Special Article

Preclinical and clinical relevance of probiotics and synbiotics in colorectal carcinogenesis: a systematic review

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Context: *Recent evidence suggests that modulation of the gut microbiota may help prevent colorectal cancer.* ***Objective:*** *The aim of this systematic review was to investigate the role of probiotics and synbiotics in the prevention of colorectal cancer and to clarify potential mechanisms involved.* ***Data Sources:*** *The PubMed, ScienceDirect, and LILACS databases were searched for studies conducted in humans or animal models and published up to August 15, 2018.* ***Study Selection:*** *Clinical trials and placebo-controlled experimental studies that evaluated the effects of probiotics and synbiotics in colorectal cancer and cancer associated with inflammatory bowel disease were included. Of 247 articles identified, 31 remained after exclusion criteria were applied. A search of reference lists identified 5 additional studies, for a total of 36 included studies.* ***Data Extraction:*** *Two authors independently assessed risk of bias of included studies and extracted data. Data were pooled by type of study, ie, preclinical or clinical.* ***Results:*** *The results showed positive effects of probiotics and synbiotics in preventing colorectal cancer. The main mechanisms identified were alterations in the composition and metabolic activity of the intestinal microbiota; reduction of inflammation; induction of apoptosis and inhibition of tumor growth; modulation of immune responses and cell proliferation; enhanced function of the intestinal barrier; production of compounds with anticarcinogenic activity; and modulation of oxidative stress.* ***Conclusions:*** *Probiotics or synbiotics may help prevent colorectal cancer, but additional studies in humans are required to better inform clinical practice.*

INTRODUCTION

Colorectal cancer has been identified as the third leading cause of death by cancer.[1] The World Health Organization estimates that, by 2030, there will be 27 million new cases of colorectal cancer worldwide and 17 million deaths due to colorectal cancer, with 75 million people living with the disease.[2] The etiology of colorectal cancer is multifactorial and involves both genetics and lifestyle factors, which can cause changes in the intestinal microenvironment that lead to colorectal carcinogenesis. This process involves chronic inflammation, increased mutation of cells exposed to carcinogens, and proliferation of dysplastic lesions.[3]

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Recently, the role of microorganisms that colonize the intestine during carcinogenesis has been the subject of increased discussion. Dysbiosis has been identified as a risk factor for colorectal cancer,[4] following observations of differences in the intestinal microbiota composition between healthy and sick individuals.[3,5] However, the complexity of the carcinogenic process precludes the establishment of a feasible link between colorectal cancer and a specific microorganism; rather, colorectal cancer is likely a consequence of host interaction with an imbalanced intestinal microbiota.[6]

The human intestinal microbiota is composed of trillions of microorganisms that inhabit and distribute themselves at specific sites, where they establish complex communities. The largest group is found in the colon (approximately 10^{11} microorganisms per gram of intestinal content). These microorganisms benefit host health locally and systemically by regulating both intestinal homeostasis and neuromuscular function of the gastrointestinal tract.[7,8]

The intestinal microbiota may be able to interfere in the carcinogenic process, owing to its capacity to stimulate the host immune response, modify the metabolism of tumor cells, and regulate cell apoptosis and proliferation.[9] Furthermore, it plays a role in the absorption and separation of bile acids, which are recognized to increase oxidative stress, promote DNA damage, and contribute to the instability of the mitochondrial membrane.[10]

The administration of probiotics is the most widely used approach to modulate the intestinal microbiota. According to the Food and Agriculture Organization of the United Nations and the WHO,[11] probiotics are "...live microorganisms, which when administered in adequate amounts confer a health benefit on the host." The term *probiotics* usually refers to lactic acid bacteria, such as *Lactobacillus* and *Bifidobacterium* (which are widely used and are Generally Recognized As Safe [GRAS] by the US Food and Drug Administration). Other organisms, however, are also used as probiotics, such as *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, and the yeast *Saccharomyces boulardii*. It is suggested that the ingestion of 10^6 to 10^{11} CFU/d is capable of reducing the incidence of colorectal cancer and other intestinal diseases.[12]

Prebiotics are nondigestible dietary ingredients that also demonstrate protective effects against cancer by selectively stimulating the growth of beneficial bacteria and the activity of the colonic microbiota.[13] Upon proliferation, probiotics promote an increase in the production of short-chain fatty acids, which are produced in variable quantities ($\approx$ 100 to 450 mmol/d). The most studied short-chain fatty acids are acetic acid, propionic acid, and butyric acid, all of which may alter the development of cancer by, for example, inhibiting cell proliferation or stimulating cell apoptosis. Furthermore, short-chain fatty acids are produced through the fermentation of prebiotics.[14,15]

The combination of probiotics and prebiotics, known as synbiotics, may be more efficient in preventing colorectal cancer than the use of either one alone. One study demonstrated that the combination of a starch-resistant prebiotic and *Bifidobacterium lactis* probiotic was capable of significantly stimulating colon cell apoptosis in rats after exposure to a carcinogenic agent.[16] There is growing interest in the development of alternatives to synthetic drugs, either to reduce the risk of adverse effects or to treat various diseases. In this context, the use of probiotics or synbiotics represents a promising strategy to decrease the risk of cancer, especially colorectal cancer, which is an aggressive type of tumor with high mortality worldwide.

Although in vitro and in vivo studies have suggested possible mechanisms through which probiotics and synbiotics protect against the development of colorectal cancer, there is little evidence of specific effects of biological responses related to colorectal carcinogenesis, especially those linked to the intestinal microbiota composition and the changes caused by colorectal cancer. Moreover, the methods and the carcinogenic markers used to define the mechanisms involved in the role of probiotics and synbiotics in colorectal cancer vary widely. Hence, this review was conducted to evaluate whether a rational basis exists for the use of probiotics and synbiotics in colorectal cancer and to investigate the main mechanisms involved in colorectal carcinogenesis. Furthermore, a critical analysis of preclinical and clinical studies was performed to identify methodological weaknesses and to aid the development of new studies.

METHODS

The protocol for this systematic review was developed in accordance with the PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) statement.[17] The PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines and the PRISMA checklist were followed to report the results of this review (see Table S1 in the Supporting Information online).

Literature search

Two authors independently searched the PubMed, LILACS (Latin American and Caribbean Health Sciences Literature), and ScienceDirect databases for clinical and preclinical studies on the protective effects

Table 1 **PICOS criteria for inclusion and exclusion of studies**

Parameter	Inclusion criteria	Exclusion criteria
Population	Adult humans (female and male); rodents	
Intervention	Supplementation with probiotics or synbiotics for prevention of colorectal cancer or colitis-associated cancer	
Comparison	Placebo; water; saline solution; food products (eg, milk, fermented milk, or yogurt), with no supplementation; standard diet for rodents, with no supplementation	
Outcomes	Reduction in incidence of tumors or preneoplastic lesions; reduction in intestinal polyps, colonic ulcers, or lesions with a high degree of dysplasia or DNA damage	
Study design	Randomized clinical trials; crossover, double-blind, and placebo-controlled or prospective studies; experimental placebo-controlled studies	In vitro studies, reviews, consensus papers, letters to editor, theses, and dissertations

of probiotics and synbiotics in colorectal carcinogenesis by consulting the Health Science Descriptors (DeCS) and Medical Subject Headings (MeSH). The following English search terms and their correspondents in Portuguese were used: neoplasms, probiotic, synbiotic, colorectal neoplasms, prevention, *Lactobacillus*, *Bifidobacterium*, and aberrant crypt foci. The logical operators "AND" or "OR" were used to combine the descriptors. Studies published up to August, 15, 2018, were eligible, and language restrictions were applied to select articles in English and Portuguese only. Additionally, the reference lists of the studies included were hand searched to identify other relevant trials.

