The Anti-Colon Cancer Effects of Probiotic and Postbiotic: A Review Article

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Abstract

Cancer, the leading cause of death worldwide, is a name for more than 100 types of diseases that evolved from normal cells and tissues in the body. Cancer cells fail to control cell proliferation and homeostasis due to mutations. These mutations arise from intensive environmental factors or alterations in genes that aid genetic instability. The immune system is the first defense line against cancer cells as it inhibits tumor development, growth, invasion, and metastasis. However, cancer cells develop many mechanisms to escape and avoid destruction by the immune system. Probiotics is a term used to describe the entire population of microorganisms that inhabit the human body. Probiotics and their metabolites stimulate the immune system and maintain gastrointestinal integrity. Postbiotics are bioactive products produced by probiotics. They facilitate cell-cell interaction and regulate signaling pathways. Postbiotics also promote health through different mechanisms. In this review, the anti-colon-cancer effects of probiotics and postbiotics have been investigated.

Keywords: Cancer, CRC, gut microbiota, probiotic, postbiotic.

Introduction

Cancer, a leading cause of death worldwide, is a name for more than 100 types of diseases that evolved from normal cells and tissues in the body (Hanahan, 2022). Tumors progress from three essential steps: 1) extensive proliferation, 2) invasion to adjacent tissues, and 3) metastasis (Hanahan, 2022). Checkpoints found in the cell fix mutations that arise from damaged DNA by either arresting the cell cycle, prolonging the time for DNA repair, or triggering the apoptosis (Loeb and Anderson, 2003). However, cancer cells fail to control cell proliferation and homeostasis due to mutations in genes that aid genetic stability such as p53, a cell cycle checkpoint protein that when mutated triggers uncontrolled cell divisions (Ames et al., 1995). Loss of heterozygosity (LOH), a loss of normal alleles in cells, is the source of genetic alterations documented in various cancers; When genetic alterations occur, they cause pre-malignant lesions that induce the monoclonal expansion of cells (Yokota, 2000). The second round of genetic alterations in the pre-malignant transforms the cells into malignants that eventually form primary tumors. Additional genetic alterations occur in aggressive and invasive tumors that metastasize to other tissues. Thus, the primary tumor must have all the genetic alterations required for its invasiveness and metastatic abelites to metastasis (Yokota, 2000). Cancer cell develops essential physiological alterations to aid malignant growth. It develops growth signals self-sufficiency where the cell stops receiving signals from surroundings and follows its guides. Cancer cells become insensitive and grow and divide regardless of the inhibitory growth signals. It escapes programmed cell death (apoptosis) and sustains an infinite replicative potential (Hanahan, 2022).

Unhealthy diet and lifestyle account for high incidents of cancer especially colorectal cancer. A diet that is low in fiber, rich in fat, and contains lots of red and processed meat changes the composition of the gut microbiota; This affects the integrity of the intestinal mucus layer leading to cancer (Pothuraju et al., 2021). However, the alteration in gut microbiota can be regulated using probiotics and their metabolites (Vrzáčková et al., 2021). In this review, the anti-colon cancer effects of probiotics and their metabolites (postbiotics) have been investigated.

Materials and methods
Research Methods
The qualitative method is utilized to analyze the nature of this paper and to achieve the research objective. The detected data is summarized and analyzed, and the review's conclusion was drawn from the collected data without comparison between the results.
Research techniques
The steps used to write this review are as the following:

At first, the aims of the study were specified, the knowledge was explained, the different elements and criteria of each section were reviewed, and a comprehensive review of the topic was provided. Then, the methodologies and research techniques were used, and finally, the expected results were explained and discussed, and the conclusions based on the collected data were discussed.

