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LITERATURE REVIEW

Exploring the Therapeutic Landscape: A Systematic Review on the Anti-inflammatory Effects of Probiotics in Colitis-associated Colorectal Cancer

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Abstrak

Latar Belakang: Kanker adalah penyebab kematian nomor dua. Peradangan kronis meningkatkan risiko kanker kolorektal; pada kolitis jangka panjang muncul CAC. Probiotik, yang memodulasi mikrobiota dan imun, berpotensi meredam lingkungan mikro inflamasi. **Objektif:** Menilai efek anti-inflamasi probiotik pada model hewan CAC. **Metode**: Tinjauan sistematis tiga basis data (PubMed, ScienceDirect, Wiley). Dari 248 artikel, 9 studi memenuhi kriteria. Risiko bias dinilai dengan SYRCLE RoB. **Hasil**: Probiotik konsisten menurunkan keparahan klinis, menekan penanda inflamasi, dan mengatur jalur terkait inflamasi, menunjukkan perbaikan proses patogenesis CAC. Namun, ukuran sampel kecil dan heterogenitas antar-studi membatasi kesinambungan temuan. **Kesimpulan**: Probiotik menjanjikan sebagai adjuvan anti-inflamasi pada CAC di model hewan. Uji pra-klinik lanjutan dan uji klinis terkontrol diperlukan untuk memverifikasi kemanjuran, keamanan, dosis, dan strain yang optimal.
Kata kunci: hewan, kanker kolorektal terkait kolitis, peradangan, probiotik, tinjauan sistematis

Abstract

Background: Colorectal cancer is closely linked to chronic inflammation; long-standing colitis confers a 2–3-fold higher risk. Modulating the gut–immune axis with probiotics may attenuate the inflammatory microenvironment of colitis-associated colorectal cancer (CAC). **Objective:** To evaluate the anti-inflammatory effects of probiotics in CAC animal models. **Methods**: We searched PubMed, ScienceDirect, and Wiley Online Library; 248 records were screened and 9 studies met inclusion. Risk of bias was appraised using SYRCLE RoB. **Results:** Probiotic treatment consistently reduced clinical severity, down-regulated inflammatory markers, and modulated key signaling pathways in CAC models. Nonetheless, evidence was heterogeneous, sample sizes were small, and dosing, strains, and formulations varied; safety reporting was limited. **Conclusions:** Probiotics show promise as anti-inflammatory adjuncts in CAC based on animal studies. Rigorous, standardized preclinical work and well-designed trials are needed to define optimal strains, dosing, and formulations, and to confirm efficacy and safety for translation.
Keywords: *animal, colitis-associated colorectal cancer, inflammation, probiotic, systematic review*

INTRODUCTION

Background

In 2018, cancer was responsible for around 9.6 million deaths, or one in every six, making it the second-leading cause of death worldwide. Men are more likely to develop stomach, prostate, lung, liver, and colorectal cancers, whereas women are more likely to develop breast, cervical, lung, thyroid, and colorectal cancers.[1] In 2020, colorectal cancer (CRC) became the third most common cancer, reaching 1.93 million new cases, and the second most common cause of cancer death (916 thousand deaths).[2] Colorectal cancer could result in up to 2.5 million additional cases by 2035.[3]

CRC is mostly caused by a combination of environmental, genetic, and age factors. Inflammatory bowel disease (IBD) has further well-known correlations with colorectal cancer.[4] Antigens and immunogens from food, bacteria, viruses, and even water are all highly exposed to the gastrointestinal tract. The immune response to these foreign antigens is typically suppressed in order to maintain gut homeostasis, which is characterized by a lack of inflammation.[5]

Chronic inflammations resulting from IBD–ulcerative colitis (UC) and Crohn's disease (CD)–are considered the mechanisms for colitis to develop into a malignancy. A 2-3-fold greater risk of colorectal cancer is associated with long-term UC and Crohn's colitis, with estimates changing based on the study, time frame, and individual risk factors. Prior studies have indicated that in Asian patients with ulcerative colitis, the incidence of colorectal cancer is 0.02%, 4.81%, and 13.91% after ten, twenty, and thirty years, respectively. Meanwhile, a study found the hazard ratio of CRC death (1.74) and incidence of CRC (1.4) in the patients with CD was higher than that of their matched controls. Individuals diagnosed prior to 40 years of age with involvement in the colon and primary sclerosing cholangitis were found to have an increased risk of colorectal cancer.[6–8]

Inflammation plays a role in the onset and progression of IBD-CRC, which is caused by a series of genetic changes that follow the "inflammation-dysplasia-carcinoma" sequence rather than the "adenoma-sequence" that is typically associated with sporadic CRC. It is widely known that several molecular mediators that contribute to IBD-CRC have a link to chronic inflammation. Nuclear factor κappa B (NFκB), a master regulator of inflammation, is activated by toll-like receptors (TLR) and tumor necrosis factor-α (TNF-α), which in turn causes the transcription of genes linked to carcinogenesis, including cyclooxygenase-2 (COX-2). Via tumor suppressor p53 pathways, inflammation causes intestinal epithelial cells to undergo apoptosis; faulty p53 signaling may be a precursor to cancer in the development of dysplasia.[9]

The first line of the body's immunological defense is the skin and mucosa. B cells and T cells are involved in the adaptive immune response in the intestinal mucosa, whereas macrophages

and dendritic cells have an innate immunological role. Macrophages have a significant role in both pathogenic processes and chronic inflammation. Moreover, the presence of macrophages indicates persistent inflammation. IBD is one of the autoimmune disorders that are linked to the polarization of macrophages. In a healthy setting, intestinal macrophages phagocytize microbes and provide antigens to stimulate T lymphocytes. An overabundance of macrophage activation causes intestinal mucosal pathological damage from physiological inflammation. [10,11]