Screening and eligibility of records

The PICOS (population, intervention, comparison, outcomes, and study design) strategy was used to identify criteria for the inclusion of studies in the systematic review (Table 1).[18] The initial selection was based on title and abstract. After screening, duplicate studies and in vitro studies were excluded. Studies that evaluated the effects of probiotics and synbiotics in the development of cancer associated with inflammatory bowel disease were also selected. Reviews, consensus papers, letters to editor, theses, and dissertations were excluded. Studies selected in this first screening were read in full and assessed for compliance with the established eligibility criteria. Studies that were not available online were requested from the authors. Selection was restricted to original studies conducted in human or murine models. Eligibility was analyzed independently by the reviewing authors, and disagreements were resolved by consensus.

Data extraction and synthesis

For preclinical studies, the following variables of interest were considered: title, authors, year, and country of publication; experimental model features (lineage, number of animals, sex, age, and body weight); research methods (shelter type, number of experimental groups, number of animals per group, presence of control group, and intervention in control group); protocol for induction of colorectal cancer/preneoplastic lesions; probiotic/synbiotic used, dose and timing of administration, and main results. The following variables were considered in clinical studies: title, authors, year, and country of publication; study aim; population features (sex, age, number of participants); experimental design (randomized, placebo-controlled, double-blind); intervention (composition of probiotic/synbiotic, dose used, frequency of administration); and main results.

Risk-of-bias assessment

The criteria set forth in the ARRIVE (Animal Research: Reporting of in Vivo Experiments) guidelines[19] were used to evaluate the experimental studies for risk of bias. These criteria are based on short descriptions of essential features of the experimental model used in the studies, such as theoretical and methodological basis, research objectives, refinement of analytical methods, statistical draw, sample calculation, and result measures.[19] To assess risk of bias in clinical studies, a checklist based on the criteria proposed by Downs and Black[20] was used. The quality score of each article was based on 13 domains and corresponded to the sum of the items evaluated, assigning a score of 1 to each criterion satisfied and a score of 0 to each criterion not satisfied. The quality of the studies was classified as poor ($\leq$ 4 of 13 points), intermediate (5–8 of 13 points), or good ($\geq$ 9 of 13 points).

RESULTS

Selected studies

Figure 1 shows a flow diagram of the literature search and selection process. Altogether, 247 articles were

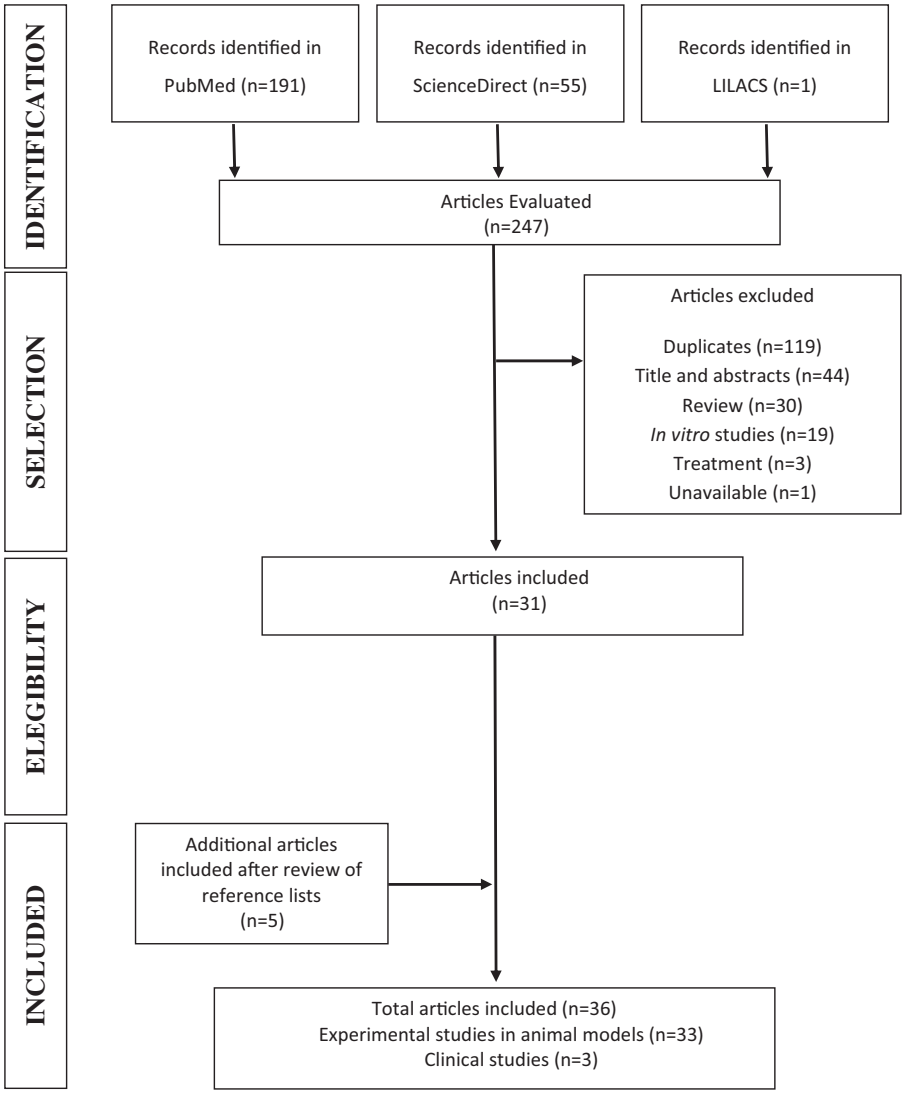

Figure 1 **Flow diagram of the literature search process**.

identified in the PubMed (n = 191), ScienceDirect (n = 55), and LILACS (n = 1) databases. Of these, 216 were excluded for the following reasons: duplicate studies (n = 119), title, abstract, or study not relevant to the topic of the review (n = 44), review articles (n = 30), in vitro studies (n = 19), studies reporting the curative effects of probiotics and synbiotics (n = 3), and studies that could not be accessed online (n = 1). Initially, 31 studies were included. After the reference lists of these studies were searched, 5 additional relevant studies were included, for a total of 36 studies. Most of the included studies (33 of 36) were preclinical studies.[21–51]

Qualitative data

The included studies were performed in 14 different countries. Most were conducted in India or Korea (n = 12),[24,26,28–30,32,34,45,48,50,51] with the rest conducted in China or the United States (n = 8)[21,23,27,31,33,38,39,46] (Table 2).[21–51] For the preclinical studies, the models used were rats (n = 18 studies)[24,26,28–34,36–38,40,41,43,44,47] and mice (n = 15 studies).[21–23,25,27,35,39,45,46,48–51] Most studies (n = 21) used male animals[22–31,35–38,40,42,43,45,47,48,51]; only 1 study used both male and female animals.[41] Interestingly, 5 studies did not report the sex of the animals,[24,34,39,46,50] and 4 studies did not mention the total number of animals used in the experiments.[23,25,27,48] The age of the animals ranged from 3 to 12 weeks, although 5 studies did not report this information.[24,32–34,46] The initial body weight of the animals was not reported in most studies.[22,24,26,27,31,33,35,37–39,41,44–51]

Preneoplastic lesions and tumors were induced with1,2-dimethylhydrazine in 16 studies,[23,24,26,28–36,40,41,43] and with inoculation of CT-26 tumor cells in 2 studies.[21,23] In 2 other studies, genetically modified animals, in which disease developed spontaneously, were

Table 2 **Characteristics of preclinical studies on the use of probiotics and synbiotics in colorectal carcinogenesis**

