, and creating acidity in the bacterial cell membrane [39] (Figure 1).

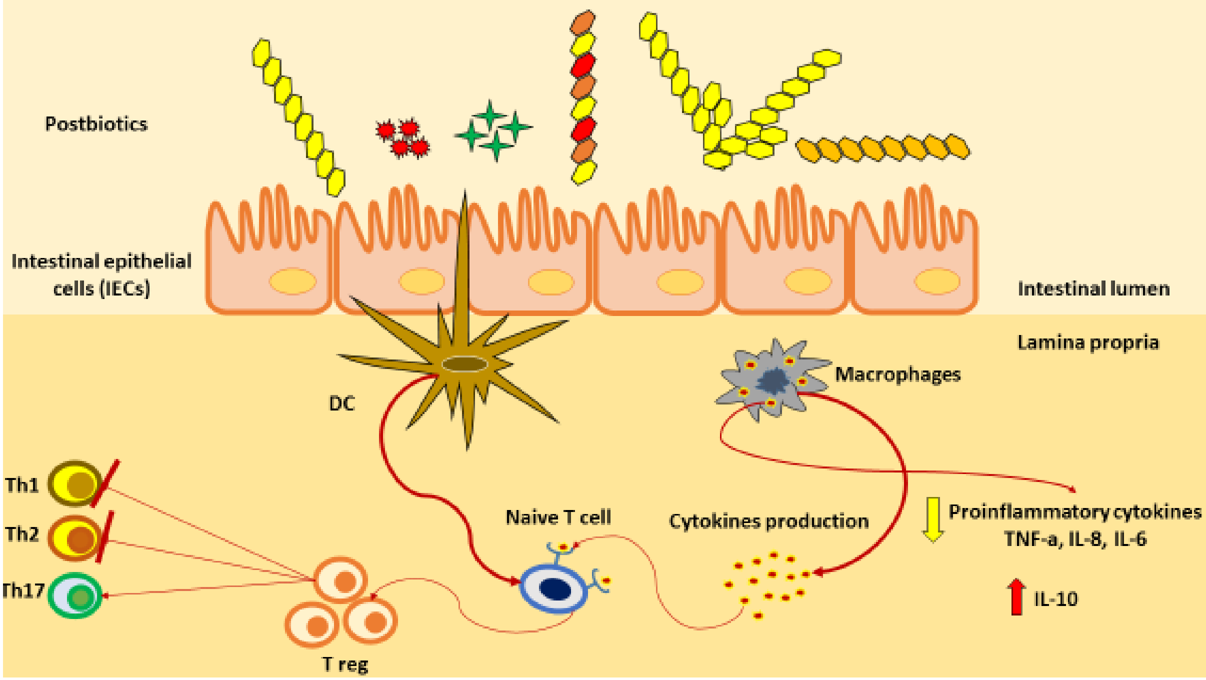

Figure 1. Antibacterial, antiviral, antioxidant, and anticancer activities of postbiotics. The postbiotics could induce activation and differentiation of DC, leading to CK production, subsequently, it mediated differentiation of naïve T cells into Tregs, which would control the excessive T cell response. Further, postbiotics could effectively stimulate the production of cytokines by macrophages which might induce homeostasis via reduction of pro-inflammatory CKs. Treg, regulatory T cell; DCs, dendritic cells; IL, interleukin; TH, T helper; TNF-α, tumor necrosis factor-alpha.