Results
Human Microbiota
Microbiota is a term used to describe the entire population of microorganisms that inhabit the human body as well as their genomes and metabolites. These microorganisms make up about 2% of the body mass and are found in many parts of the human body, such as the skin, the upper respiratory tract, the urogenital tract, the gastrointestinal tract, and more (Torres-Maravilla et al., 2021). The gut microbiota is an essential part of the digestive tracts of vertebrates, including humans. It contains about 400–1000 adherent and non-adherent bacteria species; each gram of intestinal content has 1011 bacteria (Vrzáčková et al., 2021). These bacteria are called Probiotics and involve many strains such as *Lactobacillus (L.)* and *Bifidobacterium (B.).* They form a symbiotic relationship with the host as many important body processes require the presence of Probiotics. The host offers Probiotics a constant nutrient-rich environment; in return, Probiotics provide it with tremendous benefits (Si et al., 2021). Probiotics encode more than three million genes and produce thousands of metabolites for the host, whereas a host such as humans only encodes for 23 000 genes (Valdes et al., 2018). These metabolites benefit the human body by stimulating the immune system, increasing the intestine's ability to digest and absorb food, preventing pathogen growth and evasion, and maintaining the intestinal barrier integrity (Żółkiewicz et al., 2020). In fact, the human body's most fundamental molecules are produced by the gut microbiota, such as vitamins, aromatic amino acids, and phenolic-derived molecules. These metabolites benefit the host in countless ways and facilitate the host-microbiome crosstalk (Żółkiewicz et al., 2020).

Modulating the gut microbiota was the subject of interest over the past 43 years as the number of papers reporting research in probiotics was increasing. The risks associated with the intake of probiotic metabolites are minimized as they do not contain live microorganisms. Therefore, the beneficial effects of probiotics' metabolites have been investigated in many human intervention studies (Wegh et al., 2019). Clinical studies of infant formulas containing probiotics' metabolites reported great beneficial effects on gut microbiota composition and gastrointestinal functioning.

Another report investigated the effect of probiotics' metabolites on atopic dermatitis. The severity of the disease in the intervention group was significantly reduced after two to three months compared to the placebo group (Wegh et al., 2019). Currently, many commercial products that contain probiotics or their metabolites have been formulated for their nutritional advantages for overall health in general. These probiotics include *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Streptococcus*, and *Enterococcus*, (Torres-Maravilla et al., 2021).

Gut Microbiota and CRC

The composition of microbiota differs between individuals, and their imbalance causes disease. The diversity of the inhabited microorganisms varies according to many factors such as the food that is being consumed, the environment, and the stress (Pothuraju et al., 2021). Yet not the diversity but the imbalance (dysbiosis) of microbiota negatively influences the host's overall health and causes serious problems (Hendler & Zhang, 2018). Gut microbiota plays a fundamental role in protecting the digestive tract from the harsh environment and pathogenic intruders as they maintain the mucus layers and compete with pathogens for nutrients and adherence to the intestine (Pothuraju et al., 2021). Microbial components, such as lipopolysaccharides and peptidoglycans, stimulate the secretion of mucus and the formation of a mucus layer; studies show that the mucus layer of germ-free mice noticeably differs from the mucus layer of normally raised mice due to the absence of microbiota (Pothuraju et al., 2021). Colon microbiota converts dietary fiber into short-chain fatty acids that can be used by the host as an energy source. Therefore, a healthy diet that contains an adequate amount of fiber is required to maintain colon health; Fiber deficiency force microbiota to consume mucin glycans as an energy source and severely reduces the colonic mucus layer (Pothuraju et al., 2021). In fact, Differences in mucosa-associated microbiota are observed before the appearance of cancer which indicates the involvement of gut microbiota in cancer development from a very early stage; Stage III and stage IV show higher levels of microbiota than early CRC stages (Torres-Maravilla et al., 2021). Individuals with low bacterial richness are more susceptible to metabolic disorders and diseases compared to those with high bacterial richness; The gut microbiota compositions were studied among humans with a high and low risk of colon cancer, and an increased risk of colon cancer was associated with an unbalanced level of microbiota (Rad et al., 2021). Fecal samples from colorectal cancer patients show a significant dysbiosis in probiotics composition compared to samples from normal individuals (Pothuraju et al., 2021). However, Three types of dysbiosis could be extrinsically induced:1) loss of good microbes, 2) expansion of pathogenic, and 3) loss of gut microbial diversity. All previous dysbiosis types have been linked to CRC (Torres-Maravilla et al., 2021).