Reference	Country	Animal model	No. of animals	Sex/age (wk)	Initial weight	No. of groups	No. of animals per group
Hu et al (2015)[21]	China	BALB/c mice	30	F/6–8	> 20g	3	10
Kahouli et al (2017)[22]	Canada	C57BL/6J-Apc$^{Min/J}$ mice	10	M/4	NS	2	5
Chen et al (2015)[23]	China	C57BL/6 mice	72	M/6–8	21.9–23.0 g	6	12
Kumar et al (2010)[24]	India	Wistar rats	36	M/NS	NS	6	6
Urbanska et al (2016)[25]	Canada	C57BL/6J-Apc$^{Min/+}$ mice	NS	M/7–8	20–25 g	3	NS
Mohania et al (2014)[26]	India	Wistar rats	120	M/3	NS	5	24
Arthur et al (2013)[27]	USA	IL-10$^{-/-}$ 129/SvEv mice	NS	M/7–12	NS	2	NS
Park et al (2007)[28]	Korea	F344 rats	30	M/5	185 ± 10 g	3	10
Lee et al (2007)[29]	Korea	F344 rats	18	M/5	185 ± 10 g	2	9
Mohania et al (2013)[30]	India	Wistar rats	120	M/3	22.2–23.2 g	5	24
Zhang et al (2015)[31]	China	F344 rats	24	M/5	NS	3	8
Walia et al (2015)[32]	India	Sprague-Dawley rats	36	F/NS	125–175 g	6	6
Zhu et al (2014)[33]	China	F344 rats	50	F/5	NS	5	10
Verma & Shukla (2013)[34]	India	Sprague-Dawley rats	72	NS/NS	100–150 g	12	6
Liboredo et al (2013)[35]	Brazil	Swiss mice	50	M/8	NS	5	10
Chang et al (2012)[36]	Canada	F344 rats	45	M/5	130 g	3	15
Leu et al (2010)[37]	Australia	Sprague-Dawley rats	180	M/5	NS	6	30
Kumar et al (2010)[24]	India	Rats	100	NS/10	NS	4	25
Purohit et al (2009)[38]	USA	Fisher rats	140	M/6	NS	7	20
Chen et al (2009)[39]	USA	C57BL/6JMin/+ (*Apc^{Min}*) mice	14	NS/7	NS	2	6 or 8
Sivieri et al (2008)[40]	Brazil	Wistar rats	30	M/4	90 g	3	10
Narushima et al (2010)[41]	Japan	F344 rats/RasH2 mice	29/NS	M & F/4–8	NS	3 or 4	NS
Dominici et al (2014)[42]	Italy	CD-1 mice	20	M/4–6	25–30 g	4	5
Villarini et al (2008)[43]	Italy	Sprague-Dawley rats	20	M/4–8	140–180 g	4	5
Chen et al (2015)[23]	Taiwan	BALB/cByJ mice	NS	F/4–6	20 g	4	NS
Hakansson et al (2012)[44]	Sweden	Sprague-Dawley rats	48	F/NS	NS	6	8
Chung et al (2017)[45]	Korea	BALB/c mice	50	M/4	NS	5	10
Bassaganya-Riera et al (2012)[46]	USA	C57BL/6 mice, IL-10$^{-/-}$ mice, and 129/SvEv mice	120	NS	NS	3	30 or 60
Appleyard et al (2011)[47]	Puerto Rico	Sprague-Dawley rats	45	M/6	NS	2	22 or 23
Do et al (2016)[48]	Korea	C57BL/6 mice	NS	M/4	NS	4	NS
Talero et al (2015)[49]	Spain	C57BL/6 mice	240	F/6	NS	5	10 or 20
Lee et al (2015)[50]	Korea	BALB/c mice	60	NS/6	NS	6	10
Kim et al (2005)[51]	Korea	C57BL/6 mice	15	M/6	NS	3	5

Abbreviation: NS, not specified.

used.[22,25] Table 3[21–51] shows the methods used in each of the preclinical studies. For control groups, the standard diet for rodents was used when the probiotic or synbiotic was added to the diet in freeze-dried form.[23,24,29,32,34,37,40,44,45,48] However, studies in which the probiotic or synbiotic was administered via gavage used saline solution in control groups[21–23,25,31,33,42,43,50] (Table 2).

In the experimental studies, 21 different bacterial species (8 *Lactobacillus*, 6 *Bifidobacterium*, 2 *Streptococcus*, 2 *Bacillus*, 1 *Clostridium*, 1 *Lactococcus*, 1 *Enterococcus*) and 1 fungal species (*Saccharomyces boulardii*) were used as probiotics. Of these studies, 2 used *Saccharomyces boulardii*[35,39] and 6 used the probiotic VSL#3, a concentrated mix of 7 bacterial strains.[27,45–49] In general, *Lactobacillus acidophilus* and *Lactobacillus plantarum* were used most frequently as probiotics. The probiotic was administered as a single strain of a probiotic species or as strains of multiple probiotic species in 28 studies,[21–29,31–36,38–43,46,47,49–51] combined with prebiotics in 2 studies,[37,44] or in combination with drugs in 3 studies.[30,45,48] The route of administration was oral in 16 studies,[23,24,26,27,30,33,34,38,39,41,44,45,48,50] but the form of administration (gavage or added to food or drinking water) was not specified. Ten studies reported probiotic administration via gavage.[21,22,25,31,32,40,42,43,46,51] The dose administered varied widely, with organism counts ranging from 10^6 to 10^{11} CFU/d. The duration of the intervention ranged from 5 days to 42 weeks (Table 3).

Only 3 studies in humans (n = 45 296 individuals total) were included (Table 4).[5,52,53] A total of 45 241 individuals participated in a prospective study,[52] 17 participated in a probiotic and synbiotic intervention study,[53] and 38 participated in an intervention study with different probiotics.[5] Men and women aged between 21 and 86 years were included. Two studies were crossover, randomized, controlled, double-blind studies,[5,53] and 1 study[52] was a prospective 12-year follow-up study. One of the intervention studies

Table 3 **Methods used in preclinical studies of the use of probiotics and synbiotics in colorectal carcinogenesis**

Reference	CRC induction	Control group	Probiotic/synbiotic	Method of administration	Daily dose	Duration of intervention (wk)
Hu et al (2015)[21]	CT-26 cells	Saline	*Lactobacillus plantarum*; *Lactobacillus rhamnosus*	Oral gavage	1.0×10^8 CFU; 1.0×10^9 CFU	14 d initially; after tumor induction, once weekly every 3 wk
Kahouli et al (2017)[22]	No induction; CRC developed in transgenic animals	Saline	*Lactobacillus fermentum* NCIMB 5221; *Lactobacillus acidophilus* ATCC 314	Oral gavage	1.0×10^{10} CFU (0.5×10^{10} CFU each strain)	12
Chen et al (2015)[23]	DMH (20 mg/kg), 1×/wk for 28 wk	Saline	*Clostridium butyricum*; *Bacillus subtilis*	Oral	2.5×10^8 CFU each strain	28
Kumar et al (2010)[24]	DMH (20 mg/kg), 1×/wk for 6 wk	Usual diet for rodents	*L plantarum* AS1	Oral	10^9 CFU	26
Urbanska et al (2016)[25]	No induction; CRC developed in transgenic animals	Saline	Microencapsulated *L acidophilus*	Oral gavage	Yogurt	10
Mohania et al (2014)[26]	DMH (40 mg/kg), 2×/wk for 2 wk	Buffalo milk	Dahi probiotic (Lactobacillus acidophilus LaVK2, Bifidobacterium bifidum BbVK3)	Oral	2×10^9 CFU each strain	8, 16, or 32
Arthur et al (2013)[27]	AOM (10 mg/kg), for 6 wk	NS	VSL#3 probiotic	Oral	10^9 CFU	17
Park et al (2007)[28]	DMH (30 mg/kg), 1×/wk for 6 wk	High-fat, low-fiber diet	*Bacillus polyfermenticus*	Oral (in diet)	3×10^8 CFU/1.3 g of diet	10
Lee et al (2007)[29]	DMH (30 mg/kg), 1×/wk for 6 wk	Usual diet for rodents	*B polyfermenticus* SCD	Oral (in diet)	3×10^6 CFU	10
Mohania et al (2013)[30]	DMH (40 mg/kg), 2×/wk for 2 wk	Buffalo milk	Dahi probiotic (Lactobacillus acidophilus LaVK2, Bifidobacterium bifidum BbVK3)	Oral	2×10^9 CFU each strain	32
Zhang et al (2015)[31]	DMH (30 mg/kg) for 10 wk	Saline	*Lactobacillus salivarius* Ren	Oral gavage	5×10^{10} CFU/kg of body weight	32
Walia et al (2015)[32]	DMH (30 mg/kg), 1×/wk or 2×/wk	Usual diet for rodents	*L plantarum* AdF10; *L rhamnosus* GG	Oral gavage	10^{10} CFU	16
Zhu et al (2014)[33]	DMH (30 mg/kg), 1×/wk for 10 wk	Saline	*L salivarius* Ren	Oral	1×10^{10} CFU/kg of body weight or 5×10^8 CFU/kg of body weight	15
Verma & Shukla (2013)[34]	DMH (20 mg/kg), single dose	Usual diet for rodents	*L rhamnosus* GG MTCC 1408; *L rhamnosus* GG MTCC 1408; *L plantarum* MTCC 1407; *L acidophilus* NCDC 15; *Bifidobacterium bifidum* NCDC 234	Oral	1×10^9 lactobacilli	7
Liboredo et al (2013)[35]	DMH (25 mg/kg), 1×/wk for 6 wk	Water	*Lactobacillus delbrueckii* UFV H2b20; *Bifidobacterium animalis*; *Saccharomyces boulardii*	Oral (in water)	3×10^8 CFU (except *Lactobacillus* + *Bifidobacterium* group, which received 6×10^8 CFU)	14

