

Probiotics and postbiotics in colorectal cancer: Prevention and complementary therapy

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Abstract

Colorectal cancer (CRC) is a leading cause of human mortality worldwide. As conventional anticancer therapy not always being effective, there is growing interest in innovative “drug-free” cancer treatments or interventions that improve the efficacy of established therapy. CRC is associated with microbiome alterations, a process known as dysbiosis that involves depletion and/or enrichment of particular gut bacterial species and their metabolic functions. Supplementing patient treatment with traditional probiotics (with or without prebiotics), next-generation probiotics (NGP), or postbiotics represents a potentially effective and accessible complementary anticancer strategy by restoring gut microbiota composition and/or by signaling to the host. In this capacity, restoration of the gut microbiota in cancer patients can stabilize and enhance intestinal barrier function, as well as promote anticarcinogenic, anti-inflammatory, antimutagenic or other biologically important biochemical pathways that show high specificity towards tumor cells. Potential benefits of traditional probiotics, NGP, and postbiotics include modulating gut microbiota composition and function, as well as the host inflammatory response. Their application in CRC prevention is highlighted in this review, where we consider supportive *in vitro*, animal, and clinical studies. Based on emerging research, NGP and postbiotics hold promise in establishing innovative treatments for CRC by conferring physiological functions *via* the production of dominant natural products and metabolites that provide new host-microbiota signals to combat CRC. Although favorable results have been reported, further investigations focusing on strain and dose specificity are required to ensure the efficacy and safety of traditional probiotics, NGP, and postbiotics in CRC prevention and treatment.

Key Words: Colorectal cancer; Traditional probiotics; Next-generation probiotics; Postbiotics; Gut microbiota

Core Tip: The effects of traditional probiotics, next-generation probiotics (NGP), or postbiotics in colorectal cancer (CRC) prevention and complementary therapy can be associated independently or in mutual cooperation with several mechanisms, including suppression of inflammation, enhancing apoptosis of tumor cells, restoring intestinal barrier function, competition with pathogens and by promoting healthy gut microbiota composition and function. Traditional probiotics, NGP, or postbiotics supplementation is also a potential strategy to boost the effectiveness of chemotherapy and immunotherapy, reduce the rate of postoperative complications, and improve the quality of lives of CRC patients.

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INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in males and the second in females worldwide; thus a significant change in lifestyle is required to facilitate effective CRC prevention[1]. CRC is a heterogeneous disease of the intestinal epithelium, comprising the colon, rectum, and anus. It is characterized by a dysregulated immune response, accumulation of stem cell mutations, intestinal barrier disruption, and dysbiosis, which is often regarded as an unfavorable alteration in gut microbiota composition and function. Up to 90% of CRC risk is thought to be lifestyle-dependent, primarily due to dietary or environmental factors including feeding patterns that modulate consumption of fiber, red and processed meat or alcohol consumption, and low omega-3 fatty acids and vitamin D intake. Obesity, lack of physical activity, and smoking are also significant risk factors that promote CRC-associated microbiota changes[2]. CRC is linked with microbiome alterations, which include depletion and/or enrichment in particular bacterial species that are present in CRC patients (extensively reviewed by Torres-Maravilla *et al*[3], Ternes *et al*[4], Janney *et al*[2], Fong *et al*[5], and Wirbel *et al*[6]).

The human microbiota is a complex ecosystem of bacteria, viruses, eukaryotes, and archaea, which can regulate a variety of host physiological functions including digestion, immune response, metabolism, disease pathogenesis, elimination of toxins, and biosynthesis of key compounds such as essential vitamins and cofactors. Microbiota can even modulate gut-brain axis function to alter, for example, anxiety and mood. Symbiotic bacteria that colonize the human gut can be classified into several phyla comprising Bacteroidetes and Firmicutes, followed by Proteobacteria, Fusobacteria, Actinobacteria, Verrucomicrobia, and Spirochaetes. Microbiome composition varies between healthy individuals, as well as in CRC patients[7]. CRC-associated bacteria that have been identified to date include enrichment of *Fusobacterium nucleatum*, *Enterococcus faecalis*, *Streptococcus gallolyticus*, entero-toxicogenic *Bacteroides fragilis*, *Escherichia coli*, *Peptostreptococcus* spp., and *Ruminococcus* spp. By contrast, *Lactobacillus* spp., *Bifidobacterium* spp., *Faecalibacterium* spp., *Roseburia* spp., *Clostridium* spp., *Granulicatella* spp., *Streptococcus thermophilus*, and other species of *Lachnospiraceae* family are depleted in CRC (Table 1)[2-5]. These altered microbiota signatures can potentially be used to provide future diagnostics, and their presence/absence may contribute to the pathogenesis or prevention/treatment of CRC. However, the pathophysiological role of dysbiosis in CRC still remains unclear, since microbiota changes may reflect changes in host health status and some bacteria may even confer protection as a compensatory response to disease progression. This complexity is clearly evident by the report of specific bacteria associated with tumor initiation phase (driver bacteria) whereas other bacteria are associated with tumor development during progressive stages of CRC (passenger bacteria). Driver bacteria reportedly contribute to the formation of a tumor microenvironment that is comprised of normal epithelial cells and cancer cells. In this milieu, secreted microbial metabolites trigger damage to normal host cells, thus reprogramming their metabolism to change the intestinal microenvironment and microbiome profile towards a more "CRC supportive" composition[8-10]. Potential driver bacteria include *Bacteroides fragilis*, *Escherichia coli*, *Enterococcus Faecalis*, *Bacillus*, *Bradyrhizobium*, *Methylobacterium*, *Streptomyces*, *Shigella*, *Citrobacter*, *Salmonella*, *Intrasporangiaceae*, and *Sinobacteraceae*. On the other hand, passenger bacteria occupy an existing tumor microenvironment where they are thought to either promote or inhibit CRC progression. Reported passenger bacteria include species *Fusobacterium*, *Parvimonas*, *Peptostreptococcus*, *Campylobacter*, *Streptococcus*, *Schwartzia*, *Burkholderiales*, *Caulobacteraceae*, *Delftia*, *Oxalobacteraceae*, *Faecalibacterium*, and *Sutterella*[8-11]. The host gut microbiota and immune system play important roles in CRC prevention and development. Therefore, probiotics, next-generation probiotics

