Review

GASTROENTEROLOGY

Microbiome modulation in the prevention and management of colorectal cancer: a systematic review of clinical interventions

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Abstract

Introduction. The role of probiotics/prebiotics in modulating the procarcinogenic effects of microbiota have been studied with inconclusive results. This systematic review aimed to identify the role of several studied interventions on the gut microbiota modulation in humans for the prevention and management of colorectal cancer (CRC).

Methods. We conducted a systematic search using PubMed and Cochrane Central electronic databases, identifying clinical studies published within the last 20 years. We performed a qualitative analysis of eligible studies included in our review on each of the 4 investigated topics: CRC potential biomarkers, dietary interventions, probiotic administration in non-surgical and surgical patients, respectively.

Results. A total of 54 studies involving healthy volunteers, in addition to colorectal adenoma and CRC patients were included in our qualitative synthesis. We were able to identify bacterial signatures of CRC including *Fusobacterium nucleatum* and *Clostridium butyricum*. Moreover, dietary supplementation with oligosaccharides or fibers increased short chain fatty acid-producing bacteria levels, thus inhibiting tumorigenesis. Furthermore, we have confirmed that *Lactobacilli* and *Bifidobacterium* intake modulates gut microbiota towards tumor suppression. We have also showed that probiotic intake around colectomy significantly reduces complications.

Conclusions. Bacterial metabolism is strongly linked with colonic carcinogenesis and influenced by diet. Probiotics and prebiotics can act as microbiota modulators, suppressing epithelial proliferation and reversing DNA toxicity. As adjuvants to surgery or chemotherapy, *Lactobacilli* and *Bifidobacteria* decrease complications. Improved outcomes in CRC patients can possibly be achieved through future research directed towards the benefits of bacterial agents as tumor suppressors or as treatment of oncological therapy resistance.

Keywords: gut microbiota, colonic carcinogenesis, bacterial signature, probiotics, prebiotics

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Introduction

Colorectal cancer (CRC) is the third most diagnosed form of cancer (11% of all cancers) and the second in terms of cancer-related mortality, according to 2018 global data. Although several efforts and screening programs have been put into practice for early detection of CRC through colonoscopy or fecal testing, its incidence remains rising in developed countries due to lifestyle and dietary factors [1].

The term microbiome, coined by Lederberg a couple of decades ago, refers to the genetic material of the microbiota, but the terms have been used interchangeably [2]. The human microbiota is made up of 10-100 trillion microbial cells, of which the gut microbes are the most numerous. They mostly belong to the *Bacteroidetes* and *Firmicutes phyla.*

Gut dysbiosis is constant in CRC and is correlated with diet. It has been demonstrated that gut bacteria act at different points in the health-adenoma-carcinoma continuum, mostly via the mechanism of chronic inflammation produced by their metabolites or virulence factors [3]. Although the gut microbiome varies greatly from one individual to another, as well as during the lifetime of an individual, a certain "bacterial signature" has been associated with stages of the gradual development on the healthy tissue – adenoma – polyp – CRC axis [4]. The inflammatory environment associated with high-protein, high-fat, and low-fiber diets, mediated by cytokines and reactive oxygen species, leads to dysbiosis. Subsequent decreased levels of butyrate-producing bacteria, along with other microbial imbalances, lead to the activation of oncogenic pathways and gradual progression through the stages of CRC carcinogenesis [5].

The term "bacterial driver-passenger model" was proposed, where "driver" bacteria such as enterotoxigenic *Bacteroides fragilis* (ETBF) are involved in the initial stages of carcinogenesis (mutations in the APC gene), while "passenger" bacteria, opportunistic pathogens such as *Fusobacterium* and *Streptococcus spp.* can subsequently suppress or support tumorigenesis [6]. Thus, "passenger" bacteria colonization is thought to appear as a result of local changes inflicted by driver mutations. This explains the heterogeneity of microbiome samples at different stages of disease and the role of driver bacteria as markers, with a potential benefit in early prevention.

In general, the healthy microbiota is abundant in bacteria that belong to the phylae *Bacteroidetes* and *Firmicutes*. They decrease gradually with progression to polyp and adenoma, while in the CRC stage, the abundance of *Proteobacteria* rises [7]. Thus, the proinflammatory state associates with a reduction of beneficial bacteria from genera such as *Ruminococcus, Bifidobacterium, Lactobacillus,* and *Clostridiales* [7]. Several studies reported several main species constituting the bacterial signature of CRC patients including *Fusobacterium nucleatum, Escherichia coli, Bacteroides fragilis,* and *Entreococcus faecalis* [8,9]. Other certain strains are also associated with specific points in the progression towards CRC, for example, *Oscillospira* (*Ruminococcaceae*), is depleted in the transition from adenoma to stage 0 CRC, whereas *Haemophilus* is depleted later, towards early-stage CRC [4]. Table I outlines the role and mechanism of the main bacterial species demonstrated to be involved in the process of colorectal tumorigenesis.

Table I. Role and mechanism of main bacterial species involved in colorectal tumorigenesis.

Escherichia coli [10,11]	
	• pks+ E. coli produces the toxin called colibactin • colibactin produces DNA strand breaks in colonic epithelial cells, • enhances inflammation and the production of reactive oxygen species (ROS) in early-stage CRC
Fusobacterium nucleatum [4,12–14]	

	• found in highly dysplastic adenomas at the earliest, suggesting its "passenger" role (tumor promoter)
	• acts through two virulence factors:
	  ◦ FadA drives cellular proliferation: it binds to E-cadherin, invading epithelial cells to activate the wnt/β-catenin pathway, producing an NF-κB inflammatory response, release of cytokines (IL-6, IL-8) and oncogene expression (Myc, Cyclin D1)
	  ◦ Fap2 leads to immune evasion though inhibition of NK cells
	• reduces chemosensitivity in CRC cells by inhibiting apoptosis
	• persistent from primary through metastatic stages
	• colonization associated with shorter survival

