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# Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer

Maya Raman,[1] Padma Ambalam,[2,*] Kanthi Kiran Kondepudi,[3] Sheetal Pithva,[4] Charmy Kothari, [2] Arti T. Patel,[5] Ravi Kiran Purama,[6] J.M. Dave[7] and B.R.M. Vyas[4]

[1]Department of Biotechnology; Bhupat and Jyoti Mehta School of Biosciences and Bioengineering; Indian Institute of Technology; Chennai, India; [2]Department of Biotechnology; Christ College; Rajkot, India; [3]Food Science Department; National Agri-Food Biotechnology Institute; Mohali, India; [4]Department of Biosciences; Saurashtra University; Rajkot, India; [5]SMC College of Dairy Science; Anand Agricultural University; Anand, India; [6]Department of Biotechnology; Lund University; Lund, Sweden; [7]Vrindavan Society; Rajkot, India

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Colorectal Cancer (CRC) is the second leading cause of cancer-related mortality and is the fourth most common malignant neoplasm in USA. Escaping apoptosis and cell mutation are the prime hallmarks of cancer. It is apparent that balancing the network between DNA damage and DNA repair is critical in preventing carcinogenesis. One-third of cancers might be prevented by nutritious healthy diet, maintaining healthy weight and physical activity. In this review, an attempt is made to abridge the role of carcinogen in colorectal cancer establishment and prognosis, where special attention has been paid to food-borne mutagens and functional role of beneficial human gut microbiome in evading cancer. Further the significance of tailor-made prebiotics, probiotics and synbiotics in cancer management by bio-antimutagenic and desmutagenic activity has been elaborated. Probiotic bacteria are live microorganisms that, when administered in adequate amounts, confer a healthy benefit on the host. Prebiotics are a selectively fermentable non-digestible oligosaccharide or ingredient that brings specific changes, both in the composition and/or activity of the gastrointestinal microflora, conferring health benefits. Synbiotics are a combination of probiotic bacteria and the growth promoting prebiotic ingredients that purport "synergism."

## Introduction

Cancer, the leading cause of mortality across the globe, was responsible for 7.6 million deaths in 2008 (13% of total mortality).[1,2] American Cancer Society in collaboration with National Cancer Institute, Centre for Disease Control and Prevention, North American Association of Central Cancer Registries and National Centre for Health Statistics, estimated a total of 1,638,910 new cancer cases and 577,190 deaths due to cancer in US during 2012. Colorectal cancer (CRC), prostrate, lung, stomach, liver and breast cancers are the major types of cancer that are associated with significant mortality every year. CRC is the second leading cause of cancer-related mortality and is the fourth most common malignant neoplasm in USA.[3]

*Correspondence to: Padma Ambalam; Email: Padma_ambalam@yahoo.com
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## Risk Factors Accountable for Colorectal Cancer

Rapid increase in the global burden of CRC is multifactorial, mainly attributed to certain genetic syndromes and environmental factors, such as dietary habits and life style changes, including, high meat and saturated fat consumption, chronic alcoholism, tobacco consumption and obesity.[4] CRC arise by a series of well-defined histological changes (the adenoma-carcinoma sequence), paralleled with mutational activation of oncogenes and loss of heterozygosity of tumor suppressor genes by carcinogenic chemicals and mutagens.[5,6] It occurs as a consequence of alteration in the equilibrium between DNA damage and DNA repair leading to cell progeny bearing mutagenic and/ or unrepaired DNA with mismatches that escaped during the DNA repair mechanism. (**Fig. 1**).[7,8] It originates in the inner most intestinal lining and spreads inward to the inner lining, muscle tissue and other organs, leading to metastasis. Although, procarinogens, per se, are not carcinogenic but are converted to later through a series of metabolic reactions by enzymes of cytochrome P450 family and transformed to highly reactive electrophilic compounds which could react with DNA. Moreover, the risk of CRC development depends on the potency of carcinogens, exposure rate and the genetic constitution of the individual.

Environmental carcinogens could be exogenous or endogenous in origin and linked to diet and dietary habits, life-style factors (nutrition, tobacco consumption, physical activity, etc.) and occupational hazards.[9] Polycyclic aromatic hydrocarbons (PAH), heterocyclic amines (HCA), N-nitroso compounds (NOC), mycotoxins (aflatoxins) and acrylamide are well-known food carcinogens responsible for CRC, breast and prostate cancer, as reported in preclinical and clinical studies (**Table 1** and **Fig. 2**).[10-17] These are produced from precursors during food processing methods, for instance, curing, drying, smoking, roasting, refining and fermentation and air pollution.[10,11,14] A strong correlation between regular consumption of food cooked at elevated temperatures and increased incidence of CRC was reported previously.[10] Epidemiological studies showed that HCA

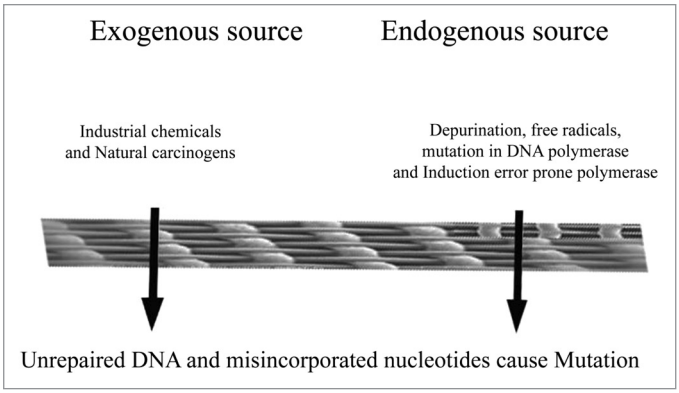


**Figure 1.** Causes of alterations in the equilibrium between DNA damage and DNA repair and unrepaired DNA escape this equilibrium.

are responsible for adenomatous polyps and the onset symptoms of CRC.[18-21] Moreover, dose-dependent relationship was found between cancer risk and the dietary exposure to compounds namely, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3,4,8-trimethyl-3H-imidazo[4,5-f]quinoxaline (DiMeIQx), 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline (MeIQx) and benzo[α]pyrene (BαP).[22] Under such inevitable situations where one is continuously exposed to food and environmental carcinogens, the antimutagenic agents could play a vital role in complete elimination of mutagens from the host system. Dietary components from natural resources such as fruits, vegetables and cereals, could be an excellent antimutagenic agents.[23] Probiotics and prebiotics gained a lot of attention as antimutagens for their noted carcinogen scavenging and elimination activity. In such scenario, a food supplement/nutraceutical, principally rich in probiotics, prebiotics and synbiotics would serve as best biological therapy in the removal of food-borne mutagens and carcinogens; thus, preventing CRC.

