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Colon Cancer Prevention through Probiotics: An Overview

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Abstract

Probiotics are live microorganisms which when administered in adequate amount confer a health benefit on the host. Beneficial aspects of probiotics include alleviation of lactose intolerance, control of diarrhoea, urogenital-infection, reduction in cholesterol level and relief from irritable bowel syndrome, antioxidant potential, and pathogen inhibition. These days research is focussing on probiotics usage in colon cancer prevention because of its positive outcomes. Scientific evidences indicate a strong association between diet, lifestyle, and changes in gut microflora composition which may initiate the onset of colon cancer. Probiotic usage in colon cancer prevention is a new direction of research and most of the studies related to colon cancer prevention are still unclear and effects are in observed form, so confirmation studies are needed in this respective area and also there is a need to standardize methodology. This review presents information about mechanism of different probiotic actions, factors contributing colon cancer risks and how probiotics are helpful in preventing colon cancer with supporting scientific based evidence and various experimental studies.

Keywords: Probiotics; Colon cancer; Colon cancer risk; Cancer prevention

Introduction

Trillions of bacteria inhabit human body and these organisms distributed at specific sites, form complex communities on the skin, mucosal surfaces, but the largest group is found in the colon (10^{11} microorganisms g^{-1} gut content). Colonic resident micro flora contributes to about 95% of the cells within the body making the colon a very metabolically active organ. Scientific evidence support that the eukaryotic host has co-evolved with their symbiont in a mutualistic relationship for their nutritional benefits from each partner. Microbes in the gut exert a significant effect on host biochemistry such as oxidation-reduction potential of luminal contents, enzymatic activity of intestinal contents, host physiology, short chain fatty acid production in the lumen, host immunology and modification of host-synthesized molecules [1-5]. There is an established association between existing microbiota and intestinal function for maintaining of homeostasis, building of balanced immunity. Any microbial alterations may lead to increased chances of the disease by mean of immune function disturbances [6]. Genetic and environmental factors also disrupt the symbiotic interaction by altering the microbial composition, distribution and the metabolic activity which may result in dysbiosis, a contributing factor for the onset and progression of several chronic diseases including cancer. Interindividual variations of micobiota of host are associated with each host genetics and environmental factors like diet, physical activity, stress, smoking, drugs, illness, and antibiotics [7-10]. However these organisms interact with the host at multiple levels to maintain its normal functions. Disruptions in this complex ecosystem crosstalk result in physiological changes associated with colorectal (colon+rectum) tumour genesis as wells as cell proliferation, programmed cell death process and immune responses [11]. Evidences have showed that modulation of the host gut microbial environment by using probiotics, (Microbial cell preparations or components of microbial cells) through ingestion or administration is a protective approach for proper maintaining of healthy gut micro-biota and also reduce the development of colon cancer risk [11-14]. In 400 BC, Hippocrates mentioned the role of the human gut in disease through statement'death sits in the bowel' [15]. Again after approximately 2000 years later, Elie Metchnikoff, by observing longevity in Bulgarian peasants, extolled the virtues of consuming fermented dairy products. In 1907, he established scientific basis for the health benefit of lactic acid bacteria in his book "The Prolongation of life" printed in 1907. He declared that some of the bacterial organisms present within the bowel served as a source of 'toxicants', harmful substances that contributed to sickness and aging. He also suggested that "Intestinal microbial dependence on the food makes it attainable to adopt measures to modify the flora in our bodies and to exchange the harmful microbes by beneficial microbes". Lactic acid fermented foods together with other cultured dairy products became the part of human diet for thousands of years and considered to have beneficial effects [16-17]. Probiotic term has undergone number of variations in its definations; the present accepted definition for probiotic is given by Joint FAO/WHO working group 2002 [12] and it is the most accepted one. According to it probiotics are defined *as "Live micro-organism which when administered in adequate amount confer a health benefit on the host ".*

Recently at International Scientific Association for Probiotics and Prebiotics (ISAPP) [18] consensus meeting on the scope and appropriate use of the probiotic term definition was worded with grammatical correction. According to it probiotics are *"Live micro-organism that, when administered in adequate amounts, confer a health benefit on the host ".*