Bacteroides fragilis [12,15]	
	• produces the ETBF toxin, mediating inflammation through a TH-17/IL-17 response, which induces colitis and promotes distal colon tumorigenesis • ETBF toxin can negatively regulate E-cadherin, activating the wnt/β-catenin pathway in a similar manner to *F. nucleatum* • converts host primary bile acids into secondary bile acids • facilitates local dysbiosis • associated with early neoplastic changes (adenoma and serrated polyps)
Enterococcus faecalis [9,12]	
	• produces genotoxic peroxide and direct epithelial cell DNA damage • associated with expression of metastasis genes

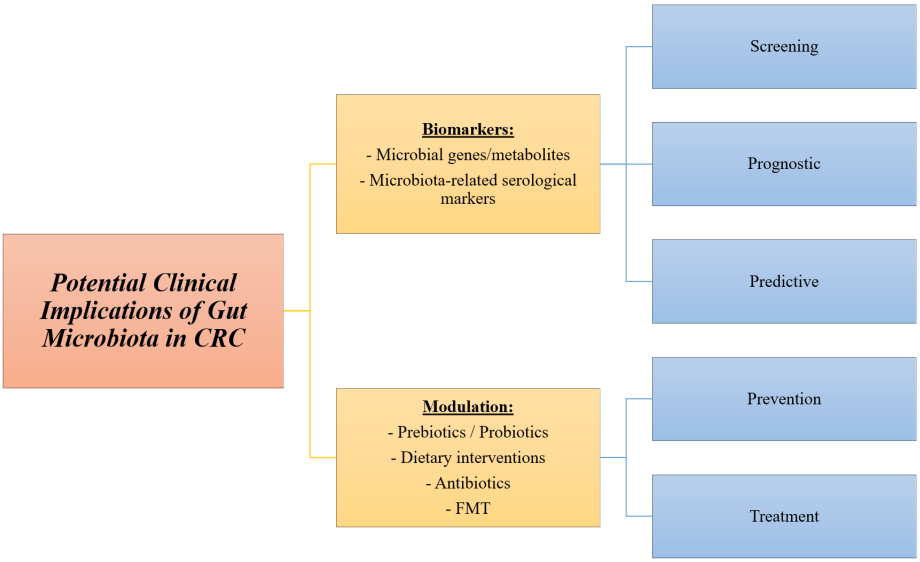

Figure 1. Potential clinical implications of gut microbiota in colorectal cancer.
CRC: colorectal cancer; FMT: fecal microbiota transplantation.

Lately, an increased interest in microbiome modulation in the prevention and management of colorectal cancer has led to multiple publications in this field. Figure 1 summarizes the potential clinical applications of gut microbiota in CRC [16]. Several bacterial species, such as *F. nucleatum*, have been proposed as prognostic biomarkers with potential applications in prevention [17]. Others have been studied for their potential to modulate microbial composition, such as *C. butyricum*, shown to favor colonization with butyrate-producing bacteria [18]. Nevertheless, results of published studies have been inconclusive with conflicting findings. Therefore, we conducted a comprehensive systematic review aiming to identify human-based evidence regarding the role of gut microbiota modulation interventions in the prevention and management of CRC.

Methods

This systematic review was written according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist 2020 [19].

Data Sources and Search Strategy

A literature search was conducted on the 30th of January 2022 using several electronic databases including PubMed/MEDLINE database and the Cochrane CENTRAL registry, using search strategies to elicit records of clinical trials or observational studies related to the use of pro/pre/synbiotics at any point in the management of colorectal cancer, as well as to the involvement of microbiota in the genesis and development of CRC. Results were filtered by type (clinical study/trial) and year (1991-2022). The following search string was used in PubMed: ("microbio*" [All Fields] AND ("colorectal neoplasms" [MeSH Terms] OR ("colorectal" [All Fields] AND "neoplasms" [All Fields]) OR "colorectal neoplasms" [All Fields] OR ("colorectal" [All Fields] AND "cancer" [All Fields]) OR "colorectal cancer" [All Fields])) AND ((clinicalstudy [Filter] OR clinicaltrial [Filter] OR clinicaltrialprotocol [Filter] OR clinicaltrialphasei [Filter] OR clinicaltrialphaseii [Filter] OR clinicaltrialphaseiii [Filter] OR controlledclinicaltrial [Filter] OR preprint [Filter] OR randomizedcontrolledtrial [Filter]) AND (cancer[Filter]) AND (1991:2022[pdat])), while a similar search was conducted in Cochrane Library. Subsequently, screening of the titles and abstracts for relevant articles, followed by full-text evaluation of selected articles based on the inclusion and exclusion criteria was performed by two investigators independently (T.D.F. and A.I.). Moreover, data extraction of eligible articles was performed by one investigator (T.D.F.) and reviewed by another (A.I.). In case of any discrepancies, a mutual consensus was reached through discussion.

From each full-text included in our review, we identified the following data: study type (randomized controlled trials, crossover trials, or observational studies), number of participants, intervention type and duration, biological products analyzed (fecal samples, serum, colon biopsies), and outcome measurements. Extracted data was summarized in tables and final data was collated and reported in the text of the manuscript. Clinical trials were grouped into 3 categories (dietary/prebiotic intervention, probiotic intervention, and perioperative microbiome modulation interventions) and qualitative analysis was performed. An additional group of observational studies were reported separately, as they identified potential CRC biological markers, either microbial or metabolic, supporting the interventions reviewed.

Eligibility Criteria

Inclusion criteria were as follows: (1) Clinical studies conducted on human subjects; (2) Articles published in English language; and (3) Examining the relationship between the gut microbiota and colorectal cancer, test strategies influencing it (e.g., dietary interventions, administration of probiotics), or describing administration benefits of probiotics at any point during the treatment of CRC.

We excluded: (1) Reviews and meta-analyses, retrospective reports; (2) Studies that focused on other gastroenterological cancers, techniques for microbiome sequencing, surgical techniques, studies on patients with hereditary CRC syndromes; and (3) Studies that lacked reports of specific bacterial strains, as well as those where the research question investigated another type of intervention (e.g., chemotherapy, immunotherapy, antibiotic prophylaxis, metabolic effects of dietary interventions).