## Gut Microbiome in CRC

In spite of CRC treatment by surgery, chemotherapy and radiotherapy, the success rate for cancer treatment is still variable, with high mortality and other adverse side-effects. There is an urgent need to find an alternative solution to this quest. More recently, studies are focused on elucidating the functional accountability of gut microbiota in colon carcinogenesis.[24-26]

Human gastrointestinal tract (GIT), from small intestine to colon, harbours a variety of bacterial species approximately containing $10^7$ to $10^{12}$ cells per gram of the intestinal content.[24,27] Babies are born with a sterile intestinal tract that gets swarmed with favorable and unfavorable microbes along with the first feed; and following the childhood, the intestinal microflora remain fairly constant until the alterations are brought by the environmental factors, life style and modified genetic set-up. Human gut microbes are broadly categorized as symbionts, commensals and pathobionts.[28]

Gut microbiota performs vital functions of the host including, immune and nutritional status, thus, assist in health maintenance.[29] Equilibrium among various gut bacterial strains and host immunity decide the occurrence of physiological (regulates the presence of resident gut microbiota) and pathological inflammation (depends on the number and virulence of the invading pathogens). Besides these, chronic inflammation profoundly triggers local immune response leading to the release of reactive oxygen species (ROS) and nitric oxide that induce DNA damage and consequently altering tissue homeostasis.[30] Cytokines produced during this process play a major role in tissue homeostasis. TNF-α, IL-6, IL-1 and chemokines induce tumor growth by promoting angiogenesis and suppressing immune-mediated tumor elimination; and, IL-10 and TGF-α acts as inhibitor in cancer establishment.[30] Thus, altered gut microbiota promote pathogenesis through chronic inflammation, immune evasion and suppression. Colonoscopic studies showed varied distribution of bacterial genera in CRC patients, based on the disease status.[31] Significant elevation in *Bacteroides/Prevotella* population were reported in cancer patients and were correlated with the elevated levels of IL-17 producing cells in the mucosa. A conspicuous difference in the microbial colonization patterns between the tumorous tissue and adjacent non-malignant mucosa suggests that CRC-associated physiological and metabolic changes recruit tumor-foraging commensal-like bacteria (*Clostridium* spp).[32] In the recent years, a great deal of research has been dedicated in understanding the role of specific microbes/microbial community/microbial molecules that confer health benefits under patho-physiological conditions. These microbes may have an apparent competitive advantage in the tumor microenvironment, in replacing pathogenic bacteria in CRC etiology. The dynamic interplay between intestinal microbial ecology (balance between favorable and unfavorable bacteria) and sporadic CRC was investigated by Marchesi et al., which might be an important lead toward the novel microbiome-related diagnostic tools and therapeutic interventions.[32]

With the fact that in colonic environment, microflora and diet are closely involved in the etiology of CRC, an intense interest has been shown toward the use of probiotics, prebiotics and synbiotics in modulating gut microbiota, host metabolism and thereby aiding in cancer prevention.[33,34] Hence the concept of probiotics, prebiotics and synbiotics, having a myriad of health-promoting effects is becoming a revolution. They have shown to alleviate lactose intolerance, lower serum cholesterol level, exert anticancer effect, improve constipation, enhance immunity, regulate obesity and relieve of vaginitis.[35,36] Studies focusing on the anti-cancerous activity of probiotics, prebiotics and synbiotics against colorectal, breast and bladder cancer in pre-clinical and clinical trials, have been reported previously.[37-39] This review is an attempt to summarize the role of probiotics, prebiotics and synbiotics in the prevention of CRC (**Fig. 3**).

## Probiotics as Anti-Carcinogens and Anti-Mutagens: Mechanism of Action

Anticancerous (ACA) and antimutagenic activity (AMA) of probiotics is due to the following:

(1) Mutagen binding, degradation and mutagenesis inhibition by probiotics

**Table 1.** Major mutagens that have been identified in fried food, such as fried beef or fish are as follows

| Short name | Chemical names | Molecular weight |
|---|---|---|
| Phe-P-1 | 2-amino-5-phenylpyridine | 170 |
| TMIP | 2-amino-n,n,n-trimethyl-imidazo{4,5-f]-pyridine | 176 |
| AαC | 2-amino-9H-pyrido-[2,3-b]-indole | 183 |
| GLU-P-2 | 2-aminodipyrido-[1,2-a:3',2'-d]-imidazole | 184 |
| Trp-P-2 | 3-amino-1-methyl-5H-pyrido[4,3-b]-indole | 197 |
| MeAαC | 2-amino-3-methyl-9H-pyrido[2,3-b]-indole | 197 |
| IQ | 2-amino-3-methyl-imidazo[4,5-f]-quinoline | 198 |
| IQx | 2-amino-3-methyl-imidazo[4,5-f]-quinoxaline | 199 |
| Trp-P-1 | 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]-indole | 211 |
| 4-MeIQ | 2-amino-3,4-dimethyl-imidazo[4,5-f]-quinoline | 212 |
| 8-MeIQx | 2-amino-3,8-dimethyl-imidazo[4,5-f]-quinoxaline | 213 |
| 4-MeIQx | 2-amino-3,4-dimethyl-imidazo[4,5-f]-quinoxaline | 213 |
| PhIP | 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]-pyridine | 224 |
| 4,8-DiMeIQx | 2-amino-3,4,8-trimethyl-imidazo[4,5-f]-quinoxaline | 227 |

(2) Prevention of nontoxic procarcinogen conversion to harmful, toxic and highly reactive carcinogens

(3) Lowering of intestinal pH by short chain fatty acids (SCFA) produced during non-digestible carbohydrate degradation

(4) Modulation and enhancement of the host's innate immunity through the secretion of anti-inflammatory molecules.