Numerous inflammatory cytokines, including TNF-α, interleukin (IL)-6, and IL-18, released by macrophages, are significant factors in ulcerative colitis. An essential mediator of the inflammatory response, IL-6 takes a direct part in both the inflammatory response and the associated damage process. IL-6 makes epithelial cells more permeable, which encourages macrophage infiltration and exacerbates ulcerative colitis development. By encouraging IκBα degradation, NF-κB p65 phosphorylation, and NF-κB nuclear transfer, TNF-α modulates the NF-κB pathway and exacerbates ulcerative colitis. These cytokines contribute to carcinogenesis and tumor growth in addition to their role in the inflammatory response. Excessive cytokine production in chronic inflammation leads to oxidative stress-induced deoxyribonucleic acid (DNA) damage and CAC carcinogenesis.[11–13] Therefore, targeting the components of inflammation may be considered a therapeutic approach to manage CAC.

A diet of dairy products, such as probiotics, is a protective factor that has been linked to a decrease in the incidence of CRC.[4] The Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) describe probiotics as live bacteria that, when administered in sufficient doses, give a health benefit to the host.[14] With a lengthy history of safety, probiotics—specifically *Lactobacillus* and *Bifidobacterium*—are regarded as generally recognized as safe (GRAS). They have demonstrated promise in the management of several cancer types, both in prevention and treatment. Before probiotics become viable cancer treatment choices, there are still a number of obstacles to be addressed.[15]

The biggest population of bacteria, approximately 3×10^{13} cells, is found in the colorectum. Microorganism-colorectal epithelium interaction plays a major role in regulating basic physiological functions, including immunological responses. Two of the main causes of microbiota-related carcinogenesis are inflammation linked to dysbiosis and the production of carcinogens. Early cancer stages have been linked to changes in the gut microbiome in precancerous adenomas. Furthermore, microbes—particularly bacteria—have a crucial role in the development of a number of illnesses, including CRC.[16–18]

CAC represents a formidable challenge at the crossroads of chronic inflammation and heightened cancer

susceptibility. Probiotics, renowned for their capacity to modulate the gut microbiota and immune responses, emerge as promising candidates for influencing the inflammatory microenvironment that is implicated in the progression of CAC. The prevalence of CAC underscores the urgency to unravel novel avenues for intervention, with probiotics positioned as agents that could potentially disrupt the intricate cascade leading to cancer development. In conclusion, this systematic review aims to synthesize the existing literature on the anti-inflammatory potential of probiotics in animal models with CAC and address the challenges and opportunities.

METHOD

Search Strategy

We searched three databases: PubMed, ScienceDirect, and Wiley Online Library, in order to obtain published studies that met the eligibility criteria. Identification of relevant studies was performed using the combination of keywords and the boolean operator to identify specific studies of interest (see Additional File 1). The combination of keywords in those three databases was different due to specific features of the database and limitations on the number of keywords. Any filters (year of publication and article type) provided in the database that may help in the search were used. This review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) method. Every citation of the included studies was imported into Mendeley Reference Manager v1.64.0 to identify duplicates and screen the papers by title, abstract, author, and year of publication.

Eligibility Criteria

We included preclinical in-vivo studies with animal models of CAC and intervened by administering any kind of probiotic without any limitations on the duration and dosage of the probiotics. The controls were given a placebo or were not administered probiotics. The expected outcomes measured in the studies were the effects of probiotics on CAC-relevant inflammatory biomarkers or the immunoregulatory properties of probiotics on CAC. This review has excluded in vitro or clinical studies; editorials, letters, case reports/series, reviews; studies that were not specifically about CAC; studies that were not assessed the anti-inflammatory effect of probiotics on CAC; studies with incomplete data and characteristics; papers not published in the past 10 years; and papers not available in English.

Study Quality Assessment

We used the SYRCLE (Systematic Review Center for Laboratory animal Experimentation) Risk Bias Tool to evaluate the risk of bias. A complete list of signaling questions is provided to aid the judgment process in assigning a low, high, or unclear risk of bias to each thing stated in the instrument. A "yes" judgment indicates a low risk of bias, whereas a "no" indicates a high risk of bias. The judgment will be "unclear" if insufficient details are supplied to correctly assess the bias.[19]

RESULT AND DISCUSSION

Results

Study Selection

We searched three databases: Pubmed, ScienceDirect, and Wiley Online Library, and obtained a total of 248 articles after screening the articles by filters in the respective databases, such as years of publication and type of papers. We found two duplicate papers and excluded 197 other articles after screening them by titles, abstracts, authors, and years. The remaining papers were assessed based on the eligibility criteria. A total of 35 articles were excluded due to some reasons: irrelevant subjects (not administered with any kind of probiotic, not exploring animal models with CAC, not analyzing the effect of inflammation on carcinogenesis), review, and bibliometric analysis. In the end, we included nine articles for our systematic review (see Figure 1).

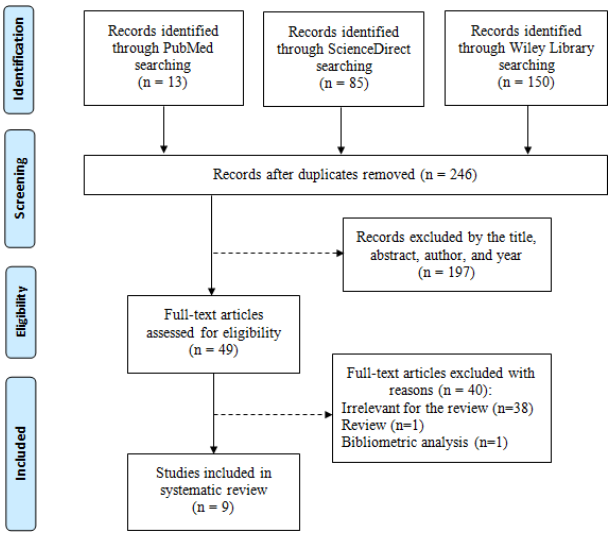


Figure 1. PRISMA Flow Diagram

Study Characteristics

We summarized the characteristics of nine studies and presented them as shown in Table 1. These included studies were published in the years 2015–2023. They were conducted in six different countries: Japan[20], China[21,24,26], the USA[22], Brazil[23], South Korea[25,28], and Taiwan[27]. It appears that the studies were primarily published in China and in East Asia generally.