Results

General results

The PRISMA flow diagram, as outlined in Figure 2, summarizes the performed search strategy. Our initial search yielded 211 records. After the removal of duplicates (n = 36), of entries in languages other than English (n = 9) and of titles unrelated to the topic (n = 18), 148 records were screened. An additional 19 of those were removed after abstract screening due to their topic being related to surgical technique only. Out of 129 reports sought for retrieval, 120 were assessed and 54 were included in the final analysis.

Half of the analyzed studies (n = 27) reported on dietary prebiotic interventions in healthy volunteers, colorectal adenoma (CRA) patients or CRC survivors. Out of the remaining half, most studies (n = 11) tested the beneficial effects of perioperative probiotic supplementation. Fewer studies focused on the effect of probiotic administration in healthy or CRC subjects (n = 8), while the remaining identified studies (n = 7) were of observational design and aimed to identify potential early fecal or sanguine biomarkers of CRC.

Microbial biomarkers of early CRC development

Microbial biomarkers were studied in stool samples, blood, and mucosal biopsies in observational studies with a median of 253 participants. A summary of outcomes is outlined in table II.

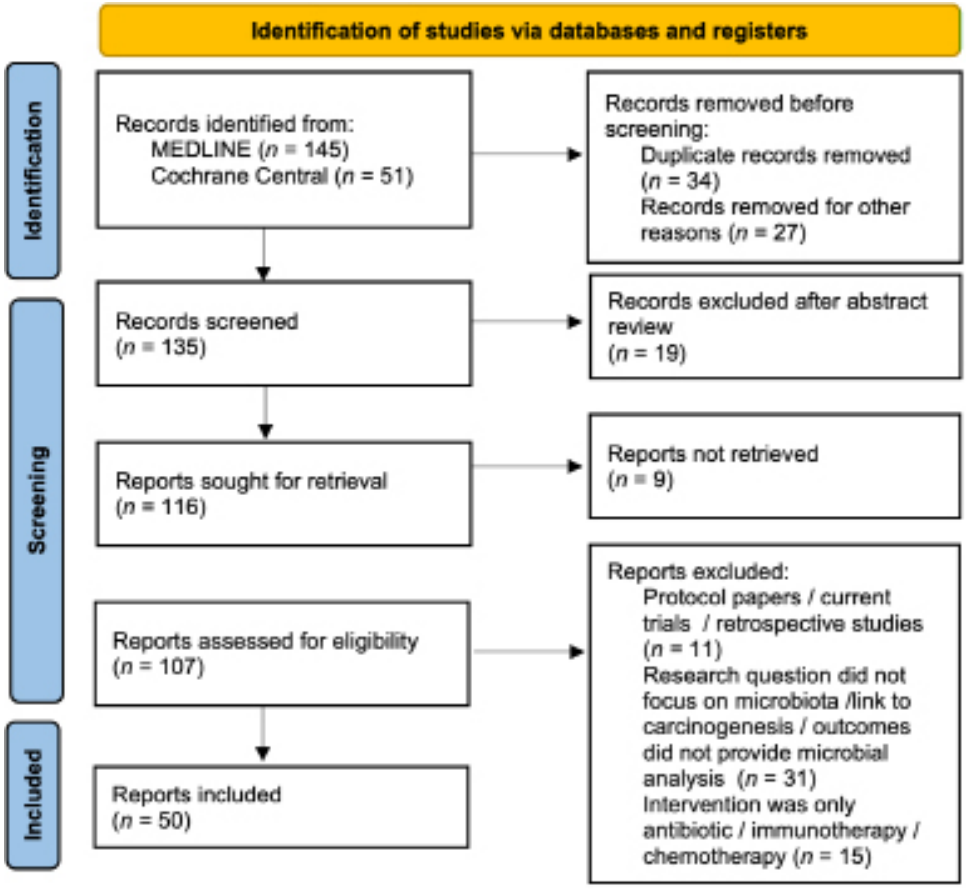

Figure 2. Description of the identification, screening, and inclusion phases using the PRISMA flow diagram.

Table II. Observational studies investigating potential CRC microbial biomarkers.

Author / Year	Intervention group	No. of participants	Method	Main findings
Xie et al., 2017 [20]	healthy controls, CRA[a], and CRC[b] patients.	781	Fecal bacterial DNA extraction	*C. symbiosum* increased gradually from adenoma to early and advanced CRC, and can be considered as a valid predictor of early CRC. Its prognostic value increases in combination with FIT[c].
Niccolai et al., 2021 [23]		45	Colonic mucosal biopsy	Elevated inflammatory markers (Th17, Th2, IL-9), as well as mucosal *Fusobacteria*, *Proteobacteria*, *Fusobacterium*, and *Ruminococcus Prevotella* positively correlate with IL-9 levels.
Kim et al., 2020 [15]		278	Fecal DNA extraction	Metabolomic signatures associate with *Firmicutes phylum*. However, they were not significant for use as biomarkers gut microbiota-metabolome, the association being stronger in female gender.
Zhang et al., 2020 [24]		253	Oral DNA extraction	The probability of pathogen co-abundance (*Fusobacterium, Treponema, Porphyromonas*), characteristic in CRC patients, was significantly higher in adenoma than control group.
Xiao et al., 2021 [22]		83	Circulating DNA analysis	Increased cell-free DNA levels of *Flavobacterium, Eubacterium rectale* and *Ruminococcus torque. Fusobacterium* levels were not found to be different between CRC patients and healthy controls.
Ma et al., 2021 [25]		249	Fecal bacterial DNA	CRC highly predictive model based on SNVs[d] in bacterial genome.
Yang et al., 2021 [21]		1038	Fecal DNA extraction	CRC microbiome different in young vs older patients, functional cellular microbiome-dependent processes support poorer prognosis of young patients.

[a]CRA: colorectal adenoma; [b]CRC: colorectal cancer; [c]FIT: Fecal Immunochemical Test; SNVs[d]: single nucleotide variants.