**Mutagen binding, degradation and mutagenesis inhibition by probiotics.** Potential probiotic strains bind the mutagens through the cell surface and peptidoglycans (sugar and protein moieties) and exert AMA and ACA.[40-44] Cellular fraction and cell wall of *Streptococcus cremoris* Z-25 binds 3-amino-1,4-dimethyl-5H-pyrido-[4,3-b]indole (Trp-P-1) and 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2).[43] The cell wall skeleton of *S. cremoris* Z-25, *L. acidophilus* IFO13951 and *B. bifidum* IF014252 binds Trp-P-1, 2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole (Glu-P-1), 2-amino-5-phenylpyridine (Phe-P-1), 2-amino-3-methylimidazo[4,5-f] quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ) and MeIQx. Similarly, the AMA of the proteolytic variant of *L. helveticus* was due to the peptides released from the fermented milk, whereas, the non-proteolytic variant did not show similar effects.[44] However, the exact mechanism of mutagen binding by peptides and its elimination was not elucidated and warrants further studies. In another study, four strains of *L. gasseri* and *B. longum* showed high binding and AMA against Trp-P-1, Trp-P-2, Glu-P-1, IQ and MeIQ.[40] Binding was dependent on the chemical nature of the mutagen, pH, bacterial strain and the complex array of polysaccharide on the cell wall receptor sites. Sreekumar and Hosono emphasized on importance of using multiple probiotics strains in removing the broad spectrum of mutagens and carcinogens.[40]

AMA of "Natto" (a *Bacillus subtilis*-fermented soybean product) against HCAs was due to the binding of HCAs to the bacterial cell-wall structures[45] that was dependent on the strain, mutagen chemical nature, pH, incubation time, metal ions, concentration of sodium chloride and alcohol, enzymes and acetylation of Trp-P-1 and IQ.[45] Similarly, aflotoxin $B_1$ ($AFB_1$) binding studies to viable, heat- and acid-treated cells of *L. rhamnosus* GG implicated the involvement of cell wall in capturing $AFB_1$ and is mainly attributed to cell wall carbohydrate components and hydrophobic and electrostatic interactions.[46] Further binding of $AFB_1$ to lactobacilli and bifidobacteria was found to be reversible.[47]

Culture free supernatants of *L. plantarum* KLAB21 (kimchi, a Korean fermented food) showed high AMA against *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), NQO, 4-nitro-*O*-phenylenediamine (NPD) and $AFB_1$.[48] AMA against MNNG was due to three secretory glycoproteins (16, 11 and 14 kD).[42] Detoxification of mutagenic compounds (AαC, PhIP, IQ, MeIQx and DiMeIQx) by different LAB was reported[49] and highest detoxification effect was observed for *L. helveticus* and *S. thermophiles*, which was seven to eight times more effective than *L. kefir* and *L. plantarum*. *L. bulgaricus* and *S. thermophilus* exhibited high AMA against 4-NQO and 2-aminofluorene than fermented milk extracts by *S. thermophilus* alone.[50] Similarly, soymilk fermented with *S. thermophilus, L. acidophilus, B. infantis, B. longum* showed higher AMA against 3,2-dimethyl-4-amino-biphenyl (DMABP) due to the production of antimutagenic molecules during bacterial fermentation of milk.[51] AMA of three *B. longum* strains in fermented skim milk against Trp-P-1 and Trp-P-2 increased with time.[52] Dose-dependent inhibition of Trp-P-1 by *B. longum* PS+ strain was due to the involvement of crude polysaccharides in binding and AMA.[52] In another study, irreversible mutagen binding and AMA by lactobacilli and bifidobacteria were attributed to the butyrate production, a SCFA, that acts at molecular level, as discussed later in prebiotics section.[53] This emphasizes the importance of viable probiotic bacteria consumption. Exopolysaccharides (EPS) produced by *L. plantarum* 301102 inactivated the mutagen, Trp-P-1.[54]

AMA of probiotic bacteria is growth phase dependent. LAB and bifidobacteria produced extracellular bioactive compounds with differential AMA against B (α) P and sodium azide (SA) at