(continued)

Table 3 **Continued**

Reference	CRC induction	Control group	Probiotic/synbiotic	Method of administration	Daily dose	Duration of intervention (wk)
Chang et al (2012)[36]	DMH (20 mg/kg), 1×/wk for 10 wk	High-fat diet	*L acidophilus* KFRI342	Oral (in diet)	2×10^9 CFU	10
Leu et al (2010)[37]	AOM (15 mg/kg), 1×/wk for 2 wk	Usual diet for rodents	Synbiotic: *Bifidobacterium lactis* + HAMS	Oral (in diet)	1×10^{11} CFU/g of diet	26
Kumar et al (2010)[24]	DMH (20 mg/kg), 1×/wk for 15 wk	Usual diet for rodents	*L acidophilus*; *L casei*; curd culture	Oral	Probiotic curd given as 30% of total diet	40
Purohit et al (2009)[38]	AOM (15 mg/kg), 1×/wk for 2 wk	Acidified milk with glucono-δ-lactone	*Streptococcus thermophilus* ST 5581; *S thermophilus* ST 5842; *S thermophilus* ST 4239; *S thermophilus* ST PH; *L delbrueckii* subsp *bulgaricus* 3984; *Lactococcus lactis* subsp *cremoris* JFR1	Oral	Diet supplemented with 30% fermented milk containing probiotic bacterial strains	30
Chen et al (2009)[39]	No induction; CRC developed in transgenic animals	NS	*S boulardii*	Oral	3×10^8 CFU/mL (water) and 6×10^8 CFU 3×/wk (gavage)	9
Sivieri et al (2008)[40]	DMH (20 mg/kg), 1×/wk for 15 wk	Usual diet for rodents	*Enterococcus faecium* CRL183	Oral gavage	10^8 CFU, 3 mL/kg of body weight	42
Narushima et al (2010)[41]	PhIP (75 mg/kg), daily for 2 wk; DMH (20 mg/kg), weekly for 20 wk	Fermented milk	*L delbrueckii* subsp *bulgaricus* 2038; *S salivarius* subsp *thermophilus* 1131	Oral	10% (vol/vol) added to fermented milk	4
Dominici et al (2014)[42]	PhIP (100 mg/kg), single dose (last experimental day)	Saline	*L rhamnosus* IMC501	Oral gavage	10^9 CFU, 10 mL/kg of body weight	10
Villarini et al (2008)[43]	DMH (15 mg/kg), single dose	Saline	*L casei*	Oral gavage	10^9 bacteria, 10 mL/kg of body weight	5 d
Chen et al (2015)[23]	CT-26 cells	Usual diet for rodents	*L acidophilus* NCFM	Oral	1×10^8 CFU/animal	2
Hakansson et al (2012)[44]	4% DSS in drinking water for 7 d (11 cycles)	Usual diet for rodents	Synbiotic: *Bifidobacterium infantis*; *Lactobacillus gasseri* DSM 16737; *L plantarum* DSM 15313; and blueberry peel	Oral	*B infantis* 2×10^9 CFU; *L gasseri* 1×10^9 CFU; *L plantarum* 2×10^9 CFU; blueberry (61 g or 122 g)	24
Chung et al (2017)[45]	AOM (10 mg/kg); 2% DSS in drinking water for 7 d	Usual diet for rodents	Probiotic VSL#3	Oral	NS	8
Bassaganya–Riera et al (2012)[46]	AOM (10 mg/kg) in wk 6, 2% DSS in drinking water for 7 d	Water	Probiotic VSL#3	Oral gavage	1.2×10^9 CFU	16
Appleyard et al (2011)[47]	TNBS (5 mg/kg), 2×/wk for 10 wk	Water	Probiotic VSL#3	Oral (in water)	5×10^9 CFU/100 g of body weight	18
Do et al (2016)[48]	AOM (10 mg/kg) and 2% DSS in drinking water for 7 d (2 cycles)	Usual diet for rodents	Probiotic VSL#3	Oral	1.3×10^6 CFU	6

(continued)

Table 3 **Continued**

Reference	CRC induction	Control group	Probiotic/synbiotic	Method of administration	Daily dose	Duration of intervention (wk)
Talero et al (2015)[49]	0.7% DSS in drinking water for 7 d	Water	Probiotic VSL#3	Oral (in water)	5×10^9 CFU/100 g of body weight	85 d (5 cycles), 170 d (10 cycles) and 255 d (15 cycles)
Lee et al (2015)[50]	AOM (10 mg/kg) and 2% DSS in drinking water for 7 d	Saline	*L plantarum* (feasible cells); *L plantarum* (unfeasible cells)	Oral	Low dose: 4×10^9 CFU/kg of body weight High dose: 4×10^{11} CFU/kg of body weight	8
Kim et al (2005)[51]	AOM (10 mg/kg) and 2% DSS in drinking water for 5 d (3 cycles)	Water	*B lactis* KCTC 5727	Oral gavage	Low dose: 2×10^9 CFU High dose: 2×10^{10} CFU	9

Abbreviations: AOM, azoxymethane; CFU, colony forming units; DMH, 1,2-dimethylhydrazine; DSS, dextran sodium sulfate; HAMS, high-amylose maize starch; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; TNBS, 2,4,6-trinitrobenzenesulfonic acid; NS, not specified.

Table 4 **Characteristics of clinical studies on the use of probiotics and synbiotics in colorectal carcinogenesis**

Reference	Study objective	Study population	Study design	No. of participants who completed study	Probiotic/synbiotic	Dose	Duration
Pala et al (2011)[52]	To investigate the association between yogurt ingestion and CRC in a multicenter cohort (EPIC-Italy)	14 178 men and 31 063 women (aged 30–86 y)	Prospective study with 12 y of follow-up	45 241	Yogurt	Yogurt ingestion varied from 0 g/d in the smaller tertile to 85 g/d (men) and 98 g/d (women) in the higher tertile	12 y of follow-up (mean, 9 y)
Worthley et al (2009)[53]	To establish the relative luminal, epithelial, and epigenetic consequences of prebiotic, probiotic, and synbiotic dietary supplementation	Men and women (aged 21–75 y)	Double-blind, randomized, placebo-controlled crossover study	17	3 groups: *Bifidobacterium lactis* only; HAMS only; and synbiotic (*B lactis* + HAMS)	5×10^9 CFU of *B lactis* HAMS (25 g/d) Synbiotic (same quantities shown above)	Each intervention was 4 wk in duration, with no washout period
Hatakka et al (2008)[5]	To evaluate the activity of the enzymes β-glucosidase, β-glucuronidase, and urease	Men (aged 24–55 y)	Randomized, double-blind, placebo-controlled crossover study	37 or 38	*Lactobacillus rhamnosus* + *Propionibacterium freudenreichii* subsp *shermanii* JS	2×10^{10} CFU of each probiotic	4 wk (each intervention)

Abbreviations: CRC, colorectal cancer; CFU, colony forming units; EPIC-Italy, European Prospective Investigation into Cancer–Italy; HAMS, high-amylose maize starch.

consisted of 3 groups: probiotic (*Bifidobacterium lactis*), prebiotic (high-amylose maize starch), and synbiotics (both)[53]; the other evaluated *Lactobacillus rhamnosus* LC705 and *Propionibacterium freudenreichii* subsp *shermanii* JS as probiotics.[5] In both intervention studies, the probiotics were available in the form of a capsule or sachet (10^9 to 10^{10} CFU/d). Each intervention lasted 4 weeks. The prospective study evaluated the ingestion of yogurt and the risk of developing colorectal cancer.[52] The results were stratified by terciles of consumption. The amount ingested varied from 0 to 98 g/d.