4. Antiviral Effect of Postbiotics

Through threatening public health, emerging viral infections are considered global issues. It has was shown *in vitro* that postbiotics exert antiviral effects when encountering enveloped viruses. We now know well that to eliminate viruses successfully, the infected cell needs to induce a pro-inflammatory immune response and develop Th1-type immunity, both of which lead to the restriction of viral replication. Such responses depend on the production of inflammatory chemokines, cytokines, and interleukins, such as TNF-α, interferons, IL-23, IL-18, and IL12, and cytotoxic activation of T-lymphocytes, NK cells, and monocytes/macrophages [40]. Probiotics and metabolites derived from them can protect against viral infections by enhancing adaptive and innate immunity. This reduces illness duration, shedding of the virus, and the number of episodes. It also enhances the generation of virus-specific antibodies and normalizes gut permeability [41]. Postbiotics can interfere with virus absorption and penetration into the host cell and inhibit several retroviral reverse transcriptases. The antiviral effect of postbiotics depends on the type of probiotic used to extract the postbiotics and the type of viruses. Many studies have been performed on the antiviral effect of probiotic metabolites (postbiotics). In a study, Firoz Anwar *et al.* (2020) showed the effect of plant-derived probiotics (Plantaricin) on SARS-CoV-2. It was observed that Plantaricin can prevent infection by modulating the immune system (by affecting T cells, producing IFN-γ, and reducing pre-inflammatory cytokines) and having a direct effect on the virus. SARS-CoV-2 enters the host cell via spike glycoprotein (S) with a high affinity for the angiotensin-converting enzyme (ACE2) receptor. Once this glycoprotein enters the body with the enzyme angiotensin 2 converter (ACE2), it causes an infection in the body. Postbiotic (Plantaricin) compounds can inhibit Covid-19 pathogenicity by binding to spike glycoprotein (S) [42]. In another study, they examined the effect of cell-free metabolites of *L. rhamnosus* on enterovirus and Coxsackievirus in the HeLa, Vero, and Hep-2 cell lines. It was found that the metabolites of *L. rhamnosus* prevented viruses from attaching to cell lines [43]. In another study, B.J. Seo *et al*. (2012) examined the effect of *Leuconostoc mesenteroides* free cell supernatant on H9N2 in SPF chicks and found that the intake of probiotic metabolites significantly increased IFN-c cytokine production and made broilers immune against H9N2[44].

4.1. Antiviral mechanism of postbiotics.

The antiviral role of postbiotics is largely seen by preventing the virus from attaching to the host cell, which prevents the early stages of viral infection. The virus is prevented from entering the host cell by blocking viral binding to the host cell receptors [45]. Another mechanism involves strengthening the immune system by postbiotics. In addition, intracellular mechanisms have been predicted by other authors, suggesting that probiotics may interfere with the stages of the viral cycle within cells [46]. Postbiotic compounds have specific antiviral mechanisms. Here we examine the antiviral mechanism of postbiotics.

4.1.1. Organic acid-based postbiotics.

Organic acids produced by probiotic bacteria are known as postbiotic compounds, which could be introduced as antiviral agents [47]. Lactic acid is one of the most important organic acids produced by probiotics which enjoys antiviral activity [48]. Lactic acid is present in two isomers, including L and D. The L-shape is more effective in preventing viral infections. Citric acid and acetic acid also prevent the infection of viruses by creating an acidic environment. The antiviral mechanism of organic acids produced by probiotic bacteria is observed through the binding of organic acids to the glycoprotein (S) of viruses, thereby preventing viruses from binding to the angiotensin-converting enzyme (ACE2) [49].

4.1.2. Fatty acid-based postbiotics.

Among the most important metabolites of probiotic bacteria are fatty acids. Fatty acids from probiotic bacteria are known as postbiotic compounds. Like other postbiotic compounds, these compounds exert many beneficial effects (such as antiviral properties). The antiviral effect of fatty acids is due to their special structures. Fatty acids are formed from a saturated and an unsaturated carbon chain attached to a carboxylic (hydrophilic) group [47,50]. Fatty acids are also recognized as potential postbiotics with considerable antiviral properties. Lauric and meristic acids are highly active against the virus growth and development [33]. Fatty acids produced by probiotics, such as organic acids, bind to spike glycoprotein (S) and prevent it from binding to the angiotensin-converting enzyme 2 (ACE2).

4.1.3. Peptide-based postbiotics.

Antimicrobial peptides are ~12-50 amino acid-long small proteins. Peptide compounds produced by probiotic bacteria exert potent antiviral effects. The use of peptide compounds against viruses is a very effective method. Studies have shown that peptide compounds produced by probiotic bacteria have excellent antiviral properties. In one study, the effect of peptides on influenza and Covid-19 viruses was assessed, where it was found that peptides prevented the proliferation of these viruses by inhibiting endosomal acidification [51].

4.1.4. Hydrogen peroxide-based postbiotics.

Hydrogen peroxide is mainly produced by all bacteria but is generally visible in aerobic cultures of catalase-negative bacteria and is the major metabolite of lactic acid bacteria [28]. Hydrogen peroxide produced by probiotics is known as a postbiotic [52]. These compounds have excellent antiviral properties. These compounds can disinfect materials because of interacting with vital constituents of bacteria, fungi, and viruses, including enzymes and nucleic acids (DNA, and RNA), thus preventing their replication and capacity of infection [53]. Therefore, the use of hydrogen peroxide from probiotic bacteria as an inhibitory compound of a virus is a good way to control viral infections.