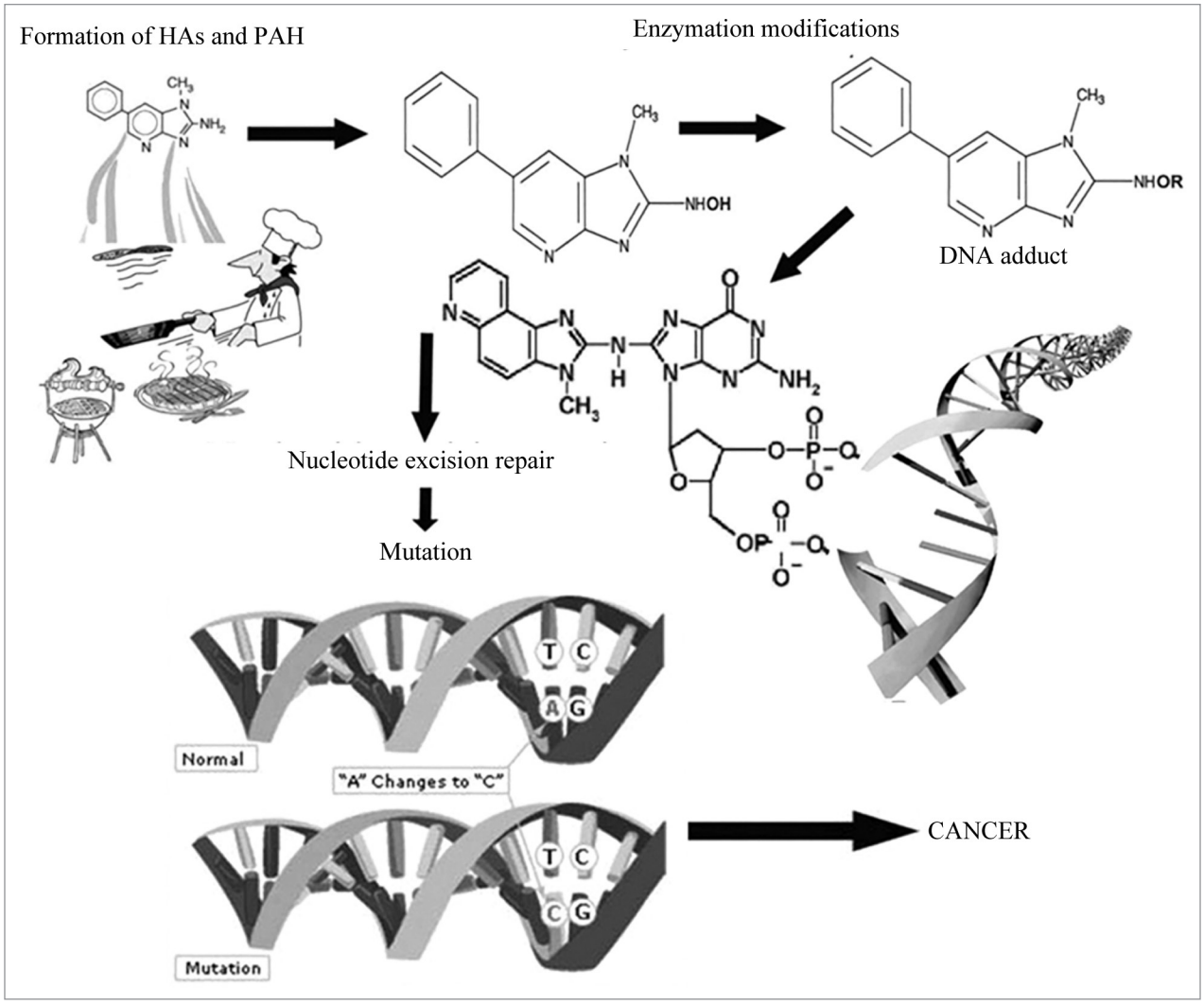


**Figure 2.** Role of food mutagens in causing cancer.

different times of growth.[55] Lactobacilli had higher AMA against B (α) P and SA in the stationary phase, whereas *B. adolescentis* ATCC 15703 exhibited higher AMA against B (α) P in the exponential phase but showed no activity against SA suggesting a strong correlation between bacterial AMA, growth phase and mutagen type.[55]

Concisely, mutagen binding by probiotic strains (lactobacilli and bifidobacteria) depends on peptidoglycans, polysaccharides, secretary glycoproteins, the growth phase and mutagen type. Further studies are required for precise understanding of the role of cell surface components of LAB and bifidobacteria in antimutagenesis. With the current advancement in molecular techniques, it is possible to accomplish mechanistic based studies to understand mutagen binding by different probiotics.

**Prevention of nontoxic procarcinogens conversion to harmful, toxic and highly reactive carcinogens.** Probiotic bacteria along with dietary ingredients helps in detoxification and biotransformation of procarcinogens and carcinogens into less toxic metabolites, thus preventing tumor formation.[56] Biotransformation of mutagens/carcinogens occur in the gut with the help of phase I and phase II enzymes and regulate the toxic, mutagenic and neoplastic effects of environmental carcinogens. These enzymes, in turn, are modulated by dietary agents.[61] Phase I enzymes causes bio-activation and phase II enzymes causes the inactivation of mutagen/carcinogen.[57] *Lactobacillus* strains from different commercial dairy products exhibited > 80% antigenotoxicity against 4-NQO.[58] Antigenotoxicity of *Lactobacillus* strains against NQO and MNNG was attributed to strain dependency. *L. casei*, *L. acidophilus*, *L. rhamnosus*, *L. delbrueckii* subsp *bulgaricus* and *L. plantarum* had higher antigenotoxicity against NQO, while *L. acidophilus* had higher activity against MNNG.[59] Antigenotoxic activities were correlated with the spectral modifications observed for procarcinogens/genotoxins after probiotic treatment. However, degraded products were not detected.[59] Strains retained their viability during and after the genotoxin exposure probably elucidating the role and necessity of viable bacteria in antigenotoxicity. Antigenotoxicity of *L. rhamnosus* IMC 501 against 4-NQO was also explored, and biotransformation and detoxification of the mutagen was evaluated.[60]