Main findings

The preclinical studies demonstrated that probiotic/synbiotic interventions provide protective effects against colorectal carcinogenesis. Of the 33 included studies, 19 (57.6%) reported a significant reduction in tumor incidence,[21,23–25,30–32,37–39,45–51] 7 (21.2%) reported a reduction in the incidence of preneoplastic lesions,[26,28,29,33–36] and 2 (6.0%) reported a reduction in both[40,41] (Table 5[21–51]). Positive findings were also reported by 2 studies that evaluated the effect of probiotic/synbiotic interventions on other outcomes such as decreased incidence of intestinal polyps, colonic ulcers, and lesions with a high degree of dysplasia.[22,44] In 2 studies, no reduction in the incidence of tumors or preneoplastic lesions as a main outcome was observed.[42,43] In both studies, the authors aimed to evaluate the effect of probiotics on direct DNA damage, modulation of oxidative balance, or change in the composition and activity of the intestinal microbiota. In both cases, probiotic use was associated with protective effects. Only 1 study reported negative effects of probiotic use, noting increased tumor penetrance, multiplicity, dysplasia grade, and adenocarcinoma invasion.[27]

It is noteworthy that, in 9 studies (27.3%) studies,[27,44–51] the objective was to evaluate the use of probiotics/synbiotics in colorectal cancer associated with inflammatory bowel disease, particularly colitis. In these studies, an inflammatory component essential for the development of colorectal cancer was observed. The protocol for induction of colorectal cancer involved exposure to the carcinogenic agent (1,2-dimethylhydrazine or its active metabolite azoxymethane) in combination with other drugs that cause colitis (dextran sulfate sodium or 2,4,6-trinitrobenzene sulfonic acid). The genetically modified animal model, such as interleukin 10 (IL-$10^{-/-}$) knockout mice, which spontaneously develop colitis (Table 3), may also be used.

The results of studies in humans showed greater variation (Table 6[5,52,53]). Pala et al[52] found an association between reduced risk of colorectal cancer development and the consumption of yogurt, while Worthley et al[53] observed no significant changes in possible markers of colorectal cancer (eg, proliferation of intestinal crypts, ammonia concentration, short-chain fatty acids, C-reactive protein, and proinflammatory cytokines) after probiotic, prebiotic, and synbiotic use. Hatakka et al[5] observed an association between an increase in fecal counts of *Lactobacillus* and *Propionibacterium* organisms and a reduction in β-glucosidase and urease activity, suggesting a protective effect of the probiotic.

Risk of bias

All included studies had relevant titles and abstracts and sufficient scientific contextualization (see Table S2 in the Supporting Information). Three studies did not include an ethics statement.[24,31,34] All studies reported the dose of the probiotic/synbiotic used, the route of administration, and the duration of the intervention. On the other hand, none of the studies specified the time of the day of probiotic/synbiotic administration, the location of administration, or the justification for the route of administration chosen. Only 4 studies provided justification for the dose used.[22,46,47,49] All studies that used genetically modified animals stated this information in the article. Only 2 studies reported previous procedures applied to the animals.[22,27]

None of the studies described how sample size was calculated. Twenty-two studies provided information on how animals were allocated to the experimental groups,[21–23,26,27,30–32,36–38,40–45,47–50] and 32 described the statistical methods used for each analysis.[22–51] All studies reported mean values and standard deviations.

Two studies reported the health of the animals before the experimental period.[32,46] Only 1 study reported a reduction in the duration of the original experimental protocol because of adverse effects.[27] Three studies provided data on the mortality rate.[27,44,47] None of the articles identified study limitations, such as constraints of the animal model used or inaccuracy of results. Only 4 articles described possible new discoveries likely to benefit other species or systems or to be relevant to human biology.[30,31,34,42]

On the basis of the score and criteria suggested by Downs and Black,[20] 3 studies included in this review were classified as being of good quality (score $\geq$ 9 points) (see Table S3 in the Supporting Information online). None of the included studies described statistical power or reported data deletion or probability values of main results. One study included a large number of individuals, but the authors did not describe whether participants included in the study were representative of the population.[52]

Table 5 **Main findings in preclinical studies on the effects of probiotics and synbiotics in colorectal carcinogenesis**

Reference	Histopathological evaluation	Immunological evaluation	Inflammatory/molecular markers	Oxidative balance evaluation	Microbial activity	Other
Hu et al (2015)[21]	25 to 30 d after inoculation with tumor cells, *Lactobacillus plantarum* group showed decreased tumor growth compared with groups that received *Lactobacillus rhamnosus* and saline	↑ $CD8^+$ cell counts; ↑ ratio of $CD4^+$ to $CD8^+$ cells; ↑ NK cell infiltration; and ↑ IFN-γ production. Increased differentiation of CD4+ cells into Th1 cells in *L plantarum* group	NP	NP	NP	↑ survival in *L plantarum* group
Kahouli et al (2017)[22]	40% ↓ in formation of intestinal polyps in groups that received *Lactobacillus fermentum* plus *Lactobacillus acidophilus* compared with CG	NP	↓ β-catenin and Ki-67 expression in probiotic group	NP	NP	NP
Chen et al (2015)[23]	↓ incidence and tumor size in *Bacillus subtilis* and *Clostridium butyricum* groups (40% and 30%, respectively) compared with CG	↓ expression of Th2 and Th17 cells; ↓ ratio of $CD4^+$to $CD8^+$ cells in peripheral blood in *B subtilis* and *C butyricum* groups	↓ activation of TLR 4/MyD88/NF-κB; ↓ expression of IL-22, survivin, NF-κB, p-ERK, and β-catenin; and ↑ expression of p21 in probiotic groups	NP	NP	NP
Kumar et al (2010)[24]	↓ incidence and tumor size in *L plantarum* AS1 group compared with CG. Effect was observed in groups that received the probiotic before or after CRC induction	NP	NP	↓ lipid peroxidation; ↓ activity of SOD, CAT, and GST in colon and plasma in probiotic groups compared with CG	NP	NP
Urbanska et al (2016)[25]	↓ adenomas and intraepithelial gastrointestinal neoplasms in probiotic groups compared with CG	NP	↓ concentrations of IL-6 and fecal bile acids in probiotic group	NP	NP	NP
Mohania et al (2014)[26]	↓ ACF, AC:ACF ratio, and mucin-depleted foci in group that received Dahi probiotic, combined or not with anti-inflammatory piroxicam, compared with CG (buffalo milk)	NP	↓ PCNA in groups treated with Dahi probiotic, whether combined with piroxicam or not	NP	NP	NP
Arthur et al (2013)[27]	↑ tumor penetrance, multiplicity, dysplasia grade, and adenocarcinoma invasion in VSL#3 groups	NP	NP	NP	↓ bacterial abundance attributed to *Clostridium* genus in microbiota	NP

(continued)