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Delivery of probiotics is considered an important issue. Though it can be delivered in form of capsules, tablets; generally a food product is considered best delivery vehicle for a probiotic microorganism to reach the GI tract of the human body in a live and active form. Upon consumption of probiotics in a range of 10^6 to 10^{11} cfu/day could be able to reduce the incidence as well as severity of some illnesses that associated with the intestine [19]. Probiotics like *Lactobacilli* and *Bifidobacteria* are considered as most successful probiotics having a long history of safe usage. In addition to this, these probiotics fall in the category of Generally Recognized as Safe (GRAS) because they are able to stay in the human body without causing harm [20]. Organisms from different genera like *Lactobacilli*, *Bifidobacterium*, *Pedicoccus Leuconostoc*, *Enterococus* and yeast such as *Saccharomyces boulardii* are recognised as probiotics [21]. Beneficial effects of probiotics include alleviation of lactose intolerance [22], inhibition of intestinal pathogens [23], control of diarrhoea [24]. Other effects studied include reduction in cholesterol level [25], urogenital infections [26], relief of irritable bowel syndrome [27], improving mineral absorption [28], enhanced immune response [29], and anti-mutagenic and anti-carcinogenic activity [30]. The aim of this paper is to present information about risk factors of colon carcinogenesis, mechanism of different probiotic actions and how probiotics are helpful in preventing colon cancer with their supporting scientific based evidence and various experimental studies etc.

Factors Contributing Colon Cancer Risk: Response

Cancer has become a globe public health problem. Worldwide colon cancer strikes more than 1 million people annually and is responsible for death of more than 500,000 person [31]. According to World Health Organization (WHO), by 2030 there will be about 17 million deaths, 27 million new cases of cancer and 75 million people living with the disease. When compared to the other types of cancers, colon cancer is found to be the most common death cause [32,33]. Various factors that may increase colon cancer risks includes (Figure 1 and 2):