In fecal samples, Xie at. al. found *Clostridium symbiosum* to be a more sensitive biomarker in the early stages (i.e., transition from healthy control to CRA, and from CRA to CRC) than *Fusobacterium nucleatum* and then either fecal immunochemistry test (FIT) or carcinoembriogenic antigen (CEA). Association of *C. symbiosum* testing was shown to increase sensitivity of FIT and CEA with up to 24% and 27%, respectively [20]. Other bacteria in the signature of CRC belonged to 4 species in the *Firmicutes phylum* (*Clostridium, Dehalobacterium, Ruminococcus,* and *Oscillospira*) and were found to correlate with several metabolic pathways, such as the endocannabinoid, secondary bile acid metabolism, or polyunsaturated fatty acids (PUFA) pathways [15]. Patient age-related differences have been described by Yang et al., a functional analysis revealing that young CRC-specific microbiota (*Fusobacterium*, *Flavonifractor* and *Odoribacter* genera) is also more supportive of proliferative and invasive cellular processes [21].

Cell-free DNA was significantly altered in CRC patients and was considered as a potential biomarker for CRC development. Xiao et al. found circulating DNA of 28 bacterial species that could differentiate CRC/CRA from healthy controls [22]. A distinctive profile was also found in colonic mucosal samples, dominated by *Fusobacterium* and *Ruminococcus*. Niccolai et al. explored the microbiota-local immune system relationship in CRC patients and reported a positive correlation between *Prevotella spp* and IL-5, known to have an anti-inflammatory role [23].

An alternative non-invasive potentially predictive method analyzed the oral microbiota and constructed a model that could differentiate between CRC/CRA and healthy control samples. *Fusobacterium* constantly co-occurred with a group of other specific bacteria and was found to be higher in CRA than in CRC patients, while *Streptococcus* was decreased in CRC [24].

Since the genetic content of closely related bacterial strains can differ significantly, Ma et al. proposed a more specific method of defining the bacterial signatures in CRC, made up of single nucleotide variants (SNVs) and shown to have high predictive accuracy. The identified CRC-characteristic SNVs were in the genomes of *Eubacterium rectale* and *Faecalibacterium prausnitzii*, but interestingly, these species were not more abundant in the patients' microbiota [25].

Microbiome modulation dietary interventions

We reviewed 26 clinical trials that tested dietary changes or supplements and 1 that examined the effect of aspirin, as summarized in table III. They included healthy volunteers, high-risk volunteers (e.g., familial risk factors or high red meat diet), and CRC patients not in the perioperative period. The interventions lasted for a median time of 6 weeks (mean = 14.6 weeks) and included a median number of 29 participants (mean = 60).

Table III. Trials investigating dietary/therapeutic interventions for microbiome modulation.

Author / Year	Intervention	Intervention group	No. of participants	Intervention duration	Main findings
Hylla et al., 1998 [26]	Resistant starch	Healthy volunteers	12	8 weeks	Decreased bacterial β-glucosidase activity, decrease in total and secondary bile acids.
Abell et al., 2008 [27]	Resistant starch	Healthy volunteers	46	14 weeks	Fecal butyrate and total SCFAs[a] significantly increased.
Humphreys et al., 2014 [28]	Resistant starch	Healthy volunteers with high red meat diet	23	8 weeks	Increased fecal butyrate, normalization of colonic mucosal cells microRNAs, but not microRNA-21
Bouhnik et al., 1996 [30]	Fructo-oligosaccharides	Healthy volunteers	20	36 days	Significant increase in *Bifidobacteria*.
Welters et al., 2002 [31]	Inulin	CRC[b] survivors	20	3 weeks	Increased butyrate, decreased *B. fragilis* in fecal samples and biopsies.
Bouhnik et al., 2004 [29]	Lactulose	Healthy volunteers	16	6 weeks	Increased fecal bifidobacterial counts.
Sheflin et al., 2015 [32]	Rice bran	Healthy volunteers	7	4 weeks	Increase in *Bifidobacterium* and *Ruminococcus.*
Brown et al., 2017 [33]	Rice bran	CRC survivors	19	4 weeks	Increased activity of fatty acid, leucine/ valine and vitamin B6 metabolic pathways.
So et al., 2021 [34]	Rice bran	High risk volunteers	49	24 weeks	Increased *Firmicutes*/ *Bacteroidetes* ratio, increased *Lactobacilli*, and *Bifidobacteria*.
Windey et al., 2015 [35]	Wheat bran extract	Healthy volunteers	18	6 weeks	Increase *Bifidobacterium*, but no change in fecal water genotoxicity (CRC risk).
Sheflin et al., 2017 [39]	heat-SRB or NBP	CRC survivors	29	4 weeks	SRB[d] or NBP[e] increased total dietary fiber intake similarly. SRB increased SCFAs in stool at 14 days but not at 28 days.
Baxter et al., 2018 [38]	Navy bean extract	CRC survivors	18	4 weeks	Modulation of potentially oncogenic pathways: glutathione, fatty acid etc. increase in fecal enterolactone and salicylate.
Lampe et al., 2019 [43]	Flaxseed lignan	Healthy volunteers	42	60 days	No change in fecal microbiome composition, high ENL[f] correlates with NF-κb inhibition.
McCann et al., 2021 [42]	Flaxseed lignan	Healthy women	252	6 weeks	Increased enterolignan production.
González-Sarrías et al., 2018 [45]	Pomegranate extract	CRC surgical pts	57	5-34 days	Decrease in plasmatic lipopolysaccharide-binding protein levels (decrease in metabolic endotoxemia).
Russell et al., 2011 [36]	HPLC vs. HPMC	Obese males	17	8 weeks	HPLC[g] diet leads to a higher increase of carcinogenic nitrosamine compounds that HPMC[h] diet.
Molan et al., 2014 [46]	First Leaf (blackcurrant extract, lactoferrin, lutein) and Cassis Anthomix 30	Healthy volunteers	30	6 weeks	Decreases beta-glucuronidase and fecal pH; increased *Lactobacilli*.
Djuric et al., 2018 [51]	Mediterranean vs Healthy Eating diet	High risk volunteers	88	6 months	No change in colonic mucosal microbiota; baseline association with serum carotenoid concentrations
Fruge et al., 2021 [50]	Green-leaf vegetables	High risk volunteers	50	12 weeks	Decrease in DNA damage markers (8-ohdg); no significant changes in microbiota.
Watson et al., 2018 [41]	Omega-3 polyunsaturated fatty acids	Healthy volunteers	22	8 weeks	Increase in SCFA-producing bacteria: *Bifidobacterium, Roseburia, Lactobacillus.*

Table III. Trials investigating dietary/therapeutic interventions for microbiome modulation (continuation).