Table 1 Overview of the most relevant bacteria related to colorectal cancer

Enriched bacteria	Depleted bacteria	Ref.
<i>Fusobacterium nucleatum</i> , <i>Peptostreptococcus</i> spp., <i>Porphyromonas asaccharolytica</i> , <i>Prevotella</i> spp., <i>Parvoimonas micra</i> , <i>Bacteroides fragilis</i> , <i>Streptococcus gallolyticus</i> , <i>Escherichia coli</i> , <i>Campylobacter</i> spp., <i>Shigella</i> spp., <i>Enterococcus faecalis</i>	<i>Blautia</i> spp., <i>Faecalibacterium prausnitzii</i> , <i>Clostridium butyricum</i> , <i>Streptococcus thermophilus</i> , <i>Roseburia</i> spp.	[4,55-57]

(NGP), or postbiotics could be used as weapons to prevent CRC, to support the treatment and to improve the clinical outcomes in CRC patients.

This minireview summarizes recent CRC findings from clinical, animal and *in vitro* studies, and discusses the efficiency of probiotics, NGP, and postbiotics in CRC prevention and therapy.

TRADITIONAL PROBIOTICS

Probiotics are defined as “live, non-pathogenic microorganisms that, when administered in adequate amounts, may confer a health benefit on the host” [12]. Probiotics have a centuries-long history of safe use as prevention and adjuvant therapy in combating human diseases. They are also promising candidates in modulating human gut microbiota composition and function in CRC patients. Traditional widely used probiotics mainly belong to *Bifidobacterium* spp., *Lactobacillus* spp. and other lactic-acid-producing bacteria, including species belonging to *Streptococcus*, *Enterococcus*, and *Lactococcus*, complemented by yeasts of the genus *Saccharomyces*. The beneficial effects of probiotics, functioning in a species and/or strain-specific manner, include sustaining a healthy microbiome, reversing dysbiosis, preventing pathogenic infections and mucosal adhesion of pathogens, stabilizing and enhancing intestinal barrier function. Probiotic bacteria may achieve these beneficial functions in part by producing anti-carcinogenic, anti-inflammatory, anti-mutagenic and other biologically important compounds such as short-chain fatty acids (SCFAs), vitamin K, or B-group vitamins [5,7,13,14].

Current research builds on a foundation of work demonstrating that gut microbiota modulation through administration of probiotics and/or prebiotics plays an important role in CRC prevention and therapy. In a randomized, double-blinded, placebo-controlled trial, 60 patients underwent surgical CRC resection, of whom 29 received the probiotic powder (*Bifidobacterium animalis* subsp. *lactis* HY8002 [1×10^8 CFU], *Lactobacillus casei* HY2782 [5×10^7 CFU], and *Lactobacillus plantarum* HY7712 [5×10^7 CFU]) and 31 placebo, for 4 wk, starting at 1 wk preoperatively. The treatment group receiving probiotic powder showed an increase in abundance of *Bifidobacterium*, *Akkermansia*, *Parabacteroides*, *Veillonella*, *Lactobacillus*, *Erysipelatoclostridium* and a reduction in bacteria associated with CRC, such as *Prevotella*, *Alloprevotella*, *Fusobacterium*, and *Porphyromonas*. Lower serum zonulin, improved postoperative bowel function, and postoperative recovery were evident in the probiotic group compared with placebo [15]. In another randomized clinical trial, a group of 31 CRC patients received probiotic supplement *Bifidobacterium longum* BB536 (5×10^{10} CFU/2 g/daily) preoperatively for 7–14 d and postoperatively for 2 wk. Attenuated postoperative inflammatory responses (high-sensitivity C-Reactive protein), reduced risk of postoperative infectious complications, and accelerated health recovery after colorectal resection were evident in the treatment group. Hospital stay was significantly shortened and correlated significantly with increased Actinobacteria and decreased Firmicutes after probiotic intervention [16]. Aisu *et al* [17] administered BIO THREE® 2 mg *Enterococcus faecalis* T110, 10 mg *Clostridium butyricum* TO-A, and 10 mg *Bacillus mesentericus* TO-A to 75 CRC patients 15 d prior to the surgery. Incidence of postoperative complications and superficial incisional infections were lower, and these health effects were as shown to associate with an increased mean proportion of beneficial *Bifidobacterium*, postoperatively, even though this organism was not administered as part of the probiotic regime. The change in microbial diversity and improved integrity of the mucosal barrier were also observed by Liu *et al* [18] after *Lactobacillus plantarum* CGMCC 1258, *Lactobacillus acidophilus* LA-11, *Bifidobacterium longum* BL-88 (2.6×10^{14} CFU/2 g/daily) administration 6 d preoperatively and 10 d postoperatively to CRC patients. The numbers of beneficial bacteria, including *Bifidobacteria* and *Lactobacilli*, increased in the probiotic group after surgery, whereas they decreased in the placebo group. By contrast, Enterobacteriales and *Pseudomonas*, were decreased in the probiotic group whereas they increased in the placebo group. Based on a number of clinical trials, the preoperative oral intake of probiotics combined with the postoperative treatment in patients who need gastrointestinal surgery is potentially recommended. Larger rigorously controlled clinical trials are required to endorse these preliminary positive outcome studies since avoidance of probiotic use has also been recommended in patients with immunodeficiency and dysbiosis. More studies and the key outcomes are listed in Table 2.