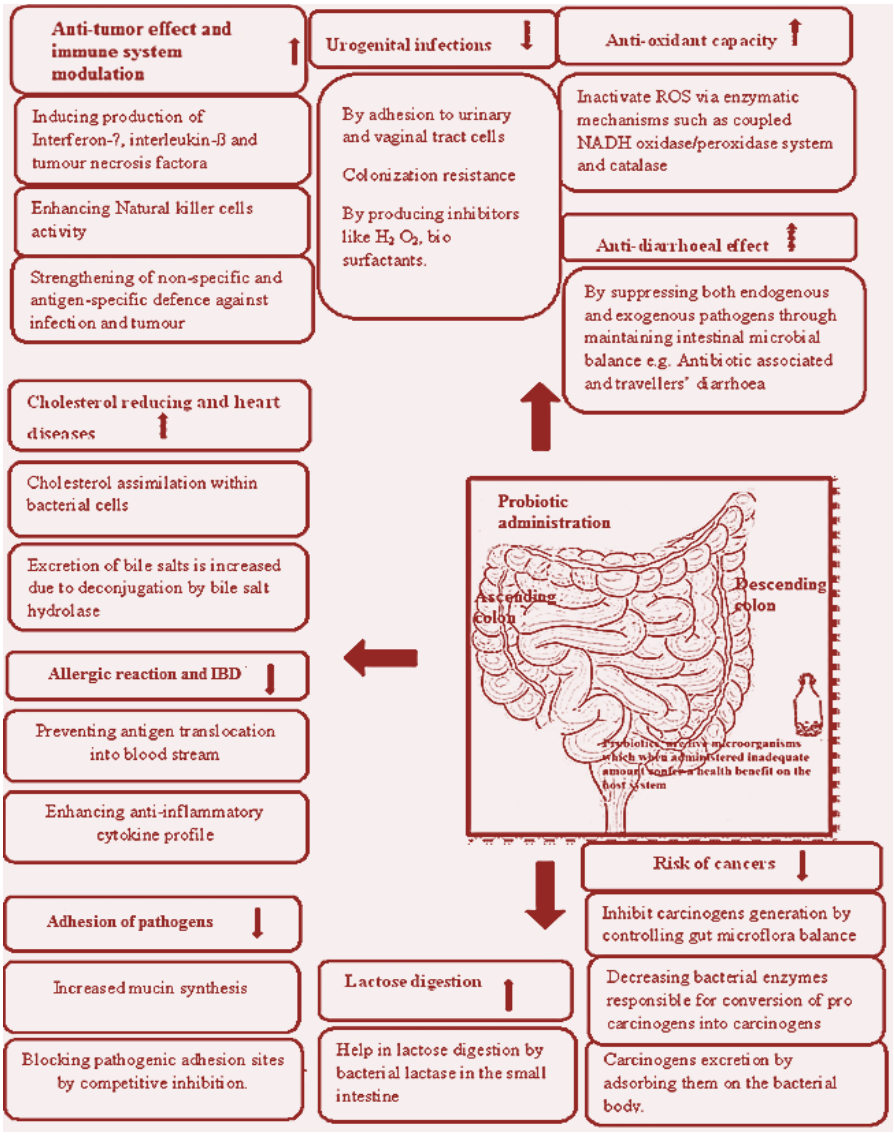

Figure 1: Mechanism of different probiotic action [86- 93,110,116-121,123,141].

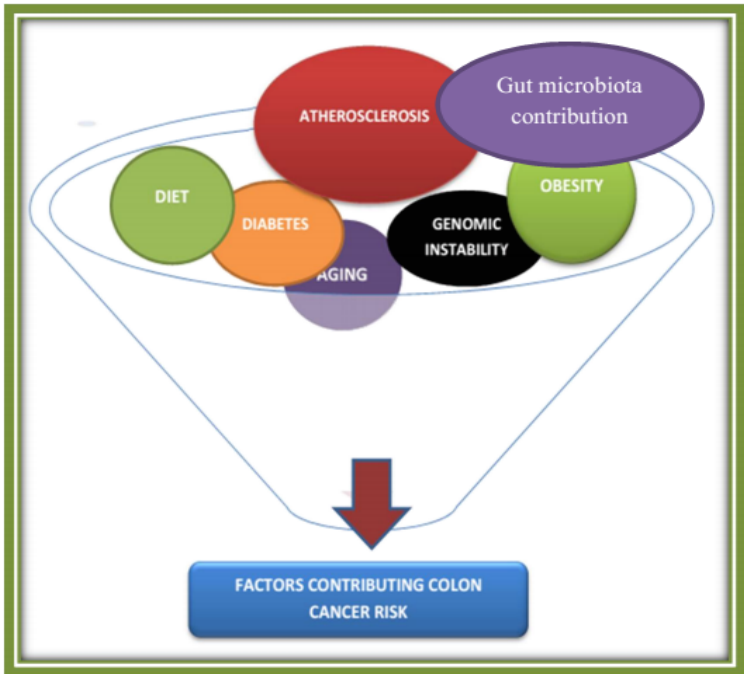

Figure 2: Factors contributing colon cancer risk.

Diet

Various studies show that diet contributes to 20%-42% in causing human cancers and for colon cancer it is about 50%-90% [34]. The very fact that 90%-95% cancers are due to lifestyle factors, environmental toxins, and infections and the rest 5% to 10% are due to genetics these factors provide a major chance for the prevention of colon carcinoma [35]. Pyrolysis products of cooked food can also initiate colon cancer [36]. Diets low in whole grain, vitamin D, fruits, vegetables, calcium, fibre and omega-3 fatty acids, and diet rich in red and processed meats, refined starches, sugar, and saturated and trans-fatty acids are closely related to an increased risk for colon cancer [37,38]. Interestingly intake of high fat favours the formation of bile acids (BA) into the duodenum and then activates bacterial 7-alpha-dehydroxylase to converts it into secondary bile acids. In several animal model studies it was observed that these bile acids, deoxycholic acid and lithocholic acids are able to promote colon carcinogenesis. Experimental studies also reported that addition of cholic acid to the rats diet, increased proliferation in colonic epithelial cells was observed [39,40]. Hence it has been hypothesized that there is a association between the diet and colon cancer, as diet has influence on composition and metabolism,creating relevant factors as a base of the disease [41].