5. The Antioxidant Effect of Postbiotics

Postbiotics enjoy particular mechanisms of defense effective in decreasing destructive effects of reactive oxygen species (ROS), which could potentially impair nucleic acids, carbohydrates, proteins, and lipids. Particularly, antioxidant enzymes, including catalase, peroxide dismutase (SOD), and glutathione peroxidase (GPx), possess significant parts in fighting against reactive oxygen species [54].

Tale 1. The following table shows the antioxidant application of postbiotics.

Probiotic strain	Type of postbiotic	Type of assessment	Method	Antioxidant effect	References
Lactobacillus plantarum RG14 ، RG11	Enzyme	As an oral supplement for lambs	2,2-diphenyl-1-picryl-hydrazyl (DPPH)	Postbiotics increase the antioxidant activity of glutathione peroxidase (GPX) in serum and rumen fluid and GPX1, GPX4, hepatic superoxide dismutase	[58]
Lactobacillus plantarum	Enzyme	As an oral supplement for broilers	By examining meat quality (pH, WHC, color and sensitivity)	Enhance the activity of antioxidant enzymes.	[59]
Lactobacillus plantarum RI11	Enzyme	As an oral supplement for broilers	By checking the quality of chicken meat	Postbiotic increases the activity of antioxidant enzymes (GPx, CAT and GSH) and decreases heat stress marker	[24]
L. plantarum RG11, RG14, RI11, RS5, TL1, and UL4	Organic acid	*In vitro*	Hydroxyl Radical Scavenging Assay (HRS)	The HRS activity was significantly higher ($P < 0.05$) for the postbiotics produced by the formulated media as compared to the control MRS medium, even though there was no significant difference among all the producer strains	[60]

The above-mentioned azimuths obtained from probiotic bacteria are each known as postbiotic. In recent years, many studies have been conducted on the antioxidant effects of postbiotics (Table 1). Studies have shown favorable antioxidant effects for these enzymes. High GPx content has been reported in two strains of *L. fermentum*, which also enjoy significant antioxidant features *in vitro* [55]. *L. plantarum*-derived postbiotics were assessed for their antioxidant features by Izuddin *et al.*, where the effect could be witnessed due to enhanced concentration of glutathione peroxidase in serum ($p < 0.05$). Furthermore, superior relief of the symptoms of Crohn's disease in mice was demonstrated for genetically modified *Lactobacillus* strains capable of synthesizing catalase or SOD, as compared to their unmodified counterparts [56]. Additionally, *Lactobacillus* strains possessing improved activity of catalase showed more successful relief of inflammation in mice with inflammatory bowel disease, as compared to strains of the same bacterium which produced SOD (compared to controls, however, both strains reduced body temperature ($p<0.05$)) [57]. It was shown in this trial that anti-inflammatory features of *Lactobacillus* strains depend on the profile of the expression of anti-oxidative enzymes for each strain. Moreover, genetically modified *Lactobacillus lactis*, which expressed catalase was capable of preventing chemically-induced colon cancer in mice [57]. To better understand the antioxidant effect of postbiotics, we here examine the antioxidant effect of enzymes produced by probiotics, each of which is known as a postbiotic compound.5.1. Antioxidant mechanism of postbiotics.

Studies on the antioxidant effect of metabolites have shown that the antioxidant effect of these compounds depends mostly on the type of probiotics selected to extract antioxidant enzymes. For example, *L. fermentum* has been shown to produce the highest amount of antioxidant enzymes. Therefore, the type of probiotics should be considered to extract more antioxidant enzymes. Probiotic bacteria produce a large number of antioxidant enzymes [61]. Here we introduce the antioxidant mechanism of these enzymes.

5.1.1. Catalase.

Catalase, as an enzyme naturally found in probiotics and other living organisms, breaks down hydrogen peroxide (H_2O_2) into oxygen and water [62] Catalase is considered a postbiotic since it is produced as a metabolite of probiotic bacteria [63]. In general, catalase is a Reductase oxidase enzyme as it suppresses ROS. It, therefore, acts as an antioxidant and protects the cell against oxidative stress through the inhibition of active oxygen species [64]. Catalase consists of four polypeptide chains, each containing more than 500 amino acids. It contains four porphyrins (iron) that allow it to react with oxygenated water. The appropriate pH for the activity of this enzyme is 4-11 [65]. Therefore, when using these enzymes as Postbiotics with antioxidant properties, the pH of the food should also be considered.