Table 1. Characteristics of the Included Studies

Author	Country	Animal Models (Age; Gender; Type)	Probiotic	Probiotic Dose (Bacteria dose; Frequency;)	Colitis Agent (Number of cycles; Type of Agent; Initiation timing)	Cancer Agent (Type of Agent; Dose and Route; Initiation timing)	Study Period	Intervention
Hiramoto (2023)[20]	Japan	8 weeks; Female; mice	*Bacillus coagulans*	200,000 dissolved in distilled water; 3 times a week	1 cycle; DSS 2% for 1 week via drinking water; Started from week 1 post AOM injection	AOM; 10 mg/kg single dose i.p.; on the first day	20 weeks	
Song (2017)[21]	China	4 weeks; Male; C57BL/6 mice	Bifico capsules (210 mg/caps) contains : • 1.0 x 10^7 CFU viable lyophilized *Bifidobacterium longum,* • 1.0 x 10^7 CFU *Lactobacillus acidophilus,* • 1.0 x 10^7 CFU. *Enterococcus faecalis*	Bifico capsules (4.2 g/kg, dissolved in 200 uL physiological saline); At least 1.2 x 10^7 CFU/d per mouse	3 cycles; 2% DSS for 7 days in a row via drinking water, followed by 2 weeks of sterile water; After AOM injection	AOM; 10 mg/kg single dose i.p.; on the first day	77 days	
Gao (2017)[22]	USA	12 weeks old;NR; *Hdc*$^{-}$*BALB/c mice*	*Lactobacillus reuteri ATCC PTA 6475*	5x10^8 CFU once per day for 7 days before AOM and followed by administration once per 3 days for 15 days	2 cycles; 2% DSS for 6 days, followed by 2 weeks of drinking water; Started immediately after AOM injection	AOM; 12.5 mg/kg single dose i.p.; on the first day	15 weeks	
Silveira (2020)[23]	Brazil	4-6 weeks; NR; C57BL/6 mice	*Lactobacillus delbrueckii ssp bulgarics*	1x10^9 CFU diluted in 200 uL PBS, 3 times a week orally during experimental period	3 cycles; 2.5% DSS for 1 week, followed by 2 weeks of normal water; Started after AOM injection	AOM; 10 mg/kg single dose i.p.; on day 0	12 weeks	
Rong (2019)[24]	China	4-5 weeks; NR; C57BL/6 mice	*Lactobacillus helveticus NS8*	100 uL of NS8 suspension (5 x10^8 CFU in sterile 1xPBS) from 3 weeks before mutagenic agent administration until the study endpoint	3 cycles; 3% DSS for 7 days, followed every 2 weeks regular drinking and repeated 2 cycles more with 2.5% DSS; After 5 days AOM injection,	AOM; 10 mg/kg single dose i.p.; on day 0	80 days	
Lee (2015)[25]	South Korea	6 weeks; NR; Balb/c mice	*Lactobacillus plantarum (*pure live*/pLp and* dietary nanosized*/nLp)*	**pLp and nLp** Low dose: 4x10^9 CFU/kg/day High dose 4x10^{11} CFU/kg/day	2 cycles; 2% DSS for 7 days and followed by 14 days of tap water; Started two weeks after AOM injection	AOM; 10 mg/kg single dose i.p.; on the first day	8 weeks	
Liu (2022)[26]	China	8 weeks; Male; C57BL/6 mice	*Clostridium butyricum*	2 x 10^8 CFU in 200 uL physiological saline, three times one week, started from the beginning of experiment until the end	3 cycles; 2.5% DSS for 5 days, followed by 14 days of normal drinking water; Started 5 days after AOM injection	AOM; 12.5 mg/kg single dose i.p.; on the first day	78 days	
Chung (2019)[27]	Taiwan	6-9 weeks; NR; C57BL/6 mice	Heat-killed *Enterococcus faecalis* strain KH2	17 mg/kg every day during the course	3-4 cycles; 2.5% DSS for 6 days, followed by	AOM; 10 mg/kg single dose i.p.; on the first day	60 days	

				experiment, starting 2 weeks before DSS administration or at the end of third DSS treatment	14 days of water; Started after AOM injection			
Oh (2020)[28]	South Korea	8 weeks; Male; C57BL/6 mice	*Lactobacillus gasseri* 505	10^8 CFU/kg/day	3 cycles; 2.5% DSS in the drinking water for one week, followed by two weeks of regular drinking water; Started after AOM injection	AOM; 10 mg/kg single dose i.p.; on the first day	11 weeks	*Cudrania tricuspidata* leaf extract-supplemented milk (1,5 g/kg/day)

AOM, azoxymethane; CFU, colony forming unit; DSS, dextran sodium sulfat; i.p., intraperitoneally; NR, not reported.

In exploring the effect of probiotics on CAC, every study used 4–12-week-old mice as animal models. Gender and number of mice, however, were not explicitly explained in most studies. The majority of studies used a single probiotic. A study by Song et al.[21] and Oh et al.[28], meanwhile, used probiotics in combination with other probiotic bacteria and prebiotics, respectively. *Lactobacillus* became the most common genus of bacteria used as probiotics administered to animals, consisting of six different species distributed in six studies[21-25,28]. On the other hand, all probiotic bacteria in the included studies were in the same group of capability—producing lactic acid—except in Liu et al.[26] which used *Clostridium butyricum*—butyric acid-producing bacteria. All probiotics were administered orally, with doses ranging from 1.2 x 10^7 to 4 x 10^{11} colony forming unit (CFU), besides two studies[20,27] that did not explain the doses in CFU.