Aging

Aging is additionally one of the factor connected with an increased risk of colon cancer [42]. Evidences exploring the results of aging on the faecal microflora have revealed that over 70 years there is a decrease in the count of Bifidobacteria and Lactobacillus and increases in clostridia occur [43,44]. The modifications that occur among microflora are thought about the result of dietary and activity changes as a results of age [45,46]. These bacteriological changes have been reported to often coincide with an rise in gastroenterological infections [47] and gut cancers.

Genomic instability

Colon cancer development is a multistage process and it involves accumulation of mutations in certain tumour suppressor genes and proto-oncogenes leading to cancer initiation [48]. The fundamental process associated is genomic instability and is related to the gene rearrangement or loss of DNA fragments, aneuploidy and loss of heterozygosis [49]. In addition, inactivation of tumor suppressor genes like APC, DCC, DPC4 and p53, along with the activation of oncogenes, of which the family of RAS genes play an important role in the malignancy appearance [50]. Adenomatous Polyposis Coli (APC) is considered as a tumor suppressor protein and it acts as an antagonist to the Wnt signalling pathway. Usually this pathway plays a key role in elevating colonocyte proliferation and suppressing caspase mediated cell death process in both humans and rodent models of experimentally induced colon carcinoma. Any gene defects in APC will usually cause an autosomal dominant premalignant disease called as Familial Adenomatous Polyposis (FAP). It is usually involved in growth to malignancy and inhibits programmed cell death process in colonocytes and thereby causes the initiation of the colon carcinogenesis [51-53].

Obesity

Now- a-day obesity has been established as a colon cancer risk factor [54]. Evidence shows that there is a strong relation between increased body mass index (BMI) and deaths related to the cancer. It is found that almost 14% of cancer death in men and 16-20% of cancer deaths in women were found to be due to obesity [55]. In humans, body mass index is some how proportional to the leptin levels and are raised in obese individuals. Leptin is the product of the ob (obese) gene, play a key role in energy expenditure. Since obesity is known to increase the risk of certain cancers, much effort has been directed at elucidating the possible role of leptin in cancer development [56-59]. However there is very less evidence for involvement of leptin in colon cancer in a clear-cut manner [60]. Leptin was shown to work as a mitogen for intestinal

epithelial cells and furthermore decreased apoptotic cell death in a cancer cell line [61]. It has been shown to induce invasion of collagen gel by cell lines derived from colonic adenomas [62]. Furthermore leptin was also shown to extend the growth and proliferation of a colon cancer cell line, as proof by BrdU incorporation and c-fos expression [63].

Diabetes

Currently investigations are going on the link between gut and type 2 diabetes. Animal based models have established a relation between altered microbial composition to the development of diabetes, obesity and insulin resistance in the host system by several mechanisms like altered fatty acid metabolism, harvesting more energy from the diet and adipose tissue and liver composition, modulation of gut peptide PYY and glucagon-like peptide (GLP)-1 secretion, lipopolysaccharide toll-like receptor-4 axis activation and increased inflammation [64]. In some studies they showed that obesity is the strongest independent determinants of insulin resistance and hyperinsulinaemia [65-68]. As the blood insulin levels increases, the levels of insulin-like growth factor binding protein-1 get decreases and it leading to increased levels of free insulin-like growth factor 1 (IGF-1). IGF-1 acts as a pro-carcinogen, each by decreasing cell death and promoting cell growth [69,70] IGF-1 is understood to be involved in the development, progression, and colon cancer metastasis [71,72].