Author / Year	Intervention	Intervention group	No. of participants	Intervention duration	Main findings
White et al., 2019 [40]	Fish oil (omega-3 polyunsaturated fatty acids)	Patients with history of CRA[c]	141	6 months	Fish oil reduced PGE2[i] levels only in pts not taking NSAIDs[j] or aspirin
Pearson et al., 2019 [49]	Ursodeodycholic acid	CRA patients	401	3 years	Lower adenoma recurrence associated with *Faecalibacterium prausnitzii* overrepresentation - sex-specific effects to men
Aslam et al., 2020 [47]	Aquamin (calcium, magnesium, other minerals)	Healthy volunteers	30	90 days	Decreased *Bacteroidetes*, *Firmicutes*; decrease in bile acid levels; increase in fecal SCFA.
Prizment et al., 2020 [48]	Aspirin 325 mg/ day	Healthy volunteers	50	6 weeks	Short-term increases in *Akkermansia, Prevotella* and *Ruminococcaceae*, as well as relative decreases in *Parabacteroides, Bacteroides*.
Shao et al., 2021 [44]	herbal formula Xiao-Chai-Hu-Tang	CRC patients	72	6 weeks	Decreased *Parabacteroides*, *Blautia* and *Ruminococcaceae*, improvement in depression symptoms.
Phipps et al., 2021 [52]	oral vs intravenous iron	anemic CRC patients	40	at least 2 weeks	On and off-tumor microbiota supports anti-inflammatory and cancer protective metabolite production in intravenous iron administration.
Yoon et al., 2021 [37]	calcium and inulin	Healthy volunteers	12	16 weeks	No difference in microbiota or SCFA concentration in inulin and calcium vs inulin or calcium alone groups after crossover administration.

SCFAs[a]: Short-chain fatty acids; [b]CRC: colorectal cancer; [c]CRA: colorectal adenoma; [d]SRB: heat-stabilized rice bran; [e]NBP: navy bean protein; [f]ENL: enterolactone; [g]HPLC: High-protein low-carbohydrate diet; [h]HPMC: high-protein moderate carbohydrate diet; [i]PGE2: Prostaglandin E2[j]: NSAIDs: Nonsteroidal anti-inflammatory drugs.
*High-risk volunteers generally defined as obese and with a high red-meat consumption

Most interventions tested the effects of fermentable non-digestible carbohydrates, such as resistant starch, fructo-oligosaccharides, or other inulin-type prebiotics on modulating microbiota and fecal metabolites involved in tumorigenesis. Resistant starches are a substrate for bacterial fermentation and result in short-chain fatty acid (SCFA) production. Similarly, rice bran is a source of fiber whose fermentation produces SCFAs: acetate, propionate, and butyrate, the latter of which is able to inhibit growth and exert anti-carcinogenic effects on colonic mucosa through their antioxidative effects and suppression of NF-κB signaling. Several studies (n = 8) with dietary supplementation of resistant starch, stabilized rice bran, wheat bran, fructo-oligosaccharides, low doses of lactulose, or inulin demonstrated consistently increased levels in fecal butyrate, *Bifidobacteria,* and decreased levels of *B. fragilis*. These findings were reported in healthy volunteers, as well as CRC survivors. Effects were significant in interventions as short as 4 weeks, but short-lived, for as little as 12 days post-intervention [26–35]. As Russell et al. demonstrated, carbohydrates confer a protective effect in a high red-meat diet [36]. At a molecular level, high butyrate production obtained by rice bran supplementation was shown to modulate favorably some of the microRNA dysregulated in CRC, namely the miR17-92 cluster, but not miR21. Dysregulation of mRNA was induced by a high red-meat diet in healthy volunteers, and reversed by butyrate due to its inhibition of histone deacetylase [28]. One crossover pilot study examining additional calcium or inulin supplementation versus both, to increase inulin absorption, showed no differences in SCFA concentration, but opposite changes in calcium or inulin supplementation versus their combination, which increases *Bacteroidetes* [37].

Navy beans, through their content of phytochemical substances and non-digestible fiber, seem to have a similar effect on fiber intake and a supplementary effect of increasing other fecal metabolites reported to have antitumoral effects (e.g. enterolactone, salicylate, piperidine), but no sustained benefit in SCFAs quantity nor modulation of microbiota [38,39].

The anti-inflammatory effects of PUFAs were demonstrated both in healthy volunteers and in CRA patients, where they increased SCFAs and the associated SCFA-producing microbiota reversibly, and reduced levels of fecal prostaglandin E2 (PGE2), respectively [40,41].

Other supplements proposed as dietary risk reduction strategies were enterolignans, phenolic plant compounds found in flaxseed, and metabolized in the colon to enterolactone and enterodiol. Enterolactone secretor profile was associated with microbial signatures and inhibition of the activation of NF-κB inflammatory pathway by lipopolysaccharide (LPS). Flaxseed supplementation did increase enterolignan levels, however, as McCann et al. demonstrated, there is high ethnic variation among microbiota associated with its production [42,43]. A Chinese study proposes the Xiao-Chai-Hu-Tang (XCHT) supplement as a way to inhibit the same pathway, a mechanism observed in vivo, in addition to its clinically proven effects as an antidepressant and gut microbiome modulator, reducing *Parabacteroides*, *Blautia* and *Ruminococcaceae* [44]. A different strategy to inhibit the lipopolysaccharide pathway activation was achieved by Gonzalez-Sarrias et al. in newly diagnosed CRC patients, where consumption of pomegranate extract decreased levels of lipopolysaccharide-binding protein, a marker of endotoxemia [45].