Azoxymethane/dextran sodium sulfat (AOM/DSS) were preferred as the agents to induce CAC in all studies. Administration of AOM was generally the same—10 mg/day single-dose intraperitoneal injection at the beginning of the experiment—except for a study by Gao et al.[22] which the AOM dose given was 12.5 mg/day. Meanwhile, varying results were shown for the administration of DSS. DSS 2–3% were administered for 1–4 cycles, followed by two weeks of regular drinking water. The total duration of the experiments collectively ranged between 8-20 weeks.

Study Quality Assesment

The risk of bias for every study included in this systematic review was assessed with the SYRCLE tool. There were ten domains that were distributed in six types of bias assessed for potential risk of bias: selection bias (allocation sequence/sequence generation, baseline characteristics, allocation concealment); performance bias (random housing, blinding); detection bias (random outcome assessment, blinding); attrition bias (incomplete outcome data); reporting bias (selective outcome reporting); and other sources of bias.[28]

As it was said that animal studies were poorly reported, the similarity is reflected in the results (see Figure 2). Most studies exhibited an unclear risk of bias in

every domain due to insufficient data to be judged. Baseline characteristics, as part of selection bias, were the domain with the highest risk of bias (11%), followed by other sources of bias (4%) and incomplete outcome data (3%). Selecting outcome data (reporting bias type) was the domain with the lowest risk of bias. A total of 33% of studies and six domains were free from 'no' judgment.

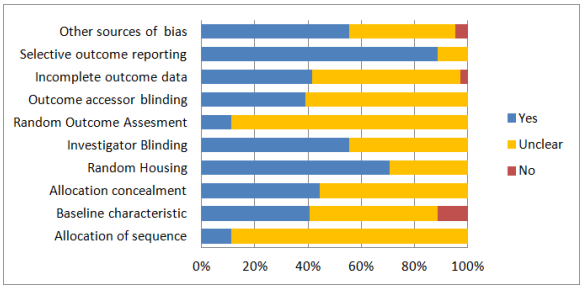


Figure 2. Risk of bias assessed using SYRCLE RoB Tools

Effect of Probiotics on Inflammatory Cytokines

mRNA Expression of Cytokines

Almost all studies showed a decrease in the mRNA expression of major proinflammatory cytokines, such as IL-6, IL-1α, IL-1β, TNF-α, and interferon (IFN)-γ, in CAC models treated with probiotics. Other proinflammatory cytokines, such as IL-23 and IL-17, also showed decreased mRNA expression. Vice versa, anti-inflammatory cytokines such as IL-10, IL-4, and TGF-β showed a decrease in mRNA expression (see Table 2).

Table 2. Antiinflammatory and anticancer effects of probiotics on CAC animal models

Author	Probiotic	Effect on inflammation compared to CAC models	Effect on Carcinogenesis	Signaling Pathway
Hiramoto (2023)[20]	*Bacillus coagulans*	• Only the B. coagulans-treated group showed significant increase in TGF-β plasma levels ($p < 0.01$). • TGF-βR1 expression in the colon increased in the AOM and DSS treatment group. • IL-6 and IFN-γ levels decreased considerably in the B. coagulans-treated group ($p < 0.01$).	There was an improvement on symptoms of colon cancer induced by AOM and DSS: • less number of tumor ($p<0.01$) • decreased shortening of colon	• PI3K, pAKT, and mTOR expression decreased • Expressions of Smad2/3, Smad4, and p21 were highly increased • CDKI expression was significantly increased • c-Myc expression increased the most in the group treated with AOM and DSS only • The upregulation of IκB expression was greater in the *B. coagulans*-treated group

				• The *B. coagulans*-treated group showed increased co-localization of NF-κB and IκB
Song (2017)[21]	Bifico capsule containing : *B. longum, L. acidophilus, E. faecalis*	• The mice in the Bifico group lost significantly less weight ($p<0.05$). • Bifico significantly reduced colon length shortening (6.1 cm vs. 5.6 cm) ($p<0.01$). • Bifico treatment partially repaired the architecture of the intestinal lamina propria. • Expression levels of TNF-α, IL-1β, IL-6, and Ptgs1 genes was decreased in mice of the Bifico group ($p<0.05$, $p<0.01$, $p<0.05$, and $p<0.001$, respectively). • Bifico treatment significantly decreased PGE2 level enhancement ($p<0.01$).	• A significant inhibitory effect on the multiplicity and size of colitis-induced tumors, with an average of 8.3 macroscopic tumors (mean diameter, 1.52 mm) per mouse in Bifico-treated animals vs 12.7 tumors (mean diameter, 1.23 mm) per mouse in the model group • A lesser of total tumor number per mouse, tumor number <3 mm, tumor number ≥3 mm per mouse, tumor average diameter ($p<0.01$, $p<0.05$, $p<0.05$, and $p<0.05$ respectively). • Mice in the Bifico group mainly manifested as crypt dysplasia and adenoma compared to model (multiple adenoma and invasive adenocarcinoma)	• The gene expression of CXCR2 showed no difference between the Model and Bifico groups. • CXCR2 ligands, such as CXCL1, CXCL2, CXCL3, and CXCL5 were downregulated by Bifico treatment. • The expression of proliferating cell nuclear antigen was significantly suppressed by Bifico treatment
Gao (2017)[22]	*L. reuteri 6475*	**Colon** • Mice with HDC-positive *L. reuteri 6475* showed the reduced relative gene expression of KC, IL-6, IL-2, TNF-α, and IL-1α in mucosa of colon ($p<0.001$), • No significant difference in IL-12, IL-23, and IFN-α expressions • Undetectable results on IL-17 expression **Plasma concentration** • *L. reuteri 6475* administration decreased concentrations of KC, IL-22, and IL-6 cytokines. • Undetectable results on IL-1β, IL-21, IL-23, epidermal growth factor cytokines. • IL-4, IL-17, IFN, IL-1α, IL-12, TNF-α, IL-10, and IL-13 showed no significant changes **Spleen and Bone Marrow** • *L. reuteri* 6475 administration significantly decreased the relative number of CD11bþGr-1þ IMCs	*L. reuteri 6475* with a wild-type allele of the HDC gene significantly reduced the number and size of colonic tumors ($p<0.01$). **PET imaging** *L. reuteri 6475* decreased the numbers of hot spots in the colon. *L. reuteri 6475* significantly decreased the abdominal FDG intensities compared with positive control mice ($p<0.05$).	**HDC and H2R expression** • L. *reuteri* can express the HCD and histidine/histamine antiporter genes in the mammalian intestines of Hdc/ mice; • HDC-positive *L. reuteri* is able to generate histamine in the gut