5.1.2. Superoxide dismutase.

As an enzyme, superoxide dismutase is capable of catalyzing and facilitating the radical decomposition of superoxide (O_2^-) into ordinary oxygen (O_2) or hydrogen peroxide (H_2O_2) molecules [59]. There are three superoxide dismutase isoforms in living organisms, which include cytosolic SOD1 (copper, zinc-SOD), mitochondrial SOD2 (Mn-SOD), and extracellular SOD3 (EC-SOD) [66], each of which plays a different role in maintaining healthy cells. For example, SOD1 protects cellular cytoplasm, SOD2 supports cellular mitochondria from free radical damage, and SOD3, as an antioxidant compound, plays an important role in creating immunity against inflammatory diseases. This enzyme protects tissues against the effects of oxidative stress with its antioxidant activity [67]. Superoxide dismutase is an essential enzyme in the antioxidant system of living organisms. Like other living organisms, probiotics possess this enzyme, which as a metabolite of probiotics, is a type of postbiotic that can be used as an antioxidant compound [27,68].

5.1.3. Glutathione peroxidases.

Glutathione peroxidases are a family of enzymes with peroxidase activity whose main biological role is to keep organisms safe from oxidative damage. Glutathione peroxidase neutralizes the effects of H_2O_2 [69]. Another key feature of glutathione peroxidase involves reducing peroxides to alcohol and hampering free radicals' formation because cellular lipid compounds are sensitive to free radicals and produce lipid peroxide by the reaction. Glutathione peroxidase has several isoenzymes that are encoded by different genes [70]. These isoenzymes are located in different parts of the cell and have different substrate properties. Glutathione peroxidase 1 is the most abundant isoenzyme found in the cytoplasm of almost all mammalian tissues and its preferred substrate is hydrogen peroxide [71]. Glutathione peroxidase 2 is an extracellular enzyme and is present in the intestinal lumen [72]. Glutathione peroxidase 3 is also extracellular and is abundant in plasma [73]. Glutathione peroxidase 4 is highly preferred to lipid hydroperoxides and is expressed in almost all mammalian cells but at deficient levels [74].

6. Anticancer Effects of Postbiotics

The anti-cancer effect of postbiotics is another favorable effect of these compounds. The anti-cancer effect of postbiotics depends on the type of probiotic chosen to extract the postbiotics, the type of postbiotic, and the type of target organ involved in cancer [75]. Many studies have been conducted on the anticancer effect of postbiotics in experimental and preclinical phases (Table 2). For example, a study has demonstrated the postbiotic role of *Lactobacillus paracasei* in cortical cancer. The anti-cancer mechanism of *L. paracasei* is associated with an inhibitory effect of cell wall protein on human cell carcinoma and cell growth [76]. Another study has examined the anticancer effect of postbiotics produced by Enterococcus on carcinogenic cells. This anticancer effect is linked to bacteriocins produced by probiotics, especially Enterocin, with the highest anticancer effect among bacteriocins. Similarly, the anticancer effect of *L. plantarum* I-UL4 on cancer cells was investigated, which showed the anticancer effect of bacteriocin produced. Bacteriocins have cytostatic and apoptotic effects against cancer cells [77].

Table 2. Potential anticancer activity of postbiotics.

Probiotic strain	Type of postbiotic	Cancer cell line	Effect (s)	Method of evaluating anticancer activity	References
Lactobacillus plantarum	Bacteriocin	Human breast cancer cells MCF-7	Decreased cell proliferation	Observation of the cytotoxic effect of postbiotics by fluorescent microscopic observation using AO / PI color reagents and flow cytometric analyzes	[78]
Lactobacillus plantarum I-UL4	Protein	MCF-7 breast cancer cell	Cytotoxicity and decreased proliferation	Via 3- (4, 5 dimethylthiazol-2-yl) -2, 5-diphenyl Tetrazolium Bromide and Trypan Blue Dye Deprivation Method.	[79]
Lactobacillus casei and *Lactobacillus paracasei*	Protein	Cervical cancer cells	Reduce apoptosis, bind to carcinogenic genotoxins and reduce proliferation	Via RT-q PCR and western blotting	[80]
Lactobacillus paracasei K5	Protein	Human colon cancer cells	Anti-proliferative activity and apoptotic effects	Employing quantitative analysis and confocal, fluorescent microscopy the adhesion properties of the postbiotics were studied	[81]