The human gut is occupied by a tremendous number of different types of microorganisms, the most abundant are bacteria. 10% to 30% only of CRC disease is due to genetic factors while the rest of the incidents are influenced by extrinsic stimuli that alter gut microbiota and induce dysbiosis (Hendler & Zhang, 2018). CRC is associated with the proliferation of certain types of bacteria such as *Fusobacterium*, *Peptostreptococcus*, *Streptococcus*, and *Ruminococcus*; and a decrease in other types such as *Lactobacillus* and *Granulicatella* (Kvakova et al., 2022). The abnormality and the abundance of the wrong types of flora affect the gastrointestinal tract significantly as it prompts the proliferation of pathogenic bacteria such as *Enterococcus*, *Helicobacter (H.) pylori*, *Streptococcus (S.)*, and reduce vital probiotics, such as *Bifidobacterium* and *Lactobacillus* (Kim et al., 2021). Pathogenic bacteria disturb host intestinal bacteria and decrease immune system efficiency by producing toxins and regulating many cellular proliferation pathways that induce tumor formation (Kim et al., 2021). The excessive presence of *Fusobacterium nucleatum* bacteria in the colon plays a vital

role in colorectal carcinogenesis as they can adhere to E-cadherin in the colon epithelial cells and activate the β-catenin signaling pathway and stimulate oncogenic and inflammatory responses (Cheng et al., 2020). The severe alterations in the composition of CRC Patients' gut microbiota cause promotions and activations of the Wnt signaling pathway which affect the development and progression of tumors by impairing immunosurveillance and increasing the proliferation and inflammation phenotypes. Alterations in specific bacterial taxa such as the increased abundance of *Enterobacteriaceae*, and *Streptococcus*, highly affect mucosal immune responses in CRC Patients as well (Perillo et al., 2020). When tumors are chemically induced in microbiota-free- animals, the progression of tumors increases significantly; This growth is revers by the introduction of the right probiotics (Alhinai et al., 2019).

The Anti-Tumor Effects of Gut Microbiota and their Metabolites

Probiotics exhibit many anticancer activities and fight cancer through many mechanisms. Many studies have indicated the safety and the effectiveness of microbiota in increasing chemotherapy efficacy, reducing the side outcomes of conventional treatments, and hindering the progression of tumor (Torres-Maravilla et al., 2021). The Probiotic *lactobacilli* inhibit the epidermal growth factor receptor (EGFR) pathway in the CRC model; this pathway results in cell proliferation, cell survival, and metastasis of tumors. Probiotics also modulate the Notch and Wnt/β-catenin pathways, which induce apoptosis and downregulate cell proliferation in HT-29 cells in vitro. Therefore, probiotics can prevent colon cancer (Torres-Maravilla et al., 2021). Probiotics such as *Lactobacillus* and *Bifidobacterium* produce lactic acid, which promotes epithelium renewal and prevents DNA damage. They also control the immune system by reducing the abundance of Th17, increasing the expression of the major histocompatibility complex-II on dendritic cells, and improving the recruitment and the cytotoxicity of natural killer cell cytotoxic T cell (Perillo et al., 2020). Probiotics can enhance the functions of dendritic cells (DCs) and natural killer (NK) cells, the innate immune cells that play a vital role in the early defense against cancer. Probiotics induce the production of critical anti-tumorigenic cytokines such as IL-18. IL-18 promotes host immunity by activating cytotoxic T cells and NK cells. This cytokine also plays many roles in repairing impaired mucosal membranes, stimulating the proliferation and differentiation of intestinal epithelial cells, and inducing the production of mucus from goblet cells (Torres-Maravilla et al., 2021). It also induces the production of the anti-bacterial peptides that hinder the development of CRC. The most important is the ability of Probiotic to combat pathogenic microorganisms that triggers CRC development; it does that by changing the pH of the surrounding, generating bacteriocins, and dropping the pro-carcinogenic enzymes

level (Torres-Maravilla et al., 2021). Moreover, the metabolic activity of microorganisms produces various bioactive compounds known as postbiotics which are extremely utilized by both the host and other microorganisms.