Table 2 Efficiency of probiotics in colorectal cancer prevention and therapy-clinical trials

Probiotic strain/synbiotics	Dose/length of the study	Trial type/sample size	Microbial changes/key outcomes	Ref.
<i>Lactobacillus rhamnosus</i> GG, <i>Bifidobacterium lactis</i> Bb12 + inulin enriched with oligofructose	10 ¹⁰ CFU and 10 g of prebiotic, 12 wk	Human prevention study-CRC patients (<i>n</i> = 15 placebo, <i>n</i> = 19 synbiotics), polypectomized patients (<i>n</i> = 19 placebo, <i>n</i> = 21 synbiotics)	↑ <i>Bifidobacterium</i> , ↑ <i>Lactobacillus</i> , ↓ <i>Clostridium perfringens</i> ; ↓proliferation rate of colorectal cells and stimulation of peripheral blood mononuclear cells (↑IFNγ, ↓IL-2)	Rafter <i>et al</i> [58]
<i>Bifidobacterium longum</i> BB536, <i>Lactobacillus johnsonii</i> La1	2 × 10 ⁷ CFU or 2 × 10 ⁹ CFU, 3 d preoperatively and 3 d postoperatively	CRC patients undergoing elective colorectal resection- Randomized double blind, placebo-controlled study (<i>n</i> = 10 placebo, <i>n</i> = 21 probiotics)	<i>B. longum</i> BB536 did not adhere to colonic mucosa only La1, ↓ <i>Enterobacteriaceae</i> ↓ <i>Enterococcus</i> , modulation of local immunity (↑CD3+, CD4+, CD8+, activity of dendritic cells); no clinical effect probiotics)	Gianotti <i>et al</i> [59]
<i>Bifidobacterium longum</i> , <i>Lactobacillus acidophilus</i> , <i>Enterococcus faecalis</i>	3 × 10 ⁸ CFU, 3 d (from -5 to -3 d) preoperatively	Single-center prospective randomized control study (<i>n</i> = 30 placebo, <i>n</i> = 30 probiotics)	↑ <i>Bifidobacterium</i> and ↓ <i>Escherichia</i> ; ↓endotoxins, D-lactic acid, serum IL-6 and C-reactive protein; ↑serum IgG and IgA; ↓postoperative occurrence of infectious complications of CRC	Zhang <i>et al</i> [60]
<i>Bifidobacterium longum</i> , <i>Lactobacillus acidophilus</i> and <i>Enterococcus faecalis</i>	1:1:1 daily 6 × 10 ⁷ CFU, 5 d	Perioperative intake of probiotics in CRC patients (<i>n</i> = 11 placebo, <i>n</i> = 11 probiotics)	↑richness and diversity of mucosal microbes, ↓ <i>Peptostreptococcus</i> , ↓ <i>Comamonas</i> , ↓ <i>Fusobacterium</i> , ↑ <i>Enterococcus</i> , ↑ <i>Proteobacteria</i> ; no clinical effect	Gao <i>et al</i> [61]
LactoLevure (<i>Lactobacillus acidophilus</i> LA-5, <i>Lactobacillus plantarum</i> , <i>Bifidobacterium lactis</i> BB-12, <i>Saccharomyces boulardii</i>)	1.75 × 10 ⁹ CFU, 0.5 × 10 ⁹ CFU, 1.75 × 10 ⁹ CFU, 1.5 × 10 ⁹ CFU, respectively, 1 d preoperatively and 15 d postoperatively	CRC patients undergoing surgery- Randomized, double-blind, placebo-controlled study (<i>n</i> = 80 placebo, <i>n</i> = 84 probiotics)	Reduction of the postoperative pneumonia rate, anastomotic leakage and surgical site infections; ↑gene expression of SOCS3; ↑circulating IL-6, TNF-α	Kotzampassi <i>et al</i> [62] (NCT02313519)
Colon Dophilus™ [<i>Bifidobacterium breve</i> HA-129 (25%), <i>Bifidobacterium bifidum</i> HA-132 HA (20%), <i>Bifidobacterium longum</i> HA-135 (14.5%), <i>Lactobacillus rhamnosus</i> HA-111 (8%), <i>Lactobacillus acidophilus</i> HA-122 (8%), <i>Lactobacillus casei</i> HA-108 (8%), <i>Lactobacillus plantarum</i> HA-119 (8%), <i>Streptococcus thermophilus</i> HA-110 (6%), <i>Lactobacillus brevis</i> HA-112 (2%), <i>Bifidobacterium infantis</i> HA-116 (0.5%)]	10 × 10 ⁹ CFU/ daily, 12 wk	Patients with CRC, concomitantly with irinotecan chemotherapy- Randomized, placebo-controlled study (<i>n</i> = 23 placebo, <i>n</i> = 23 probiotics)	Reduction in the incidence and severity of chemotherapy induced diarrhea and incidence of enterocolitis.	Mego <i>et al</i> [63] (NCT01410955)
<i>Saccharomyces boulardii</i>	7 d preoperatively	Randomized study (<i>n</i> = 18 conventional treatment, <i>n</i> = 15 probiotics)	↓mucosal IL-1β, IL-10, and IL-23A mRNA levels; no statistical impact on postoperative infection rates	Consoli <i>et al</i> [64]
ProBion Clinica (<i>Bifidobacterium lactis</i> BI-04 and <i>Lactobacillus acidophilus</i> NCFM + inulin)	1.4 × 10 ¹⁰ CFU, 7 × 10 ⁹ CFU and 0.63 g of prebiotic, 8-78 d	Prospective randomized intervention (<i>n</i> = 7 placebo, <i>n</i> = 8 probiotics)	Increased abundance of butyrate producing bacteria ↑Firmicutes, ↑ <i>Faecalibacterium</i> , ↑ <i>Eubacterium</i> , ↑ <i>Roseburia</i> ↑ <i>Lachnospira</i> ; ↓CRC associated bacteria- <i>Fusobacterium</i> and <i>Peptostreptococcus</i>	Hibberd <i>et al</i> [65] (NCT03072641)
Simbio-flora (<i>Lactobacillus acidophilus</i> NCFM, <i>Lactobacillus rhamnosus</i> HN001, <i>Lactobacillus casei</i> LPC-37, <i>Bifidobacterium lactis</i> HN019 and fructooligosaccharide)	10 ⁹ CFU and 6 g of prebiotic, 7 d preoperatively	Patients with CRC subjected to colorectal resection- Prospective, randomized, double-blind, placebo-controlled study (<i>n</i> = 37 placebo, <i>n</i> = 36 synbiotic)	Reduced inflammatory state (C-reactive protein, IL-6), reductions in morbidity, hospital length of stay, and use of antibiotics. Stimulated bowel function, decreased complications and reduced cumulative duration of antibiotic usage	Polakowski <i>et al</i> [66]
<i>Lactobacillus acidophilus</i> BCMC® 12,130, <i>Lactobacillus lactis</i> BCMC® 12,451, <i>Lactobacillus casei</i> subsp BCMC® 12,313, <i>Bifidobacterium longum</i> BCMC® 02120, <i>Bifidobacterium bifidum</i> BCMC® 02290 and <i>Bifidobacterium infantis</i> BCMC® 02129	30 billion CFU, twice daily for 6 mo	Randomized double-blind placebo-controlled trial (<i>n</i> = 25 placebo, <i>n</i> = 27 probiotics)	Reduction in the levels of pro-inflammatory cytokines, TNF-α, IL-6, IL-10, IL-12, IL-17A, IL-17C and IL-22.	Zaharuddin <i>et al</i> [32] (NCT03782428)