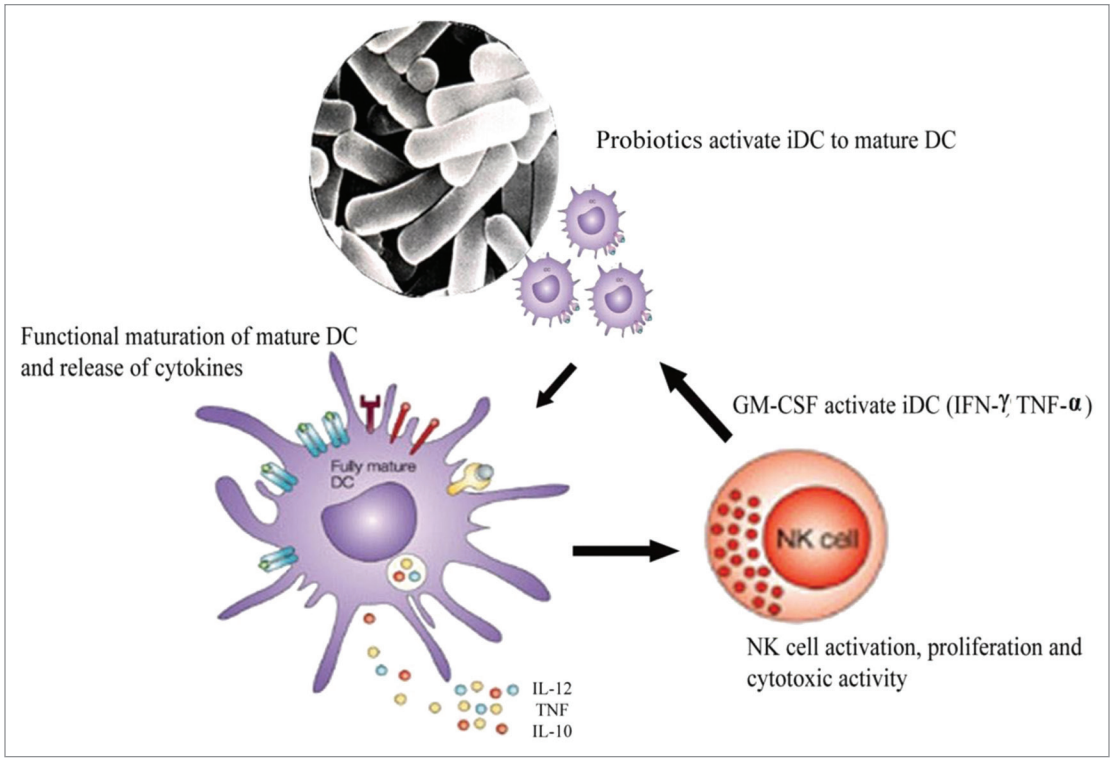


**Figure 3.** Mechanism exhibited by probiotics, prebiotics and synbiotics against prevention of colorectal cancer.

In vitro experiments showed that *L. casei* DN 114001 (Actimel strain) adsorb and metabolized IQ, PhIP and MeIQx.[61] The degree of detoxification was correlated with incubation time, cell growth and composition of the growth medium. Cells in the active growth phase showed higher activity.

Potential probiotic human strain *L. rhamnosus* 231 (Lr 231), was shown to possess antimicrobial activity against several human pathogens.[62] Further, this strain exhibited in vitro binding of MNNG and MeIQx and biotransformation; and subsequent detoxification and AMA.[63] Administration of viable Lr 231 protected rats from MNNG-induced colon inflammation.[64] Levels of azoreductase, nitroreductase activities in the feces were reduced. Glutathione reductase activity was increased while glutathione S-transferase activity was reduced in the Lr 231-fed group.[64] This was also evident in the histopathological sections of Lr 231-fed group.[64] Therefore, Lr 231 supplementation protected the animals from MNNG-induced inflammation. Safety of this strain is proved in mouse model.[65] Researchers are further investigating in detail the mechanism of biotransformation and degradation of different mutagens by Lr 231. Understanding the mechanism involved in biotransformation of mutagens/carcinogens by the probiotic bacteria may offer new ways for the management of mutagen or carcinogen-induced CRC.

**Inhibition of procarcinogen conversion to carcinogens.** Bifidobacteria and lactobacilli decreased the expression of xenobiotic-metabolizing enzymes compared with bacteroides, clostridia and enterobacteriaceae that mediate carcinogenesis through various enzymes, such as, β-glucuronidase, azoreductase and nitroreducatses.[66] Certain strains of *L. acidophilus* and *Bifidobacterium* spp lowered the activity of these enzymes and reduced the risk of tumor development.[66-69] SCFAs produced from colonic non-digestible carbohydrate fermentation enhance the growth of lactobacilli and bifidobacteria and inhibit the generation of carcinogenic products from procarcinogens by lowering enzyme levels.[66,70] Modulation of conversion of procarcinogens to carcinogens by beneficial bacteria, is yet another exciting area that needs further detailed investigation at cellular and molecular level.

**Lowering of intestinal pH.** Probiotic bacteria produce lactic acid and other SCFAs as metabolic products from non-digestible carbohydrate fermentation in the gut. These SCFAs decreases the load of pathobionts, helps in maintaining homeostasis and lower the intestinal pH.[66] It also assists in lowering solubility of bile acids and ammonia absorption and increases mineral absorption.[70-73]

**Activation of the host immune system.** The immune system is a complex cascade, acting and reacting locally at systemic level. Recently, research is focused on understanding the regulation of immune system and the interactions within and between the components of inflammatory cascades.[74] Different markers are used to explore these cascades and they are integrated to cope up with the microbial challenges from the environment and to manage common or severe infections.[75,76] Dendritic cells (DCs) and natural killer (NK) cells play a critical role in early defense against cancer.[77,78] Desmutagenic activities of different LAB strains were reported to regulate myeloid DCs maturation, polarizing the subsequent T-cell activity toward Th1, Th2 or

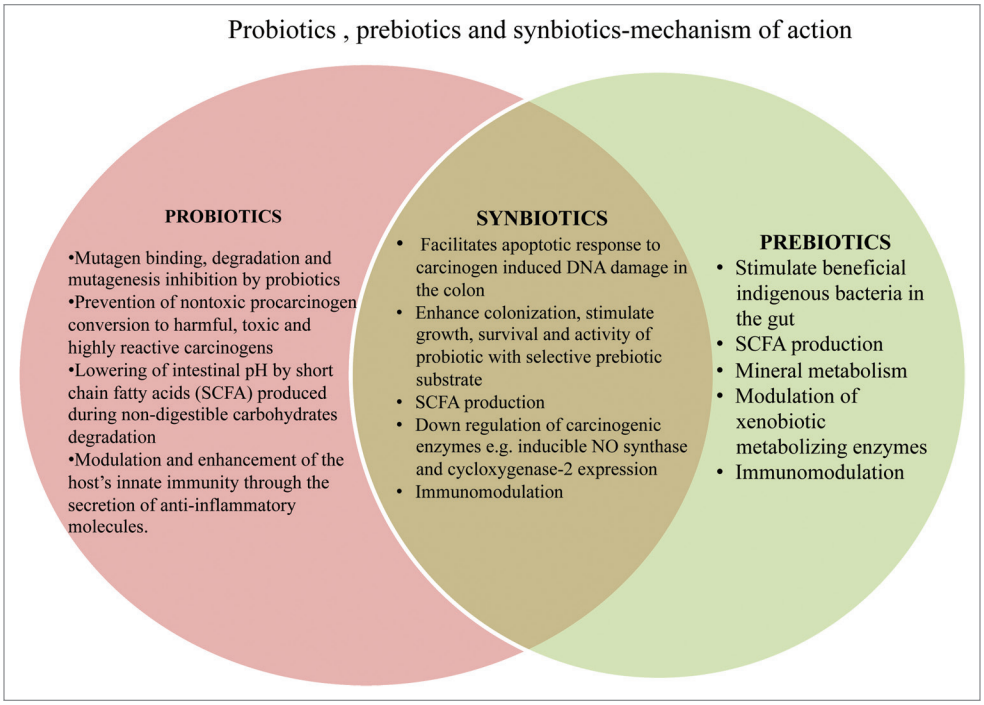


**Figure 4.** Desmutagenic potential of probiotics in preventing cancer. Interaction of probiotics with immature dendritic cells (DC) leading to activation of cascade and NK cell activation, proliferation and cytotoxic activity.