One study which investigated the microbial modulator and antioxidant effect of a blackcurrant-based extract demonstrated that it increased *Lactobacilli* and *Bifidobacteria*, decreased *Clostridium* and *Bacteroides* species, and consequently the levels of beta-glucuronidase, the high levels of which correlate with CRC [46]. Other substances which have shown promise in small studies are a mineral supplement that decreased tumorigenic bacteria and increased SCFAs in healthy volunteers, and aspirin in a daily dose of 325 mg, which produced changes in the several bacterial taxa consistent with the reduction of CRC risk [47,48]. A longer study on adenoma recurrence in relation to ursodeoxycholic (UDCA) supplementation demonstrated co-occurrence of beneficial bacteria, among which *F. prausnitzii*, and was not able to explain the probable adenoma reduction in men, and not in women [49].

Dietary changes focused on increase of vegetable consumption or Mediterranean diet, in high-risk volunteers, did not produce alterations of microbiota, although they showed an effect on reducing markers of DNA toxicity [50,51].

In anemic patients, the microbial population can be modulated by the choice of iron administration. It seems that the off-tumor microbial and enzymatic landscape favors the intravenous administration route, as it leads to an increase in SCFA-producing bacteria, as well as anti-inflammatory and anti-tumor enzymes, compared with oral iron supplementation [52].

Probiotic interventions in healthy volunteers and non-surgical CRC patients

Probiotic administration in healthy volunteers, CRC patients, and chemotherapy patients was tested for a median period of 8 weeks (mean = 8.1 weeks), on groups averaging 58.5 patients (median = 30 patients). Table IV summarizes the trials assessing dietary or therapeutic interventions for microbiome modulation.

Hatakka et al. reported that after supplementation with *L. rhamnosus* and *P. freudenreichii* in healthy volunteers, a decrease in fecal β-glucosidase activity was associated with an increase in *Propionibacteria* [53]. Another study demonstrated a significant decrease in enterotoxigenic *Bacteroides fragilis* after 8 weeks of *Bifidobacterium longum* supplemented yoghurt [54]. Association of prebiotic (resistant starch) to *Bifidobacterium* supplementation led to specific microbiota changes, significantly different from either prebiotic or probiotic administration, with an increase in *Lachnospiraceae*, among others. These were reflected at a molecular level (decrease in methylation markers); however, a causal link could not be established. Moreover, this study did not see any changes in SCFA levels nor epithelial proliferation [55].

Similar microbiome changes were reported after probiotic/symbiotic administration for as little as 5 days, namely a reduction in *Fusobacterium* and *Peptostreptococcus*, and an increase in *Bifidobacteria, Lactobacili,* and butyrate-producing species in general [56-58]. Rafter et al. demonstrated that synbiotic intervention decreased DNA damage and proliferation in colonic epithelial cells, evaluated in polypectomized patients versus CRC patients [58].

In patients undergoing chemotherapy (irinotecan, 5-fluorouracil, or capecitabine), *Lactobacilli* or combinations with *Bifidobacteria* were reported in 2 studies to significantly decrease diarrhea, compared to either placebo or to data existent in literature [59,60]. The multicentric RCT conducted by Mego et al. was terminated due to low accrual at around 1/5 of target enrollment. However, results point toward a reduction by 20% in the incidence of irinotecan-associated diarrhea under administration of a combination composed of 10 probiotic bacterial species [60].

Table IV. Probiotic interventions in healthy volunteers and non-surgical CRC patients.

Study	Treatment intervention	Patient group	No. of patients	Intervention duration	Main findings
Hatakka et al., 2008 [53]	*L. rhamnosus, Propionibacterium freudenreichii ssp shermanii*	healthy volunteers	38	8 weeks	Increased fecal counts of *lactobacilli* and *propionibacteria*, decrease in activity of β-glucosidase, but not β-glucuronidase, urease.
Worthley et al., 2009 [55]	*Bifidobacterium lactis* and resistant starch		17	4 weeks	Synbiotic combination alters microbiota significantly compared to resistant starch or *B. lactis* alone, SCFA[a] concentration or epithelial proliferation were not affected
Odamaki et al., 2012 [54]	*Bifidobacterium longum*		32	8 weeks	Significant decrease in enterotoxigenic *Bacteroides fragilis.*
Rafter et al., 2007 [58]	*Lactobacillus rhamnosus, Bifidobacterium lactis* oligofructose, inulin	CRC[b] patients and polypectomized patients	43	12 weeks	Increased *Bifidobacterium* and *Lactobacillus*, decreased *Clostridium perfringens*, decreased genotoxicity of fecal water and epithelial DNA damage and proliferation in polipectomized patients.
Gao et al., 2015 [56]	*Bifidobacterium longum, Lactobacillus acidophilus* and *Enterococcus faecalis*	CRC patients	49	5 days	Reduction in *Fusobacterium*, *Peptostreptococcus*, increase of *Enterococcus* and *Proteobacteria* in the mucosa-adherent microbiota.
Hibberd et al., 2017 [57]	*Bifidobacterium lactis, Lactobacillus acidophilus*, inulin		15	28 days	Increased butyrate-producing bacteria, decreased *Fusobacterium*.
Mego et al., 2015 [60]	*Bifidobacterium breve, B, bifidum, B. longum, Lactobacillus rhamnosus, L. acidophilus, L. casei, L. plantarum, L. brevis, Streptococcus thermopilus, B. infantis*	CRC patients under treatment with irinotecan	46	12 weeks	Overall reduction of diarrhea.
Ghidini et al., 2021 [59]	*Lactobacillus kefiri*	CRC patients under treatment with 5FU[c] / capecitabine)	76	16 weeks	Significantly reduced severe diarrhea in chemotherapy patients compared to data in literature.

[a]SCFA: short-chain fatty acids; [b]CRC: colorectal cancer; [c]5FU: Fluorouracil.