CRC: Colorectal cancer; IFNγ: Interferon-gamma; Ig: Immunoglobulin; IL: Interleukin; TNF-α: Tumor necrosis factor-alpha.

NGP

One potential approach to achieve CRC prevention and treatment is through NGP administration. As described above, the most frequently used probiotics belong to *Bifidobacterium* spp. and *Lactobacillus* spp. However, recent studies using metagenomic approaches have revealed the importance of further identification and characterization of commensal species, mainly anaerobic ones, residing in the gastrointestinal tract that play an important role in regulating the immune system and maintaining overall gut health. Growing evidence suggests that dysbiosis may contribute to CRC progression as well as several other diseases[19-22]. Although there is no official definition of NGP, it is generally defined as live microorganisms identified on the basis of comparative microbiota analyses between healthy and sick individuals/animals that, when administered with strain-specificity and in dose dependent manner, confer health benefits on the host[23,24]. Compared with healthy individuals, patients with CRC possess a different compositional structure and physiological activity of the gut microbiota with SCFAs-producing bacteria being depleted. This suggests that SCFAs-producing bacteria might potentially exhibit anti-inflammatory and anticarcinogenic properties, as well as being NGP candidates in CRC prevention and therapy. SCFAs, primarily acetate, propionate, and butyrate, are key physiological metabolites of the microbial fermentation of dietary fiber in the colon. Butyrate is the major energy source for colonocyte homeostasis, promoting growth stimulation and production of protective cytokines that maintain gut barrier integrity and function[14,25-27]. Furthermore, increasing levels of SCFAs in the gut helps to create a favorable microenvironment for beneficial bacteria by inhibiting the growth and adhesion of pathogens, and by enhancing vitamin bioavailability, mineral absorption and promoting mucosal integrity. Most butyrate-producing bacteria in the human colon belong to the Firmicutes phylum, clostridial clusters IV and XIVa, the most dominant species being *Faecalibacterium prausnitzii* and *Eubacterium rectale*, followed by *Eubacterium* spp. as well as *Anaerostipes* spp. and *Roseburia* spp. In addition to butyrate-producing bacteria, other NGP candidates with important regulatory effects on gut homeostasis include *Akkermansia muciniphila*, non-toxicogenic *Bacteroides fragilis*, *Propionibacterium freudenreichii*, and some strains of *Bacillus* spp. and *Clostridium* spp., which belong to Generally Recognized As Safe microorganisms[7,28,29].