even T-reg responses.[79,80] Probiotic-induced immune suppression of carcinogenesis is critically reviewed elsewhere; NK cells were effective against tumor cells and low NK cell activity was associated to lower cancer risk.[81] Oral administration of *L. casei* strain Shirota (LcS) to methylcholanthracene-induced tumor in mice, displaying enhanced host innate immunity by stimulating the spleenic NK cell activity; thus, leading to delayed onset of tumor development.[82] Intrapleural injection of an inactivated strain of LcS in mice improved the immune system against tumor.[83] This anti-tumor effect was reversed by using anti-TNF monoclonal antibodies, implicating the release of tumor necrosis factor-α (TNF-α), as the anti-tumor agent, signifying a cellular immune mediated effect. LAB modulated DCs, which in turn, are potent activators of NK cells.[84] DCs encountering probiotics undergo maturation, stimulating NK cells. This desmutagenic potential of probiotics has been recently addressed, and now it's evident that human monocyte-derived DCs, blood DCs, mouse spleenic and lymph node DCs were matured by IL-12 produced upon LAB induction. Later this molecule IL-12, activates NK cells to produce IFN-γ, which is in concordance with the belief that IL-12 is essential for IFN-γ production in NK cells (**Fig. 4**).[85] In addition to producing SCFA and lowering the intestinal pH, probiotics might also exert beneficial effect by macrophage activation, cytochrome p450 blocking, reduction of carcinogen generation, downregulation of Ras-p21 expression, increase of cell differentiation, inhibition of cyclooxygenase-2 upregulation and inhibition of nitric oxide synthetase.[34] Hence, both bioantimutagenic and desmutagenic activity of probiotics could be highly significant in preventing cancer; and needs further elaboration.

## Prebiotics and Cancer

"Prebiotic" is defined as "a selectively fermentable non-digestible oligosaccharide or ingredient that brings specific changes, both in the composition and/or activity of the gastrointestinal microflora, conferring health benefits."[86-90]

Among different prebiotics, fructooligosaccharides (FOS) and galactooligosaccharides (GOS) are the two frequently reported and are "Generally Regarded as Safe" (GRAS). Others include, xylooligosaccharides (XOS), isomaltooligosaccharides (IMOS), glucooligosaccharides, pectin oligosaccharides (POS), mannanooligosacharides (MOS), gentiooligosaccharides (GTO), chitooligosaccharides (CHOS), soy bean oligosaccharides (SOS) and polydextrose that are not commercially available in high purity and the safety of these oligosaccharides are yet to be evaluated.[85,887,90]

## Prebiotics: Anticarcinogens and Mechanism of Action

ACA of prebiotics is mainly due to the following properties:

(1) Stimulation of beneficial indigenous gut bacteria

(2) Production of SCFAs and lactic acid, as fermentation products

(3) Modification of gene expression in cecum, colon and feces

(4) Enhanced micronutrient absorption in the colon

(5) Modulation of xenobiotic metabolizing enzymes

(6) Modulation of immune response.

**(1) Stimulation of beneficial indigenous gut microbes.** Human gut microbiome exhibit high degree of compositional

stability and could be manipulated by prebiotics, probiotics and antibiotics.[91,92] Prebiotics positively alter the gut microbiome and its dynamics by stimulating lactobacilli and bifidobacteria that bind and eliminate carcinogens from the gut system.

Animal model studies using prebiotic feed supplements have shown a profound effect in prevention and treatment of CRC. Feeding of long-chain inulin-type fructans increased bifidogenic effect, lowered pH and modulated immunity; and reduced the azoxymethane (AOM)-induced colonic pre-neoplastic aberrant crypt foci (ACF), small intestinal and colon tumors in rodent model.[93] Similar inhibitory effects of short FOS and inulin were also reported on ACF-induced rat models.[94,95] XOS and FOS were observed to inhibit colonic ACF in dimethylhydrazine (DMH)-treated rats by lowering cecal pH and serum triglyceride concentration. It also caused gain in total cecal weight, increased bifidobacterial population and noticeable reduction in the number of ACF in the colon.[96] XOS were shown to exhibit higher bifidogenic effect and was more effective than FOS. GOS also significantly inhibited the development of DMH- or AOM-induced colorectal tumors.[97,98] In human volunteer studies, administration of 10 g trans-GOS increased the bifidobacterial count and modified the fermentative activity of colonic flora.[99]

Consumption of inulin, FOS and GOS caused a laxative effect upon reaching the large intestine where they underwent fermentation, stimulated microbial growth resulting in increased bacterial mass, fecal bulking and peristalsis.[100,101] Fecal bulking helps in cancer prevention by reducing transit time and mutagen contact time to intestinal lining.

**(2) Production of SCFAs and lactic acid, as fermentation products.** Colonic bacterial fermentation of prebiotics produces SCFA, which are the key products in maintaining gut health, intestinal morphology and function.[70,102] Commonly formed SCFA are acetic, propionic and butyric acid approximately occurring in molar ratio of 60:20:20. Butyrate serves as an energy source for colonocytes and lowers luminal pH. At molecular level, butyrate acts as histone deacetylase inhibitor, promoting epigenetic hyperacetylation of histones and non-histone proteins (regulate the expression of critical cell cycle regulators CDKN1A),[103] altering DNA-methylation, which results in enhanced accessibility of transcription factors to nucleosomal DNA, as mentioned previously.[102-107] It was also associated with induction of cell differentiation, suppressed proliferation and enhanced apoptosis, to eliminate DNA-damaged cells that might otherwise progress to malignancy both in vitro and in vivo conditions.[108-111] Lactate also improves gut health and gut-associated immune defense and increases adsorption surface area.[112] Propionate and acetate induces apoptosis in human colorectal carcinoma cell lines through the loss of mitochondrial trans-membrane potential, generation of ROS, caspase-3-processing and nuclear chromatin condensation.[102,113] Recently, SCFA has caught greater attention for its ACA and is investigated extensively.[69,113-116] Equally, ACA of circulating propionate and acetate is imprecise and needs further investigation.