Table 5 **Continued**

Reference	Histopathological evaluation	Immunological evaluation	Inflammatory/molecular markers	Oxidative balance evaluation	Microbial activity	Other
Park et al (2007)[28]	↓ ACF and AC:ACF ratio in *Bacillus polyfermenticus* group compared with CG (DMH)	NP	NP	↑ antioxidant potential and ↓ conjugated dienes in probiotic group	NP	↓ DNA damage in leukocytes in probiotic group compared with CG
Lee et al (2007)[29]	40% ↓ in preneoplastic lesions in *B polyfermenticus* group compared with CG	NP	NP	NP	NP	NP
Mohania et al (2013)[30]	↓ incidence and tumor size in groups that received Dahi, combined or not with anti-inflammatory piroxicam	NP	NP	↓ lipid peroxidation (in liver and colon) and ↑ GST activity in groups that received Dahi, whether combined with piroxicam or not	↓ β-glucuronidase activity in feces in groups that received Dahi, whether combined with piroxicam or not	NP
Zhang et al (2015)[31]	↓ incidence of tumors in *Lactobacillus salivarius* group compared with CG	NP	NP		↓ counts of *Ruminococcus* spp, Clostridiales, and *Bacteroides dorei* and ↑ counts of *Prevotella* in probiotic group	NP
Walia et al (2015)[32]	↓ incidence, multiplicity, and tumor size in *L plantarum* AdF10 and *L rhamnosus* GG groups compared with CG	NP	↓ COX-2 expression and ↓ serum concentration of total sialic acid in probiotic groups	NP	NP	NP
Zhu et al (2014)[33]	↓ ACF counts and multiplicity in groups that received low or high dose of probiotic *L salivarius* Ren compared with CG (DMH)	NP	↓ PCNA in groups that received low or high dose of probiotic	NP	↓ counts of *Bacillus* and Ruminococcaceae and ↑ counts of *Bacteroides*, Lachnospiraceae, *Prevotella*, and *Clostridium* after probiotic administration at low or high dose; ↓ azoreductase activity in probiotic groups	↑ SCFA concentrations in group that received high-dose probiotic; ↑ concentration butyric acid in low-dose group
Verma & Shukla (2013)[34]	↓ ACF in probiotic group. Percent reduction in *Lactobacillus rhamnosus* GG, *Lactobacillus casei, L acidophilus*, *L plantarum* and *Bifidobacterium bifidum* organisms was 98%, 45%, 96%, 89%, and 74%, respectively, compared with CG (DMH)	NP	NP	NP	↓ nitroreductase activity in *L casei* and *L plantarum* groups; ↓ β-glucuronidase activity in *L rhamnosus* GG and *L acidophilus* groups; ↓ β-glucosidase activity in *B bifidum* group compared with CG	NP

(continued)

Table 5 **Continued**

Reference	Histopathological evaluation	Immunological evaluation	Inflammatory/molecular markers	Oxidative balance evaluation	Microbial activity	Other
Liboredo et al (2013)[35]	↓ ACF in *Lactobacillus delbrueckii* UFV-H2b20 (55.7%) and *Bifidobacterium animalis* var *lactis* Bb12 (45.1%) groups compared with CG. Groups that received *L delbrueckii* and *B animalis* combined showed no reduction. Group that received *S boulardii* showed no reduction	NP	NP	NP	NP	NP
Chang et al (2012)[36]	↓ numbers of ACF in group that received *L acidophilus* KFRI342 and high-fat diet (41.1%) compared with CG. This difference was not significant when compared with group that received high-fat diet only	NP	NP	NP	↓ counts of aerobic bacteria and *Escherichia coli*, ↓ fecal pH, and ↓ β-glucuronidase and β-glucosidase activity in group that received probiotic and high-fat diet compared with CG	NP
Leu et al (2010)[37]	↓ incidence and multiplicity of tumors in group that received synbiotic compared with CG. ↓ incidence and multiplicity of tumors in group that received *Bifidobacterium lactis* and resistant starch. ↑ crypt height in groups that received synbiotic and resistant starch	NP	↓ number of PCNA-marked cells, per crypt, in groups that received resistant starch and synbiotic	NP	NP	↑ total concentrations of SCFA (cecum, proximal, and distal colon) in groups that received synbiotic and resistant starch
Kumar et al (2010)[24]	↓ incidence, multiplicity, and size of tumors in groups that received curd probiotic or curd culture only, compared with CG	NP	NP	NP	NP	↓ DNA damage in groups that received curd probiotic or curd culture compared with CG
Purohit et al (2009)[38]	↓ incidence and multiplicity of tumors in *Streptococcus thermophilus, L delbrueckii* subsp *bulgaricus* LB3984, and *Lactobacillus lactis* subsp *cremoris* JFR1 groups compared with CG. ↓ tumor size in *S thermophilus* (strains 5581 and.4239) and *L delbrueckii* groups	NP	↓ COX-2 activity in all groups that received probiotic	NP	NP	NP
Chen et al (2009)[39]	↓ number, size, and total superficial area of intestinal tumors in group that received *S boulardii* compared with CG. Lower dysplasia grade in *S boulardii* group	NP	↓ PCNA, phosphorylated EGFR, and p-Akt, and ↑ apoptotic cells in probiotic group compared with CG	NP	NP	NP

(continued)

Table 5 **Continued**

Reference	Histopathological evaluation	Immunological evaluation	Inflammatory/molecular markers	Oxidative balance evaluation	Microbial activity	Other
Sivieri et al (2008)[40]	↓ ACF, AC:ACF ratio, adenocarcinoma incidence, and tumor size in *Enterococcus faecium* group compared with CG	NP	↑ IL-4, IFN-γ and TNF-α concentrations in probiotic group	NP	NP	NP
Narushima et al (2010)[41]	Experiment 1: ↓ ACF and total number of ACs in yogurt group compared with CG or unfermented milk group. Experiment 2: ↓ number of tumors in yogurt group compared with CG (significant difference in male rats only)	NP	NP	NP	NP	NP
Dominici et al (2014)[42]	NP	NP	NP	NP	↑ lactobacilli counts and ↓ β-glucuronidase and *N*-acetyl-β-glycosaminidase activity in *L rhamnosus* IMC501 groups	↓ DNA damage after probiotic administration
Villarini et al (2008)[43]	NP	NP	NP	↓ SOD activity in *L casei* group compared with CG	↑ lactobacilli counts and ↓ β-glucuronidase activity in probiotic group	↓ DNA damage in probiotics groups
Chen et al (2015)[23]	↓ tumor size in *L acidophilus* NCFM group compared with CG	↓ counts of cells with MHC class I response in colon, mesenteric lymph nodes, and spleen in probiotic group	↑ apoptosis of tumor cells; ↑ caspase 3 and 9 concentrations; ↓ Bcl-2 expression; ↓ CXCR4 expression in colon, mesenteric lymph nodes, and extraintestinal metastatic tissues in probiotic group	NP	NP	NP
Hakansson et al (2012)[44]	↓ number of colonic ulcers and fewer lesions with low-grade dysplasia in synbiotic group. In groups that received probiotic only: ↓ liver lesions, parenchymal inflammatory infiltration, stasis, and bacterial translocation	NP	↓ haptoglobin blood concentration in groups that received blueberry peel and in synbiotic group	NP	↓ Enterobacteriaceae counts and ↑ lactobacilli counts in probiotic groups	↓ disease activity index in groups that received blueberry peel or synbiotic
Chung et al (2017)[45]	↓ number and size of tumors in groups that received VSL#3 combined with metformin when compared with CG	NP	↓ counts of cells positive for Ki-67; ↓ macrophage infiltration in crypts; ↑ reactivity with anti-claudin-1; and ↓ expression of cyclin D1 and Bcl-2 with combination therapy	NP	NP	↓ disease activity index with combination therapy. VSL#3 promoted AMPK and ERK activation (but combined therapy was more effective)

(continued)