Chronic oral administration of *Butyricoccus pullicaecorum* BCRC 81109 (butyrate producing bacteria) to BALB/cByJNarl male mice decreased colon tumor progression over 9 wk. This protection against CRC clinical outcomes was linked to activation of the SCFAs transporter solute carrier family 5 member 8 and/or G-protein-coupled receptor (GPR) 43[30]. Chen *et al*[25] also observed in an *in vivo* animal study that application of butyrate producing bacteria *Clostridium butyricum* ATCC 19398 (2×10^9 CFU/0.2 mL 3 times a week for 12 wk) inhibited intestinal tumor development by an increasing apoptosis of CRC cells, by modulating the Wnt/ β -catenin signaling pathway. There was also a reduction in pathogenic bacteria and bile acid-biotransforming bacteria, whereas an increase in beneficial *Lactobacillus* spp. and SCFAs-producing *Ruminococcaceae* and *Eubacterium* spp. was evident. Thus, reduction in colonic secondary bile acids increased cecal SCFAs levels and activated G-protein coupled receptors, GPR43 and GPR109A, which were mechanistically implicated. Growth of CRC cell lines (HCT-116 and SW1116) was significantly inhibited by strains *Bacillus subtilis* ATCC 23857 and *Clostridium butyricum* ATCC 19398, and by their main metabolites bacitracin and butyrate. mRNA levels of important receptors and transcriptional factors related to inflammation for example, TLR4, MYD88, nuclear factor-kappa B (NF- κ B), interleukin 22 (IL-22), and survivin were decreased and expression of p21^{WAF1} was increased after treatment of SW1116 cells with *Bacillus subtilis* and *Clostridium butyricum* NGP[31]. Purified components produced by NGP cells were also studied and inhibition of human cancer cell proliferation by controlling the cell cycle was detected. Polysaccharide A purified from *Bacteroides fragilis* NCTC9343 (non-toxicogenic) induced the production of the pro-inflammatory cytokine IL-8[32] and aspartic protease Amuc_1434 (recombinant enzyme) from *Akkermansia muciniphila* upregulated the expression of tumor protein 53, increased mitochondrial reactive oxygen species (ROS) levels and promoted apoptosis of LS174T cells[33]. Pahle *et al*[34] employed *Clostridium perfringens* enterotoxin (CPE) in CPE gene therapy to selectively target claudin-3 and claudin-4 expressing colon carcinomas *in vitro* and *in vivo* by using a translation optimized CPE expressing vector. Elevated toxicity of the optimized CPE expressing vector was evident in claudin-positive cells 48 h after the transfection, with toxicity rates of 76%–92% and rapid cytotoxic effects such as membrane disruption and necrosis. Further *in vivo* studies focused on the efficiency of NGP application in CRC are listed in Table 3 and postbiotics derived from NGP are considered below.

POSTBIOTICS

Postbiotics is an extensively researched subject that remains a largely understudied topic in CRC. Due to the phenomenal number and variety of metabolites produced by bacteria, it has been an enormous challenge to isolate and characterize the specific compound/s responsible for the therapeutic efficacy. Moreover, defining safety profiles and appropriate application doses of particular postbiotics in the preclinical and clinical settings may require regulatory guidelines and approvals[5]. The International

Table 3 Efficiency of next-generation probiotics in colorectal cancer *in vivo*

NGP strain	Application	Study/ cohort	Mechanism/effect(s)	Ref.
<i>Akkermansia muciniphila</i> MucT ATCC BAA-835	pasteurized culture 1.5×10^8 CFU/100 μ L or recombinant Amuc_1100 3 μ g (specific outer membrane protein)/2 wk before AOM injection until sacrifice	23-wk <i>in vivo</i> animal study, acute colitis was induced by AOM (10 mg/kg) (intraperitoneally) + 2% DSS (in water)-male C57BL/6J mice	Prevention of AOM/DSS-induced tumorigenesis by DNA damage attenuation, cell apoptosis and abnormal proliferation. Significant amelioration of acute colitis, relieved colon shortening and splenomegaly, delayed tumor formation and reduced expression of γ H2AX, cleaved caspase 3 and Ki67. Blunted CAC through the expansion and activation of cytotoxic T lymphocytes, indicated by TNF- α induction and PD-1 downregulation	Wang <i>et al</i> [67]
<i>Akkermansia muciniphila</i> ATCC BAA-835	1×10^8 CFU/mouse every other day (day 7-12), gavage administration	4-wk <i>in vivo</i> animal study, CRC induced by mice colon cancer cells CT-26 (1×10^6) (subcutaneously)- <i>n</i> = 70, male BALB/c mice	<i>A. muciniphila</i> colonization significantly increased inhibition rate/ anti-cancer effect of FOLFOX (from 48% to 76%) and significantly decreased marker of proliferation-Ki67 (% of positively stained cells)	Hou <i>et al</i> [68]
<i>Clostridium butyricum</i> (powder by Kexing Biopharm CO., LTD)	2×10^8 CFU/0.2 mL/3 times <i>per</i> week, gavage administration	78 d <i>in vivo</i> animal study, CAC induced by intraperitoneal AOM (12.5 mg/kg) + 2.5% DSS (in water)- <i>n</i> = 30, C57BL/6 mice	Inhibition of NF- κ B pathway and apoptosis promotion. Change in the microbiome composition-reduction of Firmicutes to Bacteroidetes ratio. Reduction of incidence and size of CRC and increase of tumor cells apoptosis. Reduction in cytokines including TNF- α , IL-6 and level of COX-2. Decrease in phosphorylation of NF- κ B and level of Bcl-2. Increase in Bax expression	Liu <i>et al</i> [69]
<i>Clostridium butyricum</i> ATCC 19398 or <i>Bacillus subtilis</i> ATCC 23857	2.5×10^8 CFU/0.3 mL/3 times <i>per</i> week for 28 wk, oral administration	28 wk <i>in vivo</i> animal study. CRC induced with DMH (20 mg/kg body weight)/ weekly (intraperitoneally)- <i>n</i> = 72, male C57BL/6 mice	Inhibition of intestinal tumorigenesis and modulation of immunity and inflammation. Reduction in tumor size and incidence. After supplementation with probiotics, mice showed decreased Th2 and Th17 expression and increased CD4/CD8 expression compared to DMH-treated mice. Reduced gene expression of TLR4-MYD88-NF- κ B, IL-22 and increase of P21 ^{waf1} and Tlr3 mRNA levels in intestinal mucosa	Chen <i>et al</i> [31]