**(3) Enhanced micronutrient absorption in the colon.** The small intestine is the principal site of mineral absorption; however, minerals are absorbed throughout the gut, exerting beneficial effects. Consumption of short- and long-chain fructans increases mineral (calcium, selenium), vitamin (vitamin D) and antioxidant absorption that aid in decreasing cancer risk and maintaining normal bowel structure.[117] Inulin, oligofructose, FOS, GOS, SOS, resistant starches (RS), sugar alcohols and difructose anhydride have positive effect on mineral absorption and metabolism.[118] The underlying interplay between prebiotic-mineral absorption and ACA are manifold and includes, increased solubility of minerals due to local production of SCFA, occurrence of SCFA-salt conjugates, augmented absorption surface, increased expression of calcium-binding proteins, degradation of phytic acid-mineral complex that liberate associated minerals, release of bone-modulating factors, such as, phytoestrogens from foods, stabilization of intestinal microflora and intestinal mucus.[117-120]

**(4) Modulation of xenobiotic metabolizing enzymes.** Xenobiotic metabolizing enzymes are the indices of carcinogenicity. They are categorised into phase I and phase II that participates in carcinogen activation and metabolism. Phase I enzymes include, cytochrome-b5, cytochrome-b5 reductase, cytochrome P450, cytochrome P450 reductase, cytochrome P450 2E1 while, phase II include glutathione S-transferase (GST), uridine diphospho-glucuronyl transferase and DT-diaphorase that reduces the activation of procarcinogens to reactive carcinogenic intermediates and its elimination from the body.[121] Modulation of phase I and phase II enzymes by dietary agents that have chemopreventive potential are explained earlier in the probiotics section.[57] Resistant starch (RS) was observed to induce glutathione transferase π in rat colon.[66] SCFA also induced glutathione transferase π as determined using Caco-2 cells.[121] LAB makes pronounced stimulation of NADPH-dependent ferrihemoprotein reductase activity (cytochrome P450 reductase) in the colonocytes.[122] Feeding prebiotics alone or in combination with horse chestnut/flaxseed reduced the β-glucuronidase activity and increased the β-galactosidase and β-glucosidase activity emphasizing the ACA of prebiotics.[123] Similar observations of reduced bacterial β-glucuronidase activity and lower amounts of toxic ammonia in faeces were reported in corn hemicelluloses-fed healthy human volunteers.[124] Arabinoxylooligosaccharides (AXOS) stimulated carbohydrate-fermenting bacteria to increase the uptake and assimilation of nitrogen and excretion of ammonia through faeces in healthy human volunteers.[125] Reduced serum ammonia levels were observed in patients with liver cirrhosis upon XOS intake.[126] This interaction of prebiotics, with gut flora and their proposed role of modulation in expression of xenobiotic metabolizing enzymes, together with its ACA, has barely been investigated and needs thorough research in order to understand and reveal the role of phase I and phase II enzymes.[57] The protective effect of fructans, prebiotics and probiotics (lactobacilli and bifidobacteria) on AOM-induced carcinogenesis could be contemplated to the downregulation of gene-expression of inducible NO-synthetase and cyclooxygenase-2.[127] Regulation of gene expression in the colon by the administration of prebiotics is yet another interesting topic that needs further research.

**(5) Modulation of immune response.** Prebiotics may indirectly exhibit immunogenic effects by influencing the intestinal

microflora and modulates immune parameters like NK cell activity, secretion of IL-10 and interferon and lymphocyte proliferation.[128] Consumption of prebiotics may modulate immune parameters in gut-associated lymphoid tissue (GALT), secondary lymphoid tissue and peripheral circulation.[129] There is a paucity of reports examining the influence of prebiotics on the GALT for the improvement of human immune system and ACA. GOS was shown to reduce colitis in Smad3-deficient mice by modulating the function and trafficking of NK cells.[130] Although, prebiotics are shown to have immunomodulatory effect, more studies are necessary to establish the mechanistic role of prebiotics-induced immunomodulation against cancer.

## Dietary Fibers and its Anticarcinogenic Effect

Dietary fibers, similar to prebiotics, possess ACA that could be attributed to mutagen binding, diluting procarcinogens and carcinogens through fecal bulking and SCFA production.[131-134] Monomeric composition and chain conformation of dietary fiber influence the rate and extent of fermentation and eventually, its ACA. Consumption of cereals, pulses, fiber-rich fruits and vegetables reduce the incidence of colorectal adenomas.[134] Protective effect of dietary fibers against DMH, HCA and PAH were reported using animal models and in vitro assays.[133,134] It was speculated that mutagen or carcinogen binding to dietary fibers or prebiotics may involve interaction between free functional groups of mutagens and dietary fibers or prebiotics and might not merely be an adsorption phenomenon.[135] Thus, dietary ingredients could help in cancer prevention by modulating biotransformation of mutagen to less toxic compounds, thereby, making carcinogens less active. However, the role of prebiotics and dietary fibers in CRC prevention needs to be confirmed in human subjects. Another leading issue is to constitute the relation between prebiotics and colonic bifidobacterial count. Most of the available data merely imply the relationship between changes in bacterial composition, reduction in ACF and carcinogenesis. However, no concrete evidence about the involvement of the growth stimulation of lactobacilli, bifidobacteria or other healthy microbial inhabitants are available so far and needs a detailed study using advance techniques to establish this relationship.

## Synbiotics and Cancer

"Synbiotics" ("syn"-together and "bios"-life) is "a combination of probiotic bacteria and the growth promoting prebiotic ingredient" that purport "synergism". Kolida and Gibson, proposed two synbiotic concepts:[136]

(1) Complementary concept: A single or combination of probiotic bacteria, selected based on the specific-desired host benefits, and prebiotics that are independently chosen to stimulate the beneficial gut microbial population. Prebiotics promote growth and activity of the ingested probiotic, but only indirectly as part of its target range.