In general, several molecular mechanisms compose in the anticancer activity of postbiotics, including diminution of the viability of the cell, regulation of immune response, control of carcinogenic and mutagenic agents, activation of the apoptotic cell death pathway, inhibition of gut dysbiosis, an increase of necrosis and apoptosis rate in cancer cells [18].

6.1. Anticancer mechanism of postbiotics.

Recently, many *in vitro* and *in vivo* investigations have shown that postbiotics possess an essential part in preventing and treating cancers of various body organs. Several molecular mechanisms with roles in the function of postbiotics were demonstrated in those studies, including regulation of immune responses, increasing apoptosis and necrosis, inhibition of carcinogenic and mutagenic factors, reducing bacterial transmission, activation of pro-apoptotic cell death pathways, and increasing tumor cell death through Autophagy [82]. Mechanism of bacterial carcinogenesis is observed by producing various toxins that disrupt the modification of the cell cycle, proliferation, differentiation, and apoptosis [83].

6.1.1. Reduction of bacterial transmission.

Different bacteria are involved in causing cancer. Colorectal cancer is a common disease caused by pathogenic bacteria (*Streptococcus bovis*, *Enterococcus spp.*, *Helicobacter pylori* and *E. coli*) [84]. Also, gastric cancer is often caused by *Helicobacter pylori*. Bacteria and their associated products can participate in the formation of the disease by developing various mechanisms, such as the induction of pro-inflammatory and carcinogenic pathways in epithelial cells, the generation of genotoxins and ROS, and the transformation of precancerous dietary agents [85]. Due to their antibacterial effects, postbiotic compounds can prevent bacteria from attaching to the intestinal epithelium and producing toxins and can prevent these bacteria from causing cancer.

6.1.2. Inhibition of mutagenic and carcinogenic factors.

Postbiotics may kill active carcinogenic components or exert effects on the function of cancer-stimulating/inactivating enzymes. Some *in vitro* experiments have shown that the anti-mutagenic property of probiotics could be attributed to the postbiotics derived from it, including secretory glycoproteins, polysaccharides, and peptide glycans, as well as to the phase of bacterial growth, ambient pH, mutant type, and related strain [78]. Probiotics and postbiotics derived from them can also bind to mutagenic agents, leading to biological deformation and consequent detoxification. Molecular mechanisms with significant parts in anti-mutagenic features of postbiotics include mutagen-binding, stimulation of chemopreventive enzymes, inhibition of enzymes that activate carcinogens, and inhibition of DNA damage [86]. Therefore, it could be proposed that postbiotics might be capable of exhibiting anti-mutagenic and anti-oxidant activities via distinct mechanisms and could accordingly be regarded as effective prophylactic agents when combatting cancer [87].

6.1.3. Activation of pro-apoptotic cell death pathways.

Cancer cells possess unregulated proliferation and growth and are capable of fighting apoptotic responses. In damaged cells, most apoptosis responses and survival mechanisms are changed adversely. The process of apoptosis is described as a major method of abnormal cell death in cancer patients because of its exceptional features (normal host response, without inflammatory response) [88]. Metabolites produced by *Lactobacillus spp*. inactivate cells by affecting cell wall components, peptidoglycans, cytoplasmic extracts, and cell-free processes which could have anti-proliferative and apoptotic responses to cancer cells. In this regard, *Lactobacillus spp* derived metabolites have been shown to have an inhibitory effect with a rapid apoptotic response and cell cycle inhibition in the S phase in HT-29 cancer cell lines [89].

6.1.4. Increased intestinal acidity.

Postbiotic components, such as FACs and organic acids (propionate and lactate) fermented by the microbial colon, are produced from active carbohydrates such as prebiotics that can acidify the intestinal environment and prevent the growth of pathogens that are effective in cancerous tumor formation. Such acids possess significant parts in cancer suppression and might potentially exert systemic and local biological effects [82]. Among postbiotics, FACs enjoys an ideal energy resource for colonocytes which potentiates the differentiation of mucosal cell, contributes to epithelial barrier function, controls apoptosis, prevents the differentiation and growth of cells, and controls glutathione S-transferase and histone acetylation pattern [90].