Perioperative probiotic interventions

Probiotics were administered in patients being operated for CRC for periods between 3 days and 1 year (median = 60 days, mean = 65.8 days). Perioperative probiotic interventions were either pre-operative, post-operative, or both. All interventions used either *Lactobacillus* or *Bifidobacterium* strains, or both, some with additional *Enterococcus faecalis*, *Streptococcus thermophilus,* or *Saccharomyces boulardii*. Table V outlines the studied perioperative microbiome modulation interventions.

The main mechanism through which probiotics influenced postoperative complications favorably was the reduction of transmucosal bacterial permeation, as demonstrated by Liu et al., who verified that *Lactobacilli* and *Bifidobacterium* for 16 days pre and post-operatively decreased the concentrations of zonulin, a regulator of intestinal permeability [61]. Thus, all probiotic combinations tested in the studies we identified had the effect of decreasing bacterial complications, reducing postoperative infectious and mechanical complications (i.e. ileus), reducing hospitalization time, and generally improving postoperative quality of life, especially for tumors with rectal localization [61-68]. Pro-inflammatory cytokines (TNF-α, IL-10, IL-12 etc.) were shown to be lowered by 6 months of *Lactobacillus* and *Bifidobacterium* intake starting 4 weeks post-surgery, without changes in diarrhea incidence [69].

Xie et al. demonstrated that microbiota modulation (increase in *Bifidobacterium* and decrease in *Bacteroides*) can also be achieved by 1 week of oligosaccharide prebiotic administration [70].

One study did not find any significant differences in bowel function scales with *L. plantarum* administration for 3 weeks in ileostomy reversal patients [71].

Table V. Perioperative microbiome modulation interventions.

Study	Intervention	Patient group	No. of patients	Intervention. duration	Main findings
Gianotti et al., 2010 [65]	*Lactobacillus johnsonii, Bifidobacterium longum*	CRC[a] surgical patients	31	5 days	*Lactobacillus johnsonii* colonized the colonic mucosa and modulated local immunity favorably.
Liu et al., 2011 [67]	*Lactobacillus plantarum, L. acidophilus, Bifidobacterium longum*		100	16 days	Reduced transmucosal permeation and bacterial translocation, decreased blood enteropathogenic bacteria, and increased fecal bacterial variety, as well as lowered postoperative complications.
Zhang et al., 2012 [66]	*B longum, L acidophilus* and *Enterococcus faecalis*		60	3 days	Inhibited overgrowth of *E coli*, restricted bacterial translocation, and decreased postoperative complications.
Liu et al., 2013 [61]	*Lactobacillus plantarum, L. acidophilus, Bifidobacterium longum*		150	17 days	Decreased bacterial translocation and perioperative infectious complications, decreased serum zonulin concentration.
Lee et al., 2014 [62]	*L. rhamnosus, L. acidophilus*		60	12 weeks	Improved quality of life (FACT scores).
Kotzampassi et al., 2015 [63]	*Lactobacillus acidophilus, L. plantarum, Bifidobacterium lactis* and *Saccharomyces boulardii*		114	16 days	Reduced bacterial translocation, decreased blood enteropathogenic bacteria and increased fecal bacterial variety, lower postoperative complications, and hospitalization time.
Bajramagic et al., 2019 [64]	*Lactobacillus acidophilus, L. casei, L. plantarum, L. rhamnosus, Bifidobacterium lactis, B. bifidum, B. breve, Streptococcus thermophilus*		78	1 year	Lower incidence of postoperative complications, statistically significant for ileus.
Polakowski et al., 2019 [68]	*Lactobacillus acidophilus, L. Rhamnosus, L. casei, Bifidobacterium lactis*		73	7 days preop	Reduction of inflammatory state (IL[d]-6 and CRP[b]), lower infectious complications, hospitalization time, and use of antibiotics.
Xie et al., 2019 [70]	Fructooligosaccharide, xylooligosaccharide, polydextrose, resistant dextrin		140	7 days	Increase in Bifidobacterium and Enterococcus, decrease in Bacteroides.
Zaharuddin et al., 2019 [69]	*Lactobacillus acidophilus, L. lactis, L. casei, Bifidobacterium longum, B. bifidum, B. infantis*	CRC postsurgical patients	52	6 months	Reduction in the level of pro-inflammatory cytokine, TNF-α, IL-6, IL-10, IL-12, IL-17A, IL-17C, and IL-22.
Yoon et al., 2020 [71]	*Lactobacillus plantarum*	CRC ileostomy reversal patients	40	3 weeks	No significant differences in bowel function score or quality of life at 1 and 3 weeks postoperatively.

[a]CRC: colorectal cancer; [b]CRP: C-reactive protein; [c]TNF-α: Tumor necrosis factor alpha; [d]IL: Interleukin.

Discussion

Lately, the interest in studying how microbiome modulation can be utilized in the prevention and management of colorectal cancer has increased significantly. Several reviews assessed the impact of gut microbiota and probiotic supplementation in CRC patients [72-74]. However, most of these reviews focused on specific evaluations or subgroups of patients, such as being limited to fecal samples or post-op patients. We believe that our systematic review was conducted in a comprehensive manner, summarizing the currently available evidence, leading to a broader understanding of the studied topic. We included 50 articles in our qualitative synthesis, of both interventional and observational designs. We showed that species such as *F. nucleatum*, *C. symbioticum, B. fragilis*, and *Ruminococcus spp.* differ in abundance in CRC patients, compared to healthy controls. Promising non-invasive biomarkers in CRC include *C. symbioticum* and *F. nucleatum.* Reductions in *Fusobacteria* and *Peptosptreptococcus* can possibly be achieved through probiotic supplementation with *Bifidobacterial* and *Lactobacilli* strains. Furthermore, probiotics and synbiotics

can be used as adjuvant in surgical CRC patients, leading to lesser complications, antibiotic use, and hastened recovery.