Silveira (2020)[23]	*Lactobacillus delbrueckii ssp bulgaricus,*	• No differences observed in body weight loss. • *L. bulgaricus*-treated mice showed a lower clinical score on the 13 and 15th days after tumor initiation. • *L. bulgaricus* reduced the DSS-induced colon shortening (p<0.001). **Cytokines** **Inflamed colon** • A reduction of at least 2-fold in the levels of the cytokines TNF-α (p<0.01), IL-1 β (p<0.05), IL-23, and IL-17 (p<0.001) in *L. bulgaricus*-treated mice. • Concentrations of IFN-γ in *L. bulgaricus* group increased (p<0.001). • No differences observed in IL-6 levels. **Tumor tissue** • A negative regulation of TNF-α and IL-1β (p<0.001), IL-17 and IL-6 (p<0.01), IL-23 (p<0.05) in mice treated with the probiotic. • An increase in IFN-γ levels in probiotic-treated group (p<0.001).	• *L. bulgaricus* and model groups of mice presented morphologically similar neoplastic lesions. • Group treated with the probiotic developed fewer (1-5 vs. 4-13) (p<0.001) and smaller tumors (total tumor volume 4,4 fold, p<0.001; and mean tumor volume 3-fold lower, p<0.05).	
Rong (2019)[24]	*Lactobacillus herveticus NS88*	• Mice treated with NS8 tended to lose less body weight after the first 7 days of DSS drinking and had a greater regain of body weight in the interval between the 1st and 2nd cycles • NS8-treated mice had significantly longer colons on day 14	• Fewer tumors than in control group • Fewer small tumors (≤ 2 mm) in the NS8-treated group (p<0.01), but the number of large tumors (> 2 mm) did not show a significant difference between	• Angiogenin and β-catenin expression levels were significantly lower in NS8-treated mice. • Cox-2 was suppressed by NS8 treatment • Significantly lower number of Ki67+
		• NS8-treated mice showed the decrease of areas with ulceration and inflammatory infiltrate and the decrease of intestinal wall thickening in the colons • Lower expression levels of IL-1β 14 days after AOM injection • Elevation of the levels of IL-10 • No significant difference on TNF-α	groups. • Lower grade hyperplasia showed in the epithelia of NS8-treated mice	proliferating cells per crypt in the colons of NS8-treated mice. • Caspase-3 activation was upregulated by NS8 treatment • IκBα phosphorylation was suppressed by NS8 treatment
Lee (2015)[25]	Nanosized and pure live *Lactobacillus plantarum*	• The administration of pLp or nLp at high and low doses suppressed AOM/DSS-induced body weight loss at 8 weeks post-AOM injection. • The administration of pLp or nLp dose dependently decreased the overexpression of TNF-α, IL-6, IL-1β, and IFN-γ	• pLp and nLp both inhibited colon shortening induced by AOM/DSS • pLp and nLp both significantly reduced colonic weight/length ratios (p<0.05) • The administration of nLp at high dose significantly reduced tumor numbers (2.4 ±0.8) compared with pLp (4.1 ± 1.1). • Mean areas of dysplasia, adenocarcinoma, or structural disruption were smaller in the pLp and nLp-treated groups than those in the AOM/DSS control group. • Dysplasia and adenocarcinoma development were rarely observed in the N-high group.	• The administration of pLp or nLp dose-dependently decreased the iNOS and COX-2 • Suppressive activities of iNOS and COX-2 were greater in the nLp-treated groups than in pLp-treated groups • The administration of pLp or nLp at low and high doses significantly increased the expression of p53, p21, and Bax • The administration of pLp or nLp at low and high doses significantly decreased the expression of Bcl-2 (P<0.05)
Liu (2022)[26]	*Clostridium butyricum* (CB)	• The body weight loss in the CB group was significantly less severe (p<0.05) • The expression of TNF-α (p<0.05), IL-6 (p<0.05) and COX-2 genes decreased (p<0.05).	• The damage of the colonic mucosal epithelium was partly improved in the CB group (p<0.05) • Lesions in mice in the CB group mainly manifested as adenoma and crypt dysplasia	• The decreased expression of p-IκBα (p<0.05) and NF-κB in the colon tissue of CB group • The expression of Bcl-2 (p<0.05) and p65 (p<0.05) were reduced