Postbiotic

The postbiotic term is used to refer to the nonviable probiotic and its metabolites such as short-chain fatty acids (SCFAs), functional proteins, polysaccharides, cell lysates, and microbial cell components (Rad et al., 2021; Wegh et al., 2019). Postbiotic bioactive products assist host-microbial symbiosis and crosstalk by inducing probiotic growth, facilitating cell-cell interaction, and regulating signaling pathways. They promote health through different mechanisms as they either directly influence the signaling pathways of the body or indirectly manipulate the metabolism and the composition of the intestinal microflora (Perillo et al., 2020). Postbiotics can be obtained through chemical and mechanical lysis of bacterial cell cultures to produce various postbiotics and metabolites

such as enzymes, polysaccharides, SCFA, vitamins, phenols, and cell wall fragments (Żółkiewicz et al., 2020).

Types of Postbiotics

Cell-Free Supernatant (CFS) is a type of postbiotic that can be acquired directly from bacterial or yeast cell cultures. After centrifugation of the microbe's cell culture, the supernatant is removed and filtered. CFS can be acetate, lactate, butyrate, propionate, and more. CFSs produced from different cultures contain active metabolites that possess many activities such as antibacterial, antioxidant, anti-infectious, anti-inflammatory, antitumor, and anti-inflammatory activities (Rafique et al., 2023).

The second type of postbiotic is exopolysaccharides (EPSs). It is biopolymers that are produced by microorganisms during their growth. EPSs are used as stabilizing, and water-binding agents in the food industry. Studies showed that EPSs interact and stimulate the proliferation of immune cells, and enhance the phagocytic ability of macrophages. EPSs also increase the concentration of IgA in the intestinal mucosa (Żółkiewicz et al., 2020).

Microorganisms also, produce essential enzymes as a type of postbiotic. These enzymes are used as a defense mechanism against reactive oxygen species (ROS) that damage proteins, lipids, carbohydrates, and nucleic acids. Glutathione peroxidase (GPx), for instance, plays a vital role in reducing ROS (Żółkiewicz et al., 2020).

Fragments of the bacterial cell wall also consider a type of postbiotic. They are immunogenic components that elicit an immune response when encountering immune cells. The bacterial lipoteichoic acid (LTA) is one of the Gram-positive bacterial components that is released into the environment and induces the production of cytokines. LTA from Lactobacillus and Bifidobacteria stimulates the response of the skin's mast cells to fight bacterial and viral infections (Żółkiewicz et al., 2020).

Another type of postbiotic is short-chain fatty acids (SCFAs) which are produced during the fermentation prosses of the plant polysaccharides by the intestinal microbiota. There are many recognized SCFAs such as propionic, acetate, and butyric acids. They mainly help in forming a barrier between the gut epithelium and the outer surrounding. Butyrate is an essential fatty acid that aids the renewal of the intestinal epithelium as well as regulating the expression of mucin (Pothuraju et al., 2021). It also suppresses the body's immune system by inducing food tolerance inducing the expression of immunosuppressive cytokines (Rafique et al., 2023). For human colonocytes, Butyrate is their main energy source. It also stimulates apoptosis in colon cancer cells, maintains the balance of oxygen in the gut, and prevents gut microbiota dysbiosis (Valdes et al., 2018). In the muscles and the liver, acetate, and propionate are transferred and used as substrates for the mitochondrial oxidation (Vrzáčková et al., 2021). Acetate is an abundant metabolite that is consumed in cholesterol metabolism, appetite regulation and is required for the growth of other bacteria (Valdes et al., 2018).

Bacterial lysates, a type of postbiotics, are produced by the degradation of Gram-positive and Gram-negative bacteria. Studies show that the oral administration of bacterial lysates stimulates DCs in the Peyer patches of the small intestine. The activated DCs consequently stimulate the innate immune system and promotes the secretion of IgA (Żółkiewicz et al., 2020).