Antherosclerosis and colon cancer link

Latest research in obesity noted that adipose tissue is considered as an active endocrine organ and they produce different kinds of the bioactive molecules characterized as adipokines [73,74]. It is well evident that as the obesity (adiposity) level increases, there is impairment in the levels of anti-inflammatory/adipokines expression, especially adiponectin (Adiponectin is a peptide with 244 amino-acids, secreted from adipose tissue) thereby it will lead to an increased levels of pro-inflammatory as well as atherogenic adipokines. These discussed pro-inflammatory/atherogenic adipokines include Resistin, tumor necrosis factor (TNF-α), Interleukin-6, Macrophage Chemoattractant Protein (MCP) -1 etc. Therefore the aforementioned adipokines can able to contribute the initiation and progression of atherosclerosis in a number of ways, such as regulating the endothelial cell function (which is to be considered as an initial onset event in atherosclerosis), vascular inflammation and formation of plaques [75,76]. In addition, in another study it is noted that hypoadiponectinemia is closely concerned with inflammatory atherosclerosis signifying that to maintain the usual vascular wall in non-inflammatory state sufficient levels of adiponectin is needed [77]. Low levels of adiponectin also play a role in causing obesity linked malignancies risks which include endometrial, prostate, breast and more specifically colon cancer [78]. Evidences based studies supporting that adiponectin can inhibit colon and rectum cancer through the activation of adenosine monophosphate-activated protein kinase followed by mammalian target of rapamycin (mTOR) pathway. Any deficiency in adiponectin can contribute inflammation induced colon cancer [79,80].

Gut microbiota contribution

Intestine is composed of over 1000 different bacterial species and the microbial population is heterogeneous in nature. The microbial density within large intestine is 12-fold beyond that in the small intestine. So there is an estimated 12-fold increase in cancer risk in the large intestine compared with the small intestine [81]. It has been revealed that microflora resident in colonic region are able to convert harmless compounds into metabolites that causes inflammation or tumourigenisis [82]. The microflora in intesitinal region can contribute to carcinogenesis by producing enzymes like β-glucosidase, β-glucuronidase,nitroreductase and azoreductase [83]. The most common pathogens associated with the production of β-glucuronidase are *E.coli* and *Clostridium perfringens* [84]. These faecal enzymes may hydrolyse glucuronide, a compound that is needed to detoxify foreign compounds and produces cancer causing aglycones in intestinal lumen [85].

Role of Probiotics in Colon Cancer Prevention

Research has been conducted to explore the role of probiotics in colon cancer prevention. How gut microbiota influence the development of colon cancer is unclear but the gut micro biome contributes colon cancer through initiation of inflammation [94,95]. Researchers suggested that the prevention of colon cancer might occur through intervention of synbiotics (prebiotic+probiotic) that allow certain substantial changes in the gut micro biota [96]. According to Roberfroid [97] prebiotics are defined as *"These are the non-digestible food ingredient that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health."*

But recent and well accepted definition for prebiotic was agreed at the 2010 Meeting of the International Scientific Association for Probiotics and Prebiotics (ISAPP) [98]. According to it prebiotics are defined as *"A dietary prebiotic is a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health."*

Upon fermentation of these prebiotics by beneficial microorganisms under anaerobic conditions they produce Short Chain Fatty-acids (SCFA) like butyrate, acetate, propionate of varying quantities. At the end, it results in a decrease in pH and thereby preventing overgrowth of pathogenic bacteria, which are pH sensitive in nature and it was based broadly on in vitro studies. These short chain fatty acids usually act as a source of carbon for colonocytes and they carry out important metabolic activities like modulation of bioactive food components, vitamin synthesis by intestinal microbiota. Its function shapes the host intestinal anatomy and also gut mucosal immune system [99-101]. Among these short-chain fatty acids, butyrate is found to play a defensive role in DNA oxidative damage induced by H_2O_2. It may also decrease the altered cell proliferation and induce programmed cell death process in altered cells [102-105]. Evidences have shown that four probiotic microorganisms *Lactobacillus salivarius* (L.salivarius) *FP25*, *L. salivarius FP35*, *Pediococcus pentosaceus FP3* and *Enterococcus faecium FP51* exhibited anti proliferative properties. The proposed mechanism was given to the synergic induction by directly adhering to colon cancer cells and triggering bio production of butyric and propionic short chain fatty acids [106]. In one clinical trial, that underwent a 12 week double-blind placebo-controlled, randomized test with 37 colon cancer patients and 43 polypectomized patients, a synbiotic composition of (*Lactobacillus rhamnosus* GG, *Bifidobacterium lactis* Bb12+oligofructose enriched inulin) resulted in an increase of Lactobacilli & Bifidobacteria and decrease of *C. perfringens* in the gut microbiota. Further, the production of interferon (IFN) -γ was increased by peripheral blood mononuclear cells (PBMC) and reduced colorectal proliferation was observed in the cancer patients [107]. Animal and human studies showed that fructooligosaccharides (FOS) are able to act as good substrates for the bifidobacteria spp because of β-fructoisidase activity and for *E. coli* and *C. perfrigens* they act as bad substrates. FOS feeding is also associated with a rise in population of lactobacilli and bifidobacteria and decreases