Table 5 **Continued**

Reference	Histopathological evaluation	Immunological evaluation	Inflammatory/molecular markers	Oxidative balance evaluation	Microbial activity	Other
Bassaganya–Riera et al (2012)[46]	↓ adenomas and adenocarcinomas in groups that received VSL#3 or CLA compared with CG	↑ $CD4^+$ cells in mesenteric lymph nodes in healthy group that received VSL#3. ↑ in percentages of $CD4^+FoxP3^+$ cells and $CD4^+CD44^+CD62L^+$ LP T cells in VSL#3 group induced to CRC	↑ CD36 and PPAR-α expression in groups treated with VSL#3 or CLA. VSL#3 increased expression of angiostatin in distal colon. Both VSL#3 and CLA groups showed increased TNF-α expression compared with CG	NP	NP	↓ disease activity index groups that received VSL#3 or CLA. However, VSL#3 was more effective in reducing inflammation
Appleyard et al (2011)[47]	↓ total score in macro- and microscopic damage in VSL#3 group compared with CG. No animals that received VSL#3 developed cancer, being observed only a high grade of dysplasia	NP	↑ expression of angiostatin and vitamin D receptor in proximal colon in VSL#3 group, with greater expression observed in normal tissue than in carcinoma	NP	NP	Positive correlation between colon dysplasia index and diversity of microbiota in VSL#3 group
Do et al (2016)[48]	↓ tumor formation and macrophage infiltration in colon of groups that received VSL#3 alone or VSL#3 combined with BSZ	NP	↓ expression of MIP-1β, MCP-1, IL-6, IL-10, IL-11, IL-17, IL-22, and pSTAT3 in groups that received VSL#3 alone or in combination with BSZ. ↓ Bcl-2 expression and ↑ Bax expression in group that received combination therapy	NP	NP	↓ disease activity index in groups that received combined therapy
Talero et al (2015)[49]	↓ tumor incidence and macroscopic damage in colon of VSL#3 group compared with CG	NP	↓ positive PCNA in VSL#3 group before or during cancer induction. ↓ TNF-α, IL-1β, IL-6, and COX-2 expression in VSL#3 group	NP	NP	↓ disease activity index in VSL#3 group
Lee et al (2015)[50]	↓ adenocarcinoma development, dysplasia, and structural disruption area in group treated with *L plantarum* compared with CG	↑ fecal IgA concentrations in probiotic group	↓ expression of TNF-α, IL-6, IL-1β, IFN-γ, iNOS, COX-2, and Bcl-2; ↑ expression of p21, p53, and Bax in probiotic group	NP	NP	NP
Kim et al (2005)[51]	↓ tumor incidence, tumor size, and inflammatory cell infiltration in colon of animals supplemented with *B lactis* compared with CG	NP	Probiotic inhibited IκBα degradation, suppressed NF-κB activation, and ↓ COX-2 expression	NP	NP	NP

Abbreviations and symbols: AC, aberrant crypt; ACF, aberrant crypt foci; AMPK, AMP-activated protein kinase; Bcl-2, B-cell lymphoma 2; BSZ, balsalazide; CAT, catalase; CG, control group; CLA, conjugated linoleic acid; COX-2, cyclooxygenase 2; CRC, colorectal cancer; CXCR4, C-X-C chemokine receptor 4; DMH, 1,2-dimethylhydrazine; EGFR, epidermal growth factor receptor; ERK, extracellular signal-related kinase; GST, glutathione *S*-transferase; IFN, interferon; IgA, immunoglobulin A; iNOS, inducible nitric oxide synthase; IL, interleukin; MCP, monocyte chemoattractant protein; MHC, major histocompatibility complex; MIP-1β, macrophage inflammatory protein 1β; PCNA, proliferating cell nuclear antigen; NF-κB, nuclear factor κB; NK, natural killer; NP, not performed; p-Akt, phosphorylated Akt; PCNA, proliferating cell nuclear antigen; p-ERK, activated extracellular signal–regulated kinase; PPAR-γ, peroxisome proliferator-activated receptor γ; pSTAT, phosphorylated STAT; SCFA, short-chain fatty acids; SOD, superoxide dismutase; TLR, Toll-like receptor; TNF-α, tumor necrosis factor α; VSL#3, probiotic mixture; ↑, increased; ↓, reduced.

Table 6 **Main results of clinical studies on the effects of probiotics and synbiotics in colorectal carcinogenesis**

Reference	Histopathological evaluation	Immunological evaluation	Inflammatory/molecular markers	Oxidative balance evaluation	Microbial activity	Other
Pala et al (2011)[52]	NP	NP	NP	NP	NP	↑ survival in *L plantarum* group. Yogurt consumption associated with significant reduction in CRC development
Worthley et al (2009)[53]	Intervention did not alter crypt proliferation or cell height	NP	No significant difference in (A) short-chain fatty acids or ammonia in feces, or (B) levels of C-reactive protein or cytokines in serum	NP	↑ counts of Lachnospiraceae members. Fecal pH did not differ between groups	NP
Hatakka et al (2008)[5]	NP	NP	NP	NP	↑ fecal counts of *Lactobacillus* spp and *Propionibacterium* spp. ↓ β-glucosidase activity and urease activity	NP

Abbreviations and symbols: CRC, colorectal cancer; NP, not performed; ↑, increased; ↓, decreased.

DISCUSSION

The prevention of colorectal cancer improves quality of life and reduces healthcare costs. Despite the heterogeneity of the studies included in this review, the findings confirm the protective effect of probiotic and synbiotic consumption against colorectal cancer. Several protective mechanisms were identified: modulation of the composition and metabolic activity of the intestinal microbiota; reduction of inflammatory mediators; induction of tumor cell apoptosis or inhibition of tumor cell proliferation; modulation of the immune response; improvement of the intestinal barrier function; production of compounds with anticarcinogenic activity, and reduction of oxidative stress.

Most of the studies included in this review were preclinical studies performed in murine models, likely because barriers still exist in human studies, especially those that are well controlled. For a study to assess the ability of probiotics/synbiotics to decrease the risk of colorectal cancer, an experimental design with a long period of follow-up is required, as in prospective studies, which generate high costs.

To induce preneoplastic lesions or tumors, most of the preclinical studies used the drug 1,2-dimethylhydrazine or its active metabolite (azoxymethane), which are carcinogenic compounds widely used in experimental studies of colorectal cancer.[54] These drugs are highly specific, leading to the initiation and promotion of carcinogenesis in a dose-dependent manner.[55] The doses used for induction vary, although the azoxymethane dose is usually lower than that of 1,2-dimethylhydrazine, since azoxymethane is the metabolically active form of the drug.

A wide variety of probiotics were included in the studies, with the genus *Lactobacillus* used most often. However, there is no consensus in the literature supporting the use of a specific probiotic to reduce the risk of colorectal cancer.[6] Similarly, the dose of probiotic is still undefined. According to Galdeano and Perdigón,[56] counts between 10^8 and 10^9 CFU are sufficient to promote stimulation of the immune system specifically. The dosages used in the studies included in this review varied widely (between 10^6 and 10^{11} CFU/d), making it impracticable to suggest a specific dose.

These findings indicate that different factors, such as inflammation and increased oxidative stress, contribute to the establishment of colorectal cancer, causing profound changes in the tumor microenvironment. Thus, the aim of therapy with probiotics and synbiotics is to interfere in the inflammatory and oxidative process as well as in the genetic, epigenetic, and morphologic alterations that occur during carcinogenesis.