There is a "bidirectional crosstalk" between the microbiota and the immune and epithelial growth pathways, confirming that gut bacteria can modulate and even drive colorectal carcinogenesis in humans [23]. The existence of a distinct microbial profile and cytokine signature of CRC patients exhibit promise for early screening purposes. A better understanding, in vitro, of mechanisms by which bacteria are involved in tumorigenesis together with sequencing techniques that verify their characteristic presence in human CRC stool samples has led authors to propose bacteria such as *C. butyricum* and *F. nucleatum* as early biomarkers. More recent studies support the role of *F. nucleatum* noninvasive marker potential, proposing a shift in its role and considering it a "driver" bacteria according to the 2012 "driver-passenger model". Mainly, due to the fact that it is increasingly found in the oral mucosal microbiome of patients with colorectal adenoma versus patients in carcinomatous stage [6,24].

However, variability of CRC specific microbiota is high in human studies and strongly linked with genetic and environmental factors. Also, genetic similarities between species have recently led Ma et al. to propose more specific bacterial signatures, made up of 3 SNPs that are specifically associated with CRC. The strains whose genomes the SNPs belonged to were interestingly not more abundant in fecal samples, suggesting the high sensitivity of this method [25].

Most interventions we identified focused on dietary supplementation to modulate microbiota towards raising the levels of butyrate, acetate, and total SCFAs. However, the results were generally limited to a few days or weeks post-intervention. There is correlation between SCFA levels and certain microbial profiles (i.e. a higher *Firmicutes* /*Bacteroidetes* ratio and lower *Clostridium* levels) [75], as well as potential of using SCFA stool levels as a marker for CRC. Nevertheless, interventions need to be locally appropriated due to microbiota variations across racial groups [75,76]. While a significantly longer "healthy eating" diet intervention (6 months), noted no significant alterations in gut microbiome, there are indications from animal models that changes in the microbiome could become irreversible in the long term, after several generations [51]. The need for strengthening human-based evidence on diet as a microbiome modulator is listed among the sustainable development goals for 2025 in nutrition research [77].

There is great potential for tumorigenetic pathway modulation by probiotics, such as *L. acidophilus* and *L. plantarum* [8]. Synbiotics are reportedly more efficient than probiotics in reducing epithelial proliferation and the increased efficacy of interventions seems to be linked to host microbial profile, which is less altered in early carcinogenesis [58]. Decrease in bacteria involved in tumor development was consistently proven with probiotic administration, but metabolic and genetic mechanisms are not yet well known.

Perioperatively, prebiotic administration is recommended, as there is definite proof that it reduces local and systemic complications, hospital stay, and antibiotic use. A reduction in bacterial translocation is a consistent outcome of the included trials. However, it is difficult to propose a standard duration and timing of perioperative probiotic intake, as they differ widely in bacterial combinations used, additional outcomes measured, and their duration ranges from 3 days, preoperatively, to 1 year, postoperatively.

There are also a few other emerging applications of probiotics during CRC management, which provide future directions for research. For example, the role of probiotics as an adjuvant to immunotherapy, which has been demonstrated in vitro and in animal models, where *L. acidophilus* improved the efficacy of anti-CTLA-4, but such interventions have not yet, to our knowledge, been used in human therapy [78,79]. Moreover, microbiome modulation has shown potential in alleviating resistance to chemotherapeutical treatment in animal models, where gemcitabine degradation by gammaproteobacterial was inhibited by *Bifidobacterium pseudolongum* [80].

The main limitation of this review is its focus on human studies only. Recent and ongoing research is more abundant in exploring the role of gut bacteria in colorectal carcinogenesis through in vitro and animal model studies. There is a focus on preclinical research on the preventive potential of certain bacterial strains – for example, the ability of *C butyricum* to inhibit tumor proliferation in human in vitro cultures via its action on the Wnt/β-catenin signaling pathway [18], or that of Scutellaria barbata (a Chinese herbal medicine used as an adjuvant to chemotherapy) to inhibit the same pathway in mouse cultures [81]. There is great potential for research in the direction of microbe-based therapies, as suggested by Li et al., who have recently shown the favorable effects of β-galactosidase secreted by *S. thermophilus* in the inhibition of CRC growth and modulation of microbiota [82], or as Bullman et al. recently discovered when they obtained a reduction in mouse tumor size using antibiotics to eradicate *F. nucleatum* [14].

Nevertheless, our systematic review has several important strengths. We believe that the role of microbiome modulation in the prevention and management of CRC is of important clinical significance. This is due to the increasing trend of CRC incidence, in addition to the associated morbidity, mortality, and economic burden. We conducted a comprehensive search using several electronic databases, and summarized the currently available evidence in a systematic manner. We did not limit our search to a specific type of bacteria that might be related to CRC, or a specific group of CRC patients. Having a large number of included articles in this systematic review allowed us to evaluate studies involving participants from different backgrounds,

as well as multiple interventions, making our results more generalizable. Moreover, we also highlighted several gaps in evidence that need to be addressed in future research.

Conclusions and future directions

Gut microbiota individual and geographic variability is high. However, a bacterial signature of CRC patients has been identified, comprised of species such as *F. nucleatum, C. symbioticum, B. fragilis,* and *Ruminococcus spp.*, with mechanisms of pathogenicity partially elucidated. Of these, *C. symbioticum* and *F. nucleatum* show promise as early CRC non-invasive biomarkers.

There is robust evidence to support dietary supplementation with oligosaccharides or insoluble fiber that ferment to increase the level of SCFAs, especially butyrate, which in turn, inactivates inflammatory and cell proliferation pathways, decreasing tumorigenesis.

Probiotic supplementation with *Bifidobacterial* and *Lactobacilli* strains in primary, secondary, and tertiary prevention of CRC is efficient in modulating microbiota towards a reduction in *Fusobacteria* and *Peptosptreptococcus,* and thus in DNA-damaging metabolites, with a more prominent effect in polypectomy patients than in advanced carcinoma.

As adjuvant in surgical CRC patients, probiotics and synbiotics can be firmly recommended, as they are proven to decrease complications, reduce antibiotic use, and accelerate recovery.

Further areas of exploration suggested by preclinical studies are the applicability of various probiotic bacteria in decreasing resistance to chemotherapy and immunotherapy, as well as direct antitumoral effects of bacteria such as *S. thermophilus* or *C. butyricum*.

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