AOM: Azoxymethane; CAC: Colitis-associated cancer; CRC: Colorectal cancer; DMH: 1,2-dimethylhydrazine; DSS: Dextran sulfate sodium; FOLFOX: Oxaliplatin, fluorouracil and calcium folinate; IL: Interleukin; NF- κ B: Nuclear factor-kappa B; TNF- α : Tumor necrosis factor-alpha.

Scientific Association for Probiotics and Prebiotics (ISAPP) offers expertise in microbiology, microbial physiology, gastroenterology, nutrition, food science and pediatrics. ISAPP recently provided the clear definition and scope of postbiotics to include "preparation of inanimate microorganisms and/or their components that confer a health benefit on the host" [35]. Postbiotics, which exert desired physiological effects to the host, include inactivated microbial cells or cell components (cell surface proteins, endo- or exo-polysaccharides, peptidoglycan-derived muropeptides and teichoic acids) or important metabolites secreted by gut microbiota through a fermentation process or released under certain conditions such as a change in intestinal environment or after lysis (SCFAs including acetate, propionate and butyrate; enzymes; bacteriocins; reuterin; acetoin; organic acids, *etc*) [5,35,36]. Therefore, the isolation and characterization of new postbiotics is a growing field and requires careful biochemical characterization of beneficial mechanisms. Supplementation with postbiotics, can in some cases be an effective and safer strategy to prevent and/or treat diseases, compared with ingestion of viable probiotic bacteria [5].

Microbial metabolites undoubtedly play an important role in CRC pathogenesis. Certain postbiotics exert antitumor activity, including selective cytotoxicity against tumor cells suggesting their therapeutic potential (Figure 1) [5]. For example, SCFAs are well-known inhibitors of epigenetic enzymes histone deacetylases, which play a central role in gene regulation; thus, SCFAs have the ability to induce cell cycle arrest, and/or apoptosis in many cancer cell lines [37]. Cell-free supernatants (CFS) of different *Lactobacillus* and *Bifidobacterium* strains have been shown to induce apoptosis or inhibit proliferation of CRC cell lines [38-40]. Chen *et al* [41] demonstrated that supernatants of *Lactobacillus johnsonii* BCRC17010 and *Lactobacillus reuteri* BCRC14625 strains in high concentrations were able to damage HT-29 cell membranes causing elevated lactate dehydrogenase release. A recent study has reported a potent selective cytotoxicity effect of postbiotic metabolites from *Lactobacillus plantarum* strains *via* anti-proliferative effects and induction of apoptosis in HT-29 cells whilst sparing the normal cells [42]. Cousin *et al* [43] showed that metabolites from *Propionibacterium freudenreichii* ITG-P9, namely propionate and acetate, had induced intrinsic apoptosis of CRC cells, *via* the production and release of SCFAs acting on mitochondria. Moreover, CFS or SCFAs in combination with Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL), increased the pro-apoptotic gene expression (TRAIL-R2/DR5), decreased the anti-apoptotic gene expression of FLIP and XIAP in HT-29 cancer cells and enhanced the cytotoxicity in CRC cells compared to human healthy intestinal epithelial cells. Further control studies are required to delineate specific molecular targets in these models since enhanced toxicity to fermentation induced acidic pH shifts remains a potential protective mechanism.

As inflammation is undeniably linked to carcinogenesis, any postbiotic that inhibits inflammation is also an important candidate acting as anti-tumor agent. It was shown that *Lactobacillus rhamnosus* GG-

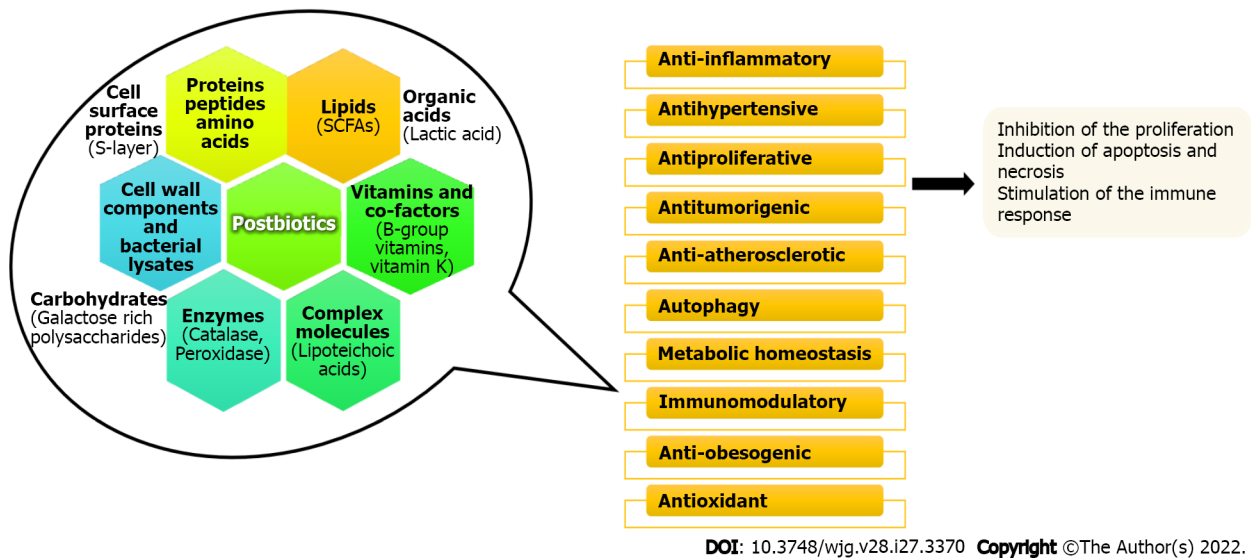


Figure 1 Examples of postbiotics and their proposed activity in patients with colorectal cancer. SCFAs: Short-chain fatty acids.