			• The mean size of neoplasm was 1.49 (± 0.22)mm in the model group and 1.20 (± 0.17)mm in the CB group (P < 0.05)	• Expression of Bax were increased
Chung (2019)[27]	Pretreatment with heat-killed *E. faecalis*	• Reduced DSS-induced weight loss and diarrhea scores, but not in NLRP3-deficient mice • Longer colons compared to control (8.5 cm vs 7.6 cm), but not significantly different in NLRP3-deficient mice • Reduced colon levels of mature IL-1β	• Decreased the weight loss because of AOM/DSS • Significantly lower diarrhea scores • Had longer colons (8.0 cm vs. 7.3 cm) • Inhibited the number of AOM/DSS-induced colon tumors per mouse	• Via NLRP3-manner • Decreased colon levels of cleaved (activated) caspase-1
Oh (2020)[28]	*L. gasseri 505* (LG) and *Cudrania tricuspidata* (CT); LG+CT = FCT	• *L. gasseri 505* inhibited weight loss from DSS treatment, but combination with CT showed a slightly higher protective effect on weight loss at week 11. • mRNA expressions of TNF-α, IFN-γ, IL-1β, and IL-6 were gradually decreased by *L. gasseri 505*, but were highly suppressed up to a level similar to the normal control group by combination with CT • mRNA expressions of IL-4 and IL-10 gradually increased in the *L. gasseri groups* and were the highest in the combination with CT group.	• LG, CT, and FCT significantly prevented AOM/DSS-induced colonic shortening • LG, CT, or FCT suppressed neoplastic development, with the FCT group highly suppressed the effects. • The dysplasia and structural disruption were reduced in the LG and CT groups. • FCT group showed relatively well-preserved crypt structures and further resolved histological appearance. • FCT rarely observed dysplasia and adenocarcinoma development .	• Expression of MUC2, TFF3, occludin, and ZO-1 recovered • The FCT group showed the highest mRNA expression level in MUC2, Occludin, and ZO-1. • The mRNA expressions of such as p53, p21, and Bax, were gradually increased in the LG, CT, and mainly in FCT groups. • BCL-2 and BCL-xL, gradually reduced in the LG, CT, and FCT groups. • The mRNA expression and protein production of β-catenin and NF-κB reduced significantly • IκB-α gradually increased • Reduced iNOS and COX-2 mRNA expression, mainly if combined with CT

However, several cytokines that play a role in the inflammatory process with both pro- and anti-inflammatory effects seem to show varying results. For example, both IFN-γ in inflamed colons and colons with tumors had increased mRNA expression[23], while a study by Lee et al.[25] showed the opposite results. Another example of this decrease in expression also occurs in IL-22. Furthermore, a study by Gao et al.[22] showed some specificity for certain cytokines, such as the absence of significant differences in IL-12, IL-23, and IFN-γ mRNA expression, as well as undetectable changes in IL-17 mRNA expression in CAC animal models treated with probiotics. In addition, several studies[23,24] also showed no significant effect on the proinflammatory cytokine IL-6, especially in the inflamed colon. However, the protective effect of probiotics against increased IL-6 expression appears in colons with both inflammation and tumors.[23]

Level of Cytokines

Several studies[20,22,24] also analyzed the effects of probiotic administration on plasma cytokine levels in animal models of CAC. The results exhibited varied results. The pro-inflammatory cytokine IL-6 is known to reduce plasma levels in the group treated with probiotics. Meanwhile, plasma levels of other pro-inflammatory cytokines such as IL-1b seemed to decrease, but on the other hand, the decrease was not detectable.[22] This

inconsistency also occurred in anti-inflammatory cytokines such as IL-10, where Rong et al.[24] found an increase in IL-10 plasma levels, while in a study by Gao et al.[22], IL-10 did not provide a significant protective effect. Some proinflammatory cytokines (IL-1a, IL-17, TNF-a, IL-12, and IFN-γ) and other anti-inflammatory cytokines (IL-4 and IL-13) did not appear to be significantly affected by probiotics.[22]

Effects of Probiotics on Body Weight Loss

Six of nine studies[21,23-24,26-28] assessed comparative weight loss in animal models after being induced by CAC agents and treated with probiotics. Most studies showed an inhibitory effect of probiotics on weight loss in the progression of CAC. Some studies even provided additional information: a study by Oh et al. [28] found that the inhibition of weight loss that occurred was greater if probiotics were also combined with prebiotics (*Cudrania tricuspidata*); and Rong et al.[24] found that greater weight regain in CAC animal models in certain weeks was present in the group treated with probiotics. However, Chung et al.[27] showed that the effect of ameliorating weight loss might not be found in probiotic-treated animal models if they did not have certain genes, like NLRP3 (NLRP3-deficient mice). In addition, Silveira et al.[23] found that administration of probiotics did not significantly inhibit weight loss in animal models of CAC.

Effect of Probiotics on Shortening Colon

Seven of the nine studies[20-21,23-25,27-28] included an assessment of the protective effect provided by probiotic administration in animal models in preventing colonic shortening during CAC progression. All of these studies showed less extensive shortening of the colon in animal models with CAC in the probiotic group compared to CAC models without probiotic treatment. Furthermore, the study of Chung et al.[27], as related to inhibition of weight loss, showed that administration of probiotics alone was not sufficient to produce the expected significant protective effect regarding colon shortening in NLRP3-deficient mice.

Regulatory of pathway

Several gene expressions were assessed in the included studies. In three studies, nuclear factor kappa B (NFκB) was found to have decreased expression. On the other hand, the inhibitor of nuclear factor kappa B (IκB) experienced increased expression and decreased phosphorylation.[20,26,28] Several tumor suppressor gene expressions, such as p53 and p21, also increased.[20,25,28] BCL-2-associated X protein (Bax) is also known to have increased expression in studies that have analyzed it.[25-26,28] Meanwhile, COX-2 decreased in several studies.[24-26,28] Some consistent increases in gene expression were also seen in Smad2/3, Smad 4, the cyclin-dependent kinase inhibitor (CDKI), histidine decarboxylase (HCD), histidine/histamine antiporter gene, histamine, caspase-3, MUC2, Occludin, and zonula occludens (ZO)-1. Meanwhile, decreased gene expression also occurred in phosphoinositide 3-kinase (PI3K), pAKT,

mammalian target of rapamycin (mTOR), c-Myc, prostaglandin-endoperoxide synthase 1 (Ptgs1), CXCR2 ligand, proliferating cell nuclear antigen, angiogenin, b-catenin, B-cell lymphoma-2 (BCL2), as well as decreased NOD-like receptor protein 3 (NLRP3) inflammasome activation.