(2) Synergistic concept: Specific host beneficial probiotics are selected and the prebiotic component is chosen to specifically enhance the survival, growth and activity of the selected probiotic strain(s). However, the prebiotic may also increase the levels of resident gastrointestinal beneficial microbiota of the host.

An ideal synergistic synbiotic supplement should contain an appropriate single or multi strain probiotic and suitable mixture of prebiotics, where the later selectively favors the former and produce additive or synergistic effect.[136,137] It should favor the multiplication of the endogenous beneficial bacteria and reduce the number of cancer-promoting bacteria.

## Synbiotics: Anticarcinogens and Mechanisms of Action

Anticarcinogenic effect of synbiotics is ambiguous and is still under debate. The possible mechanisms could be the following:

(1) Facilitating apoptotic response to carcinogen-induced DNA damage in the colon

(2) Enhancing colonization, stimulate growth, survival and activity of probiotics in the presence of selective prebiotic substrate

(3) Increase SCFA production, anti proliferative activity of synbiotics and downregulation of inducible NO-synthase and cycloxygenase-2 enzymes, involved in colon carcinogenesis

(4) Immunomodulation

(5) Modification of colonic bacterial ecosystem, leading to an overall improvement in metabolic activity of the colon and cecum.

Diets rich in olive oil and extracts from freeze-dried fruits and vegetables considerably reduce the intestinal adenomas in mice indicating that calorie restriction and diet modulation would affect the intestinal microbiota and prevent carcinogenesis.[138] *B. longum* with a derivative of inulin ("Raftiline HP") also brought similar beneficial changes in cecal physiology and bacterial metabolic activity, reduced tumor risk and the incidence of an AOM-induced putative pre-neoplastic colonic lesions in the rodent model.[139] Combination of *B. lactis* and resistant starch (RS) facilitated the acute apoptotic response to a genotoxic carcinogen (AARGC) and colonic fermentative events in rat model.[140] RS serves as a metabolic substrate for *B. lactis* to exert its pro-apoptotic action. Same research group later in another study reported the protective effect of *B. lactis* and RS individually and their synbiotic combination (*B. lactis* and RS) in AOM-induced CRC in rodent models.[141] Fermentation events were altered by the inclusion of RS into the diet. This supports the complementary synbiotic concept and proves the superior preventive strategy of synbiotics over prebiotic or probiotic alone.[136,141]

Fructans and synbiotic combination containing fructans with *B. lactis* (Bb12) and *L. rhamnosus* GG minifies the AOM-induced colorectal adenomas and carcinogenesis by increasing SCFA production, lowering proliferative activity and the expression of GST placental enzyme pi-type, inducible NO-synthase, cyclooxygenase-2 enzymes, involved in the pathogenesis of CRC.[127] Anti-tumorigenic effect of synbiotic combination, oligofructose-enriched inulin, *L. rhamnosus* and *B. lactis* was due to immune-modulation, and it was demonstrated that peripheral blood mononuclear cells (PBMC) and Peyer's patches (PP)

were the primary tissues that were specifically affected by prebiotics.[142] Moreover, prebiotic supplementation alone induced significant immune-modulation in the intestine, whereas probiotic supplementation was primarily effective when provided as a component of synbiotic. These studies stressed the significance of synbiotic containing prebiotics and probiotics in CRC treatment. Same group reported that AOM treatment significantly reduced NK-cell like cytotoxicity in control, probiotic and prebiotic supplemented groups. In synbiotic supplemented group, NK cell like cytotoxicity in PP was prevented compared with control rats.[143] Additionally, synbiotic and prebiotic supplemented groups stimulated IL-10 production and reduced interferon-γ production in PP. Largely, synbiotic supplementation in carcinogen-treated rats modulated immune function in the PP and coincided with a reduced number of colon tumors by GALT modulation.[143]

ACA of FOS together with *Bifidobacterium* strain and lactitol in conjunction with *Lactobacillus*, were also reported in vitro and in vivo models.[144]

Beneficial effect of synbiotics against CRC is postulated based on the studies in animal model. Till date, only one human study showing protective effect of synbiotic against colon cancer is published.[145] A synbiotic preparation containing oligofructose enriched inulin, *L. rhamnosus* GG (LGG) and *B. lactis* Bb12 (BB12) were able to reduce the risk of colon cancer in 12-week randomized, double-blinded, placebo-controlled human study. Synbiotic intervention reduced genotoxin exposure and resulted in increased bifidobacteria and lactobacilli and reduced *Clostridium perfringens*. This intervention also reduced the colorectal proliferation and the capacity of fecal water to induce necrosis in colonic cells and improved epithelial barrier function in polypectomized patients. Synbiotic consumption reduced IL-2 secretion by peripheral blood mononuclear cells (PBMC) and increased the production of interferon-γ.[145]

With the studies done so far, synbiotics seems to be far superior to probiotics or prebiotic alone in preventing or treating CRC. Combination of *Lactobacillus* and bifidobacteria strains together with oligosaccharides (GOS, FOS and inulin) gave greater results compared with pro/prebiotics given individually. However, knowledge on the compatibility of strains in a multistrain synbiotic combination, minimum effective dose to impart the desired health benefits without any side effects and the appropriate biomarkers in the in vivo trials, are lacking. Studies on the synergistic effects of probiotics and prebiotics with the above discussed observation need to be studied in detail for the effective development of synbiotics.

## Conclusion

Knowledge available so far based on in vitro and animal-based studies indicate that probiotics, prebiotics and synbiotics are an ideal choice for the prevention of carcinogenesis. With the advancement in molecular techniques and elucidation of gut microbiome, it is possible to understand the definite mechanism of probiotics, prebiotics and synbiotics, individually or collectively, as anticarcinogenic agent that might answer many unsolved queries and will open new avenues and strategies for cancer prevention, based on the dietary intervention. In conclusion, prevention and treatment of CRC using probiotics, prebiotics and synbiotics need a thorough investigation and more detailed study with human clinical trials and evidences.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest have been disclosed.

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