derived protein p40 can play a role in the prevention of CRC by suppressing intestinal epithelial inflammation, inhibiting epithelial cells apoptosis and by promoting IgA production[44-46]. CFS derived from several other probiotic strains, such as, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus* GG and *Bifidobacterium breve*, were able to downregulate inflammation, exhibit antioxidant activity or maintained intestinal barrier integrity[47-49].

To date, only a few animal studies have been performed to evaluate the effectiveness of postbiotics in CRC prevention and therapy *in vivo*. The stage is now set to expand this work with the use of translational *in vivo* models and clinical trials, which are essential to demonstrate efficacy. Sharma and Shukla [50] observed that CFS from *Lactobacillus rhamnosus* MD 14 MH656799 containing acetamide, acetate, propionate, butyrate, thiocyanic acid and oxalic acid attenuated early colon carcinogenesis in Sprague-Dawley rats ($n = 36$). The protective mechanism was linked to reduced fecal procarcinogenic enzymes, oxidants, aberrant crypt foci, vis-à-vis downregulating oncogenes (β -catenin, K-ras, Cox-2, NF- κ B) and upregulating tumor suppressor p53 gene leading to an almost healthy colon histology. De Moren de LeBlanc *et al*[51] evaluated the effect of the enzyme catalase as a postbiotic from catalase-producing *Lactococcus lactis* htrA-NZ9000 on the prevention/regression of 1,2-dimethylhydrazine (DMH) induced CRC in BALB/c mice ($n = 180-210$). Catalase-producing *Lactococcus lactis* increased catalase activity in DMH-treated mice and reduced H_2O_2 levels compared with the control group. Using the histopathological grading scale of chemically induced CRC, mice that received catalase-producing *Lactococcus lactis* had significantly less colonic damage and inflammation (2.0 ± 0.4) compared to control animals that received non-catalase-producing *Lactococcus lactis* (4.0 ± 0.3) or placebo-treated animals (4.7 ± 0.5). Increased antioxidant activity reduced levels of H_2O_2 and ROS involved in CRC onset and progression.

There are also promising results from studies of postbiotics derived from NGP. Recently, numerous *in vitro* studies showed that supernatant from SCFAs-producing bacteria, such as *Butyricoccus pullicaecorum* BCRC 81109[30], *Clostridium butyricum* ATCC 19398[25], *Propionibacterium freudenreichii* TL142[52], *Propionibacterium acidipropionici* CNRZ80, *Propionibacterium freudenreichii* subsp. *freudenreichii* ITG18, *Propionibacterium freudenreichii* subsp. *shermanii* SI41[53] suppressed CRC cells proliferation and induced apoptosis. The same results were documented by Zhao *et al*[54], where single strain CFS from human *Bacillus* strains BY38, BY40, BY43, BY45 exhibited inhibitory effects on the proliferation of CRC cells in a dose-dependent manner through the induction of cell apoptosis. These results suggest that NGP could represent novel and promising anti-tumor agents against CRC. Further *in vitro* studies focused on the activity of postbiotics derived from different probiotic strains in CRC cell lines are listed in Table 4.

CONCLUSION

Traditional probiotics have utility in the management of CRC as adjuvant treatment, mainly to reduce postoperative complications and to alleviate the side effects of chemotherapy. Antitumorigenic mechanisms of probiotics include the modification of intestinal microbiome, improvement of intestinal barrier integrity, immune potentiation and maintaining gut homeostasis. However, it is well known that the efficiency of probiotics is strain specific. The available clinical data indicate that CRC patients most