7. The Multifunctional Perspectives of Postbiotics

Postbiotic compounds may have multiple functions [91] (Figure 2). Postbiotics with antioxidant properties (enzymes) can have anti-proliferative and pro-apoptotic effects on cancer cells. It was shown that some *Bifidobacterium* and *Lactobacilli* are capable of inducing enhanced activity of anti-oxidative enzymes or modulating circulatory oxidative stress protecting cells against damage induced by carcinogens [92]. Fatty acids produced by *Lactobacillus* can heal the gut by reducing pro-inflammatory cytokines. Fatty acids can also prevent *E. coli* O157: H7 enterohaemorrhagic infection [93]. Some EPS generated via Lactobacillus strains isolated from fermented Durian fruit enjoy antioxidant and antimicrobial features. The antioxidant feature of an EPS (uronic acid) derived from *Lactobacillus helveticus* was attributed to its capability for binding to iron ions, to which the antioxidant characteristics of green tea could also be attributed [17,94].

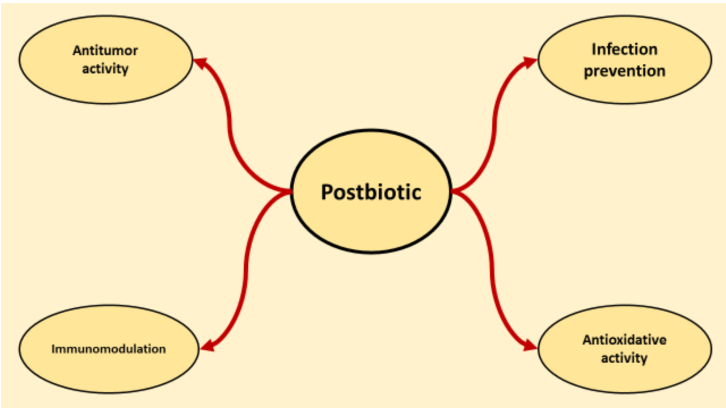

Figure 2. The multifunctional perspectives of postbiotics.

Exopolysaccharides are capable of interacting with Dectin-1 receptors and activating them on macrophage surfaces. Resultantly, β-glucans might improve cellular immune responses against bacteria, viruses, parasites, and cancer cells [95]. Abdelkarim Mahdhi *et al*. (2017) investigated the antibacterial and antioxidant effects of EPS from *L. plantarum, in vitro*. This study found that EPS produced by *L. plantarum* prevents the growth of this bacterium through oxidation of linoleic acid, lipid peroxidation, and biofilm formation by *Staphylococcus aureus* and *Salmonella typhimurium* [96,97]. Taken together, it could be concluded that each of the postbiotic compounds can have numerous functions. Despite the studies on several effects of postbiotics, the mechanism of action of multiple effects of postbiotics has not yet been determined. Therefore, it is necessary to conduct future studies to determine the mechanism of various effects of postbiotics.

8. Conclusions

Pathogenic microorganisms, carcinogenesis, and oxidative stress threaten public health. Many treatments are used to control the risks in this regard, which cost much money each year. Postbiotics, as metabolites or non-living forms of probiotics with many health effects. Postbiotics have been used in recent years for various purposes, including assessing their antimicrobial, anticancer, and antioxidant activities. Excellent results have been reported in this regard. Each postbiotic compound (proteins, fatty acids, peptides, bacteriocins, enzymes, organic acids, and hydrogen peroxide) has specific antimicrobial, antioxidant, and anticancer mechanisms. Postbiotics are achieved through the activity of probiotic bacteria (fermentation) or are produced on a laboratory scale. Previous research has shown that postbiotics have clinical (safety), technological (sustainability) and economical (low costs of production) advantages over probiotics. They are therefore safe alternatives to probiotics. Due to their unique properties, postbiotics can be used as promising tools to control pathogenic microorganisms, as well as a novel bio-preservative agent in the formulation of functional foods. Studies on the antimicrobial, antioxidant, and anticancer effects of postbiotics have been conducted extensively both *in vivo* and *in vitro*. Many metabolic and clinical studies are needed to arrive at new types of postbiotics, safe doses, and compound chemical structures.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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