Effect of Probiotics on Tumor Number and Size

It seems consistent that the tumor number and tumor size in the included studies were affected by the protective effect of probiotics on the progression of CAC. Six studies[20–24,27] demonstrated a significant reduction in tumor numbers and five studies[21-23,25,26] showed a significant reduction in tumor size in animal models of CAC treated with probiotics. Moreover, one study also proved this effect through positron emission tomography (PET) imaging.[22] However, there were differences in the number of tumors between tumor size groups in two studies[21,24], which were the significant[21] and non-significant[24] effects related to the reduction in the number of tumors measuring >2—3 mm.

Discussion

CRC became the third most frequent malignancy and the second leading cause of cancer-related mortality. Chronic inflammation caused by colitis is thought to be one of the pathways that lead to cancer. Inflammation contributes to the onset and progression of CAC, which is generated by a series of genetic changes that follow the "inflammation-dysplasia-carcinoma" sequence compared to sporadic CRC.[2,9] In this systematic review, the included studies were mostly performed in developed countries, specifically in China (East Asia). The familial risk of CRC in the East Asian population may be contributed by some novel variations paired with common variant genes. Red or processed meats, preserved foods, saturated or animal fats, cholesterol, spicy foods, and high-sugar foods are dietary components that have been identified as risk factors for colon cancer in Asians.[29,30] Physical exercise and obesity are two significant contributors as well. Furthermore, both tendencies are undergoing changes in Asian countries. In addition, alcohol consumption and cigarette smoking habits that start to merge in the Asian population have been shown to increase the risk of colorectal cancer, yet the link between smoking and colorectal cancer in Asians is less obvious.[31] On the other hand, the course duration of the colitis—UC—has a great role in the incidence of CRC in Asian countries, but the differences on a regional level were not significant.[32]

AOM, a metabolite of 1,2-dimethylhydrazine (DMH), is the most widely utilized chemical to induce CRC. Its high carcinogenicity results in a wide range of alterations in critical genes that code for components of many intracellular signaling pathways. The administration of DMH or AOM causes epithelial neoplasia (abnormal crypts—abberant crypt foci/AFC) in the colon, which develops to

adenoma and finally adenocarcinoma.[33] Meanwhile, DSS is an agent with a non-genotoxic pro-inflammatory nature used in mouse models of acute and chronic colitis. The CAC models were initially introduced by Tanaka et al. in 2003, when male mice were intraperitoneally injected with a single dose of 10 mg/kg AOM and 2% DSS solution for 7 days. All of the mice had acquired colon adenocarcinomas by the week of 12.[34] This model has proven to be exceedingly convenient, reasonably affordable, and very reproducible, and it is commonly utilized in studies of colitis-associated carcinogenesis. Despite its extensive use, the model with AOM/DSS-induced CAC has not been standardized. Furthermore, various mouse strains are sensitive to AOM/DSS in varying degrees. The C57B/L6 and Balb/c mice are moderately sensitive and exhibit a lower incidence of colon cancers. For example, Balb/c mice had a 100% incidence of colon cancer, while C57BL/6N mice had 50%, compared to another strain. This is most likely why C57BL/6 and Balb.c mice were the two most commonly used mouse model in the included studies.[33,35-36] Furthermore, the duration and doses of AOM/DSS exposure vary in many studies, leading to a complicated comparison between the experimental results.[33]

Lactobacilli were the most frequently used as probiotics to treat CAC animal models in the included studies. Lactobacillus is a gram-positive anaerobic bacteria without spores. Lactobacillus is classified as a member of Firmicutes, the class of Bacillus, the order of Lactobacillales, and the family of Lactobacillus. It has the ability to break down other carbohydrates, including glucose, into lactic acid. *Lactobacillus* is composed of several species.[37], of which six different species were used in the studies reviewed here. The similarities between the bacteria used in this study were that they were in the same group: gram-positive and acid-producing bacteria. *Bacillus coagulans, Bifidobacterium longum, Enterococcus faecalis, and Lactobacillus sp.* can all produce lactic acid, which causes the intestines to become more acidic, inhibits the growth of dangerous bacteria, increases intestinal motility, and stimulates immune cells. *Clostridum butyricum,* meanwhile, may produce butyrate, providing substrates for epithelial cells energy, anti-inflammatory activity, and protection for colonocytes from DNA damage.[20,26,38]

In CAC, chronic inflammation cannot be separated from the course of the disease. Inflammatory mediators—pro- and anti-inflammatory cytokines—are playing a big role in this situation. As expected, the administration of probiotics was shown to be effective in suppressing the activity of pro-inflammatory cytokines and elevating the role of anti-inflammatory cytokines. IL-1β is part of the interleukin-1 family. Activated macrophages produce this cytokine, which is further proteolytically digested by caspase 1 to become active. IL-1β promotes cell proliferation, differentiation, and apoptosis while also increasing the expression of proinflammatory factors