Table 4 Efficiency of postbiotics on cancer cells *in vitro*

Probiotic strain	Derived postbiotic	Cell line	Mechanism/effect(s)	Ref.
<i>Lactobacillus casei</i> ATCC334	CFS (ferrichrome)	Caco-2/bbe, SKCO-1, SW620	In CFS, ferrichrome subsequently identified as the responsible molecule that induced apoptosis <i>via</i> JNK-DDIT3 signaling axis, thus having tumor-suppressive effect and exerted minimal effect on normal intestinal epithelial cells	Konishi <i>et al</i> [70]
<i>Lactobacillus rhamnosus</i> MD 14 MH 656799	CFS (acetate, butyrate, propionate, acetamide, thiocyanic acid, and oxalic acid)	Caco-2, HT-29	CFS with metabolites exhibited both anti-genotoxic and cytotoxic potential against CRC cells	Sharma <i>et al</i> [71]
<i>Bifidobacterium adolescentis</i> SPM0212	CFS	Caco-2, HT-29, SW480	CFS significantly inhibited the proliferation of cancer cells	Kim <i>et al</i> [39]
<i>Lactobacillus fermentum</i> KCTC 3112	CFS	CCD18-Co, HCT-116, HT-29	Induction of cancer cells apoptosis by CFS up-regulating Caspase-3, Bax, Bak, Noxa, and Bid mRNA expressions	Lee <i>et al</i> [38]
<i>Lactobacillus casei</i> (ATCC 334), <i>Lactobacillus rhamnosus</i> (GG ATCC 53103)	CFS	HCT-116	Anti-metastatic effects of high molecular weight fractions	Escamilla <i>et al</i> [40]
<i>Lactobacillus acidophilus</i> ATCC 4356, <i>Lactococcus lactis</i> ATCC 11454, <i>Lactobacillus casei</i> ATCC 334, <i>Lactobacillus reuteri</i> ATCC 55148, <i>Saccharomyces boulardii</i> ATCC MYA-796	CFS	HT-29	Downregulation of the expression of PGE-2 and IL-8 in cancer cells by metabolites of probiotics. CFS differently modulated IL-1 β , IL-6, TNF- α , and IL-10 production by human macrophages, suggesting a peculiar anti-inflammatory activity	De Marco <i>et al</i> [48]
<i>Lactobacillus casei</i> ATCC 393	Sonicated-cell suspension	CT26, HT-29	Inhibition of cancer cells proliferation and induction of apoptosis	Tiptiri-Kourpeti <i>et al</i> [72]
<i>Lactobacillus reuteri</i> PTCC 1655	Sonicated-cell suspension	HT-29-ShE	Anti-metastatic and anti-proliferative effects	Maghsood <i>et al</i> [73]
<i>Lactococcus lactis</i> PTCC 1336	Nisin, cell wall, cytoplasmic extract of nisin	SW480	Anti-proliferative effects, associated with the decreased expression of cyclin D1 in SW480 cell line	Hosseini <i>et al</i> [74]
<i>Pediococcus pentosaceus</i> FP3, <i>Lactobacillus salivarius</i> FP25/FP35, <i>Enterococcus faecium</i> FP51	SCFAs (butyrate and propionate)	Caco-2	Significant proliferation inhibition of Caco-2 cells and activation of apoptosis	Thirabunyanon and Hongwittayakorn [75]
<i>Streptomyces levis</i> ABRINW111	Extracted metabolites	SW480	SW480 growth inhibition, increased Caspase-3 and reduced Ki67 expression in a concentration/time-dependent manner; subG ₁ phase (apoptosis) increased by metabolites and cell cycle arrest in G ₁ , G ₂ /M and S phase; p53 gene expression followed SW480 cells treatment significantly	Faramarzi Azimi Maragheh <i>et al</i> [76]
<i>Streptomyces</i> sp. MUM256	MUM256 extract	HT-29, Caco-2	Antioxidant properties, cytotoxicity against CRC cells by reduction in viability and induction of apoptosis (depolarization of mitochondrial membrane potential and arrest in subG ₁ phase)	Tan <i>et al</i> [77]
<i>Clostridium butyricum</i> ATCC 19398	SCFAs	HCT-116, HCT-8, Caco-2	Suppression of the Wnt/b-catenin signaling pathway and modulation of the gut microbiota composition.	Chen <i>et al</i> [25]

CFS: Cell-free supernatant; CRC: Colorectal cancer; IL: Interleukin; SCFAs: Short-chain fatty acids; TNF- α : Tumor necrosis factor-alpha.

often benefit from combined administration of strains *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium lactis*, and *Bifidobacterium longum*. Use of their combination or in combination with other species is more effective than individual supplementation. Nevertheless, consideration of each CRC patient's health status is still strictly recommended before administering viable probiotics. The gut microbiota is emerging as a contributing factor in the etiopathology of CRC. It is necessary to consider gut microbiota-drug interactions, including composition and metabolic activity of gut microbiota, which can both positively and negatively affect the outcome of CRC therapy. And even though research in this area is still in its infancy, it can be assumed that future clinical treatment and prevention of CRC will focus on supplementing the microbiome with commensal species (NGP candidates) that are predominantly anaerobic. Recent studies indicate that SCFAs-producing bacteria, especially butyrate producers, such as *Akkermansia muciniphila*, *Propionibacterium freudenreichi*, and *Butyricicoccus pullicaecorum* belong to

beneficial NGP that may have applicability in CRC therapy. Furthermore, it was discovered that strains previously defined as potential pathogens appear to possess probiotic properties when these lack key virulence factors, for example non-toxicogenic *Bacteroides fragilis* NCTC9343 has positive effects on patient's health. A significant disadvantage of NGP is, above all, their safety as this has not yet been sufficiently confirmed in animal and clinical studies. Safety validation is of particular importance before administering NGP to oncology patients. Although NGP research is experimentally demanding, emerging data shows great potential. Therefore, it is necessary to continue and explore new possibilities of NGP use in the therapy or prevention of diseases, including CRC, especially through clinical trials. Supplementation with postbiotics should be favorable in CRC therapy, because postbiotics have the ability to stimulate immune responses, inhibit cancer cell proliferation, induce apoptosis and necrosis, and they can shape microbiome composition in CRC patients. The advantage of postbiotics is that they do not pose a risk of unwanted infection to the patient, although screening for product contamination will be important. Moreover, it is possible to accurately determine and verify administered doses of a particular postbiotic. However, this emerging research area currently lack *in vivo* or clinical data to assess feasibility. In conclusion, the administration of traditional probiotics, NGP or postbiotics, supported by various experimental studies, is an efficient complementary therapeutic approach to combat CRC. A protective effect of probiotics and postbiotics against CRC onset is also indicated, however, lifestyle changes are recommended as a first line of defense in CRC prevention.

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