Postbiotic and Cancer Prevention

The biological role of gut-probiotic and their derived postbiotics have been proven in safeguarding gut health and function as they improve the immune system response and possess antioxidative and antiproliferative properties. Because of that, many studies investigated the potential role of postbiotics in treating cancer (Rad et al., 2021). The efficiency of postbiotics from many pro-

biotic strains in preventing in many cancer cell growth in vitro has been investigated (Kvakova et al., 2022). Supernatant from *Lactobacillus acidophilus* cell cultures shows anti-inflammatory and antioxidant effects on many cells, such as macrophages, epithelial cells of the intestine, and neutrophils. It exerts its effects by lowering the release of pro-inflammatory cytokines and increasing the release of anti-inflammatory cytokines (Kvakova et al., 2022). Studies show that *L. casei* and *Lactobacillus rhamnosus GG's* supernatants prevent the invasion of colon cancer cells in vitro, reduce oxidative stress in vivo, and possess direct antitumor activities. *Lactobacillus Plantarum* supernatants also help in the maturation and morphological structure of the intestinal barrier (Żółkiewicz et al., 2020). The supernatant of *Bifidobacterium bifidum* suppresses the proliferation of human colon cancer cell line SW742 in vitro (Bahmani et al., 2019). CFS from *Bifidobacterium adolescentis SPM0212* inhibits three colon cancer cell lines: Caco-2, HT-29, and SW-480. CFS from *Lactobacillus plantarum* inhibits tumor progression in high-fat diet-fed C57BL/6-APCMin/+mice by boosting the immune system, downregulating the expression of NF-κB and Wnt, and keeping the gut microbiota composition balanced (Vrzáčková et al., 2021). Tumor-induced mice showed a significant reduction in tumor size when treated with postbiotics as it suppressed pro-inflammatory expression of cytokine, including IL-6, TNF- , and IL-17, COX-2, and NF-kB (Perillo et al., 2020). Supernatants from yeast cultures show similar activities to the bacterial cell supernatants as they have anti-inflammatory, antioxidant, wound healing, and regeneration of the intestinal barrier activities (Żółkiewicz et al., 2020). Folate, a metabolite produced by the gut microbiota, is absorbed by colon cells and helps in the synthesis, reparation, and methylation of DNA. Folate also shows antioxidant and antitumor activity (Żółkiewicz et al., 2020).

The Pleiotropic Effects of Postbiotics

A high concentration of SCFAs in the colon helps in increasing the synthesis of the intestinal mucus, reducing the levels of pro-inflammatory cytokines, inhibiting the adherence of pathogenic bacteria, preventing DNA damage, and stimulating the growth of protective bacteria (Hendler & Zhang, 2018). Postbiotics also play a role in regulating the gut microbiota because it has similar efficacy to probiotics but with fewer side effects. Paraprobiotics, non-viable microbial cells, and postbiotics are all terms used to describe bioactive compounds produced by the gut microbiota (Wegh et al., 2019). In a study done by Faghfoori and his collogues (2021) the apoptotic effects of some *bifidobacteria* species and their supernatant on colon cancer cell lines were assessed. all studied *bifidobacteria* supernatant significantly decreased the cell viability of the colon cancer cells compared to the control, increased BAX gene expression, and downregulated BCL-2 expression (Faghfoori et al., 2021).

Postbiotics have immunomodulatory, anti-cancer, anti-infection, and anti-oxidant activities. These pleiotropic effects of postbiotics make them a suitable therapeutic and preventive strategy in modern medicine. The immunomodulatory effects of Postbiotics are well-known as some types activate immune cells, induce cytokines production, and inhibit inflammation (Kvakova et al., 2022). In the intestine, small-chain fatty acids, such as butyrate and propionate, stimulate the differentiation of regulatory T cells and improve the formation of peripheral Tregs, respectively. Propionate also has antitumor activity as it provokes apoptosis in gastric cancer cells. SCFAs have the ability to make epigenetic modifications that alter the expression of oncogenes and suppressors genes (Żółkiewicz et al., 2020). In a study, the supernatant of *L. rhamnosus GG* increases the expression level of ZO-1 which improves the structure of tight junctions, and the expression level of MMP-9 which plays a role in cancer invasion. Thus, exposure to postbiotics is found to reduce tumor cell invasion in many in vitro models. Some postbiotics can prevent infection as they seal the intestinal barrier to prevent

pathogens' adhesion, alter the expression of host genes, and modify the local environment. Postbiotics also play a role in lipid metabolism and decrease the risk of cardiovascular incidents. Propionate, as an example, can decrease cholesterol precursors and lower cholesterol levels (Żółkiewicz et al., 2020).