The association between chronic inflammation and malignant disease is well documented in inflammatory bowel disease.[57] Individuals with chronic inflammatory bowel disease, such as Crohn disease and ulcerative colitis, are at high risk for developing colorectal cancer.[58] In this review, studies that evaluated experimental models of colitis-associated colorectal cancer[27,44–51] also demonstrated a protective effect of probiotics or synbiotics, which resulted in a reduced incidence of tumors and decreased systemic and tissue inflammation. Probiotics/synbiotics stimulate the production of anti-inflammatory cytokines, reduce the production of proinflammatory cytokines, such as tumor necrosis factor, interleukin (IL) 1β, IL-6, IL-8, IL-12, and IL-17, and suppress the expression of cyclooxygenase 2.[25]

Arthur et al[27] observed a contradictory result in their study, in which the incidence of colitis-associated colorectal cancer was greater in IL-10 knockout mice after treatment with the probiotic VSL#3. In their study, increased concentrations of proinflammatory and immunologic mediators were observed. Adequate colonization of the microbiota is essential for the maturation and appropriate stimulation of the immune system, which protects the host against pathogens.[59] Microorganisms and their metabolites interact with immune cells through Toll-like receptors and nucleotide-binding oligomerization domain–like receptors. In turn, the immune cells begin to release cytokines that regulate the adaptive and innate response.[60,61] *Bacteroides fragilis*, for example, induces cancer by mechanisms that depend on the Th17 response, which is suppressed after administration of anti-IL-17 antibodies.[62–64] In addition, both chronic inflammation and the contact of pro-oxidant and carcinogenic agents with the intestinal lumen are directly related to an increase in oxidative stress and the production of free radicals. Exposure to these agents may lead to redox imbalance and DNA damage, contributing to the development of colorectal cancer.[65,66] Individuals with cancer have higher plasmatic and tissue concentrations of oxidative products when compared with healthy individuals.[67]

The proliferation of adequate numbers of beneficial bacteria, such as catalase producers, in the gut is thought to lead to increased antioxidant capability and protection against free radicals. Moreno et al[68] observed a reduction in hydrogen peroxide concentrations in rats induced to develop colorectal cancer and subsequently fed catalase-producing *Lactococcus lactis* (10^9 CFU/d, for 16 weeks). The administration of *Lactobacillus fermentum* increases the expression of superoxide dismutase and the glutathione complex (oxidized glutathione, glutathione peroxidase, and glutathione reductase), important phase II enzyme group of the biotransformation

process, which play an important role in phase II biotransformation reactions.[57,58,63,69,70] Furthermore, many prebiotics are rich in phenolic compounds that have antioxidant and anti-inflammatory activity, which may protect biomolecules such as DNA, lipids, and proteins against damage caused by free radicals.[68]

The beneficial effects of probiotics and synbiotics stem from their ability to modulate the composition and activity of the intestinal microbiota and to prevent colonization by pathogenic microorganisms. Rafter et al[71] evaluated 37 individuals with colon cancer and 43 polypectomized individuals who received a synbiotic for 12 weeks. The synbiotic contained inulin and oligofructose as a prebiotic and *Bifidobacterium lactis* Bb12 and *Lactobacillus delbrueckii* subsp *rhamnosus* GG as probiotics. They observed a significant change in the composition of the intestinal microbiota, ie, an increase in counts of *Bifidobacterium* and *Lactobacillus* organisms and a reduction in counts of the pathogen *Clostridium perfringens*. Furthermore, the function of the intestinal barrier improved.[71]

Pathogenic bacteria may produce carcinogenic agents through the activity of enzymes such as β-glucuronidase, β-glucosidase, azoreductase, and nitroreductase. These enzymes generate cytotoxic and genotoxic metabolites, such as polycyclic aromatic hydrocarbons, secondary bile acids, aglycones, aromatic heterocyclic amines, and *N*-nitroso compounds.[72] In addition, they increase the carcinogenic activity of cancer-inducing drugs.[54,73,74] The effect of β-glucuronidase administered in combination with a colorectal cancer–promoting drug was evaluated in 6 of the studies in this review.[5,30,34,36,42,43] The use of a probiotic or synbiotic may inhibit the activity of the enzymes mentioned above, and a reduction in the incidence of aberrant crypt foci is strongly correlated with a decrease in β-glucuronidase activity.[58,75]

The consumption of prebiotics, such as fructooligosaccharides and inulin, is associated with increased counts of *Lactobacillus* and *Bifidobacterium* organisms. These probiotics produce the enzyme β-fructosidase, which is responsible for the fermentation of fructooligosaccharides.[76] As a result, the availability of fermentable substrate contributes to the selective growth of beneficial bacteria. Upon fermentation, prebiotics produce short-chain fatty acids, mainly acetic, propionic, and butyric acids, which represent an important source of energy for the colonocytes, Short-chain fatty acids increase mucus production and promote the proliferation of healthy cells, thereby contributing to the adequate functioning of the intestinal barrier.[77–79]

Butyric acid has been widely studied as a protective agent against colorectal cancer and has been shown to play a role in protecting against oxidative DNA damage; regulating the balance between proliferation, differentiation, and apoptosis of the colonocytes; regulating the activity of Bcl-2, Bax, and caspases 3 and 7[80,81]; stimulating the production of anti-inflammatory cytokines such as IL-10[79]; and reducing the production of inflammatory cytokines by inhibiting the activation of nuclear factor κB and cyclooxygenase 2.[81] Recently, it has been shown to inhibit histone deacetylase, leading to chromatin condensation and transcriptional repression.[82,83] The capacity of butyrate and other histone inhibitors to promote or suppress tumoral growth is associated with hyperactivation of the Wnt/β-catenin pathway. This upregulation of Wnt signaling is related to the induction of apoptosis, although the mechanism is not yet fully understood.[84–86]

In experiments with HCT-116 tumor cells treated with sodium butyrate at a concentration of 5mM, changes in the expression of over 1000 genes related to the Wnt/β-catenin pathway were observed.[86] It is possible that the constitutive activation of this pathway, caused by mutation in the adenomatous polyposis coli (*APC*), β-catenin (*CTNNB1*), or axin (*AXN1*) genes, is the initiating event of colorectal tumorigenesis.[69]

Review studies are characterized by large amounts of evidence, since they allow multiple studies to be evaluated while still accounting for the variability between individual studies. This work examines the effects of probiotic and synbiotic use in colorectal cancer. The selection of literature was based on widely recommended and approved practices for systematic reviews. Moreover, risk of bias was assessed in accordance with the ARRIVE guidelines[18] and by adapting the quality evaluation criteria of Downs and Black,[20] which allows publication bias to be tested individually and, later, collectively. The risk-of-bias analysis clearly demonstrated that aspects related to the experimental design of individual studies had been neglected. Thus, there is a need to improve both the experimental design and the current guidelines for the reporting of animal experiments to ensure an adequate level of scientific evidence.

Finally, the methods employed and the parameters used for evaluation are extremely heterogeneous, with all studies reporting different measures. Interestingly, most articles did not report whether the study results were applicable to other species and systems, including humans. Considering the experimental model used in most studies and the relevance of colorectal cancer to the world's population, the translation and applicability of results to the treatment of humans is pivotal for future probiotic and synbiotic studies.

CONCLUSION

The development of cancer is related not only to genetic alterations but also, more importantly, to

environmental factors. The study of the intestinal microbiota is critical for increasing current knowledge about the prevention of colorectal cancer, since modulation of the intestinal microenvironment may alter the body's response to carcinogenic stimuli. The scientific evidence from in vivo studies demonstrates that the use of probiotics and synbiotics can reduce the incidence of preneoplastic lesions and tumors in animal models. In addition, it may delay the progression of cancer associated with inflammatory bowel disease. Although the protective effect likely depends on the bacterial species and specific fermentable substrates, there is still no consensus in the literature about the type of microorganism or the fermentable substrate to be used, the optimal dose, or the duration of treatment. There is also a need to improve the reporting of preclinical studies, which requires a collective effort from authors, journal editors, reviewers, and financial organizations to ensure the reproducibility, reliability, and generalization of evidence. Considering the promising results of in vivo studies and the lack of evidence of potential adverse effects associated with the use of probiotics and synbiotics (except when contraindicated), clinical studies must be prioritized in future research.

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Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website.

Table S1 **PRISMA checklist**

Table S2 **Risk-of-bias analysis (conducted according to ARRIVE guidelines) of experimental studies on the effects of probiotic and synbiotic use in colorectal carcinogenesis**

Table S3 **Risk-of-bias analysis of clinical studies on the effects of probiotic and synbiotic use in colorectal carcinogenesis**

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