such as TNFα, IL-6, IL-8, IL-17, COX-2, and prostaglandin E2 (PGE2). [39] In a mouse model of CAC, IL-6 can enhance epithelial cell proliferation and the growth of tumor-initiating cells, and inhibiting IL-6 is an important strategy to limit carcinogenesis in CAC. [40,41] That is why those factors were consistently attenuated by probiotic administration. Both COX-2 and COX-2-derived PGE2 have been shown to activate CXCR2 ligands in the intestinal mucosa and malignancies.[42] Treatment with Bifico, which consisted of *B. longum, L. acidophilus,* and *E. faecalis*, significantly reduced COX-2 expression in colon tissues and PGE2 levels in serum, and it might be that COX-2 is one of the mediators of the course of the disease on the CXCR2 signaling axis.[21] Regulating inducible nitric oxide synthase (iNOS), which generates nitric oxide (NO), is critical for controlling inflammation in intestinal epithelial cells. Pro-inflammatory cytokines IL1-α, IFN-γ, and TNF-α increased iNOS gene expression and protein synthesis. However, anti-inflammatory cytokines like IL-4 and IL-13 decrease both mRNA expression and protein production, implying that inflammatory cytokines have a role in controlling iNOS production.[43,44]

NF-κB, a transcription factor, plays a key role in cancer by regulating cell proliferation, differentiation, apoptosis, migration, and angiogenesis. Overactivation of the NFκB pathway is a key characteristic of CRC, regulating inflammation, cell proliferation, and apoptosis. NFκB can activate genes that regulate cell death, including anti-apoptotic BCL-2, BCL-xL, and pro-apoptotic Bax. Liu et al. discovered that transcription of BCL-2 was reduced in the probiotic group while transcription of Bax increased. Probiotics may enhance CRC apoptosis by inhibiting the NF-κB signaling pathway, resulting in decreased Bcl-2 and increased Bax levels.[26,45,46] Furthermore, probiotic administration boosted the expression of IκB, a nuclear IκB family protein activated by the transforming growth factor (TGF)-β signaling pathway. The canonical pathway involves NFκB/Rel protein binding to IκB and inhibiting it. TGF stimulates the IkB kinase (IKK), which phosphorylates IκB. When IκB is phosphorylated, it ubiquitinates and releases the NF-κB/Rel complex. The released NFκB regulates cytokine expression, including TNF-α, IL-6, IL-1, and IFN-γ, and contributes to cancer progression.[20,47,48]

Probiotics raise TGF-β levels, which interact with TGF-βR1 and activate Smad2 and Smad3. Smad2/3, together with Smad4, constitute a Smad complex. The Smad complex enhanced p21, a CDKI, while decreasing c-Myc expression. p21 is an important component in the cell cycle because it attaches to the cyclin-CDK complex and controls the transition of the G1/S cell cycle. That process inhibits CDK activity and the cell cycle. Meanwhile, c-Myc functions as a positive regulator. Probiotics may prevent cancer growth by boosting TGF-β levels, leading to increased p21 expression and decreased c-Myc expression.[20,49,50] The activation of the tumor suppressor gene, p53, causes the transcriptional upregulation of p21, which

results in the arrest of the cell cycle in the late G1 phase as well as apoptosis by regulating Bax and BCL-2 expression. Even the inactive or dead nanosized probiotics reduced CAC development more than the live ones, causing greater cell cycle arrest and cancer cell apoptosis via the p53-dependent pathway.[25,51]

Another pathway was proven from the studies with gene-modified mice in developing colitis into a cancer.[22] Adult mice lacking functioning mammalian histidine decarboxylase (HDC), the enzyme that converts L-histidine to histamine, were more vulnerable to CAC due to the role of HDC in oncogenesis.[52,53] When it came to a lack of endogenous histamine, AOM/DSS therapy significantly boosted the number of IL-6 and CD11bþ Gr-1þ, the immature myeloid cells (IMC). Probiotic *L. reuteri* has the capability to produce histamine. It has been correlated with decreased production of human TNF.[54] *L. reuteri* decreased intestinal inflammation by targeting the histamine H2 receptor (H2R). H2R, located in the human intestinal epithelium, is thought to play a significant role in mammalian cell responses to histamine generated by luminal gut bacteria.[55,56] In addition, another pathway is the NLRP3 inflammasome. NLRP3 inflammasomes are complexes of cytoplasmic multiproteins that may mediate the maturation of pro-inflammatory cytokines, such as IL-1β.[57] Improper activation of the NLRP3 inflammasome may induce inflammatory disease. A study by Chung et al.[27] showed that pretreatment with probiotic *E. faecalis* ameliorates the IL-1β-dependent inflammations that occured in CAC progression.

The AOM/DSS administration causes weight loss and bloody diarrhea, followed by the formation of numerous colon cancers as manifestations. The exact location of the tumor along the colon length is based on the strain of mouse.[58] Mice that are treated with AOM/DSS develop tumors that are histologically similar to CAC in humans and are usually located in the medial or distal colon. They are well-known to be tubular adenomas or moderately differentiated tubular adenocarcinomas. There may be invasion into the submucosa, muscle, and even serous membranes. AOM-only treatment frequently results in adenomas, whereas AOM/DSS administration may initiate a full course of colon oncogenesis, extending from the initial proliferation of crypts to the eventual development of colon cancer.[52] The results showed that probiotic administration was considered to attenuate the severity of CAC progression and the progression itself. This study has not explored the changes in gut microbiota in animal models with CAC progression after being treated with probiotics. The effects of combining probiotics with others, such as prebiotics, were also not well specified. The next review can consider this to gain further knowledge regarding the benefits of probiotics in the development of colorectal cancer, which originates from an inflammatory process (colitis).

CONCLUSION

We considered this study to be a refresher in exploring information regarding the anti-inflammatory benefits of probiotics against CAC among the many studies about probiotics associated with sporadic CRC. We concluded that the administration of probiotics provided promising benefits related to their anti-inflammatory effects in the development of CAC at the animal research level. This can be a strategic basis for further research considering the same benefits that were provided by probiotic treatment in human CAC cases.

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