Mechanisms of postbiotics as anti-cancer agents

Postbiotics are microbial metabolites that when provided as a functional food or food supplement, promote health and play roles in the prevention and treatment of cancer as they induce apoptosis. Studies showed that many types of postbiotics can distinguish normal from cancerous cells. They control cancerous cells by preventing angiogenesis and increasing apoptosis. On the other hand, they modulate normal cells by regulating their proliferation (Kvakova et al., 2022). Postbiotics showed antiproliferative activities against many cancer cells line such as colon cancer cells (HCT116 and HT-29), leukemia cells (THP1), and cervical cancer cell lines. Postbiotic correspondingly regulate the expression of many genes. They increased the expression of early apoptotic-promoting genes and decreased the proinflammatory cytokine (Rad et al., 2021). Postbiotics induce intrinsic and extrinsic apoptosis in human cancer by increasing the expression of proapoptotic proteins such as Bad, Bax, caspase3, caspase8, and caspase9, and decreasing Bcl-2 (Kim et al., 2021). Postbiotics from *L.rhamnosus GG* as well as *L. paracasei IMPC2.1* inhibit the proliferation of human colon cancer DLD-1 and human gastric cancer HGC-27 cell lines. In vitro, flow cytometry analysis showed an induced level of apoptosis in the human cancer cell lines H129, MCF-7, HEK293T, HeLa, HT1080, and H129 after postbiotic treatment extracted from *Brevibacillus spp* (Kim et al., 2021). Studies on the exopolysaccharides (EPSs) that are produced by many probiotic bacteria indicate their promising therapeutic outcome on cancer. EPSs induce cytotoxicity in tumor cells, stimulate the immune response, and inactivation of inflammatory pathways. The effects of Lactobacillus EPSs were tested on the colon cancer cells (HT-29) and were found to stimulate apoptosis by increasing the expression of Caspase 3, Caspase 9, and BAX and decreasing the levels of Bcl-2 (Torres-Maravilla et al., 2021). *Lactobacilli* postbiotics control the expression of inflammatory mediators and reduce tissue inflammation (Cicenia et al., 2014). The proliferation of the CRC cell lines HCT-116 and SW1116 was significantly inhibited when incubated with the *Bacillus subtilis* and *Clostridium butyricum* strains, or by their byproducts such as butyrate and bacitracin; this drop in cells growth was also associated with a decreased mRNA level of vital inflammatory genes such as TLR4, nuclear factor-kappa B (NF-κB), and interleukin 22 (IL-22) (Kvakova et al., 2022). The Cell-free supernatant of *Lactobacillus acidophilus* decreased the antiproliferative activities of the HT-29 cell line by inducing the intrinsic pathway-dependent-proptosis which was confirmed by the increase of Bax/Bcl-2 gene expression ratio (Baghbani-Arani et al., 2020). The cell-free supernatants from lactobacillus acidophilus also inhibit the proliferation and invasion of the human colon carcinoma cell line (caco-2 cells) by decreasing the expression level of MMP12, a protein that involves in cancer metastasis, and increasing the expression of MMP-9, a protein that digests all extracellular matrix proteins, (Shokati et al., 2021). As studies indicated, the cell membrane components of Lactobacilli, such as peptidoglycan, cell-wall polysaccharides, and membrane-associated proteins, are vital effector molecules that act directly on the host cells (Teame et al., 2020).

Conclusion

Cancer is one of the leading causes of death worldwide. Cells in affected people fail to maintain normal cell proliferation and homeostasis due to mutations. Tumors are formed by the accumulation of these mutations and the escape from the immune system. The gut microbiota is an essential

part of the digestive tracts of vertebrates as they form a symbiotic relationship with the host and facilitate essential body processes to maintain gastrointestinal health. However, the composition of these microorganisms differs between individuals, and their imbalance causes disease. The abundance of the wrong types of flora promotes the proliferation of pathogenic microorganisms that cause CRC. The biological and anti-colon cancer impacts of the gut-probiotic and their derived postbiotics have been discussed in this review.

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