the population of pathogens such as *C. perfrigens*. The reason behind is bifidobacteria can able to produce acetic acid and lactic acid will lead to decreases in intestinal pH, which restricts further the proliferation of pathogens and other putrefactive bacterias which are concerned with faecal enzyme like nitroreductase, decarboxylase etc in stools with a deep impact on the metabolism of carcinogenic substances like N-nitroso compounds, phenolic products of tyrosine and tryptophan and metabolites of biliary steroids etc. Hence FOS can able to enhance bifidobacteria and could act as a protective factor against colon cancer [108]. In another study rats associated with human feaces fed with active diet containing synbiotic mixture of dietary inulin (5%w/w) and *Bifidobacterium longum;* and they observed 55% lower fecal β-glucoronidase activity and 30% lower ammonia concentrations, when compared to the control rats [109]. In addition, some strains of Lactobacillus especially *Lactobacillu casei strain shirota* were shown to have anti-tumor effect, upon administration of Lcs intrapleurally into the tumour carrying mice. It has been noted that they induce the production of cytokines like TNF-α (tumour necrosis factor-α), IL-1β (Interleukin-1β) and Interferon γ (Interferon-α) thereby tumour growth was inhibited and increased the survival rate [110]. Colon tumorigenesis is a process that involves activating mutations in proto-oncogenes as well as inactivating mutation in tumour suppressor genes like p53, Adenomatous Polyposis Coli (APC) gene. These genetic events lead to changes in signal transduction pathways which are involved in regulation of various processes like apoptosis, cell proliferation and differentiation. Many cell communication pathways are also associated with colon tumorigenesis like APC,Beta-catenin protein [111]. Probiotic studies have clearly shown that microencapsulated *Lactobacillus acidophilus* preparation upon oral administration at a range of (10^9-10^{11} cfu/ml) daily reduces the tumor size, multiplicity and cancer progression in mice model. This study showed that probiotic could be able to modulate the gene expression of APC in colonocytes [112]. Recent studies explored that *L.casei* and *L. rhamnosus GG* cell-free supernatants (CFS) are able to inhibit colon cancer cell invasion by influencing levels of the tight junction protein zona occludens-1 (ZO-1) and matrix metalloproteinase-9 (MMP-9) activity in cultured metastatic human colorectal carcinoma cells [113].

Colonic region of humans contain very diverse mixture of bile, mucus, desquamated epithelial tissue cells, various microorganisms and their fermentation products, undigested or unabsorbed food and their respective metabolic products like metals, salts, toxins, mutagens, carcinogens, and dissolved gases. It is assumed that enteric membrane is consistently challenged with diet- and other oxidants and carcinogens that are derived from bacterial source. Chronic exposure of such difficult conditions might then cause uncontrolled free radicals generation, building redox imbalance, and DNA damage, which can affect intestinal metabolic physiological conditions and thereby contribute cancer as an endpoint [114]. As it is know that cancer can initiate from the epithelial cells that line the bowel. These cells divide rapidly with a high metabolic rate and it might to be responsible for increased oxidation of DNA [115]. Another hopeful approaches of preventing colon cancer is by decreasing the levels of H_2O_2 (hydrogen peroxide) as generally it involved in the development of various aspects of tumours like tumour progression,enhanced proliferation when compared to normal ones and increased spreading of cancer cells in colonic region. These types of processes can be modulated by increasing the levels of activity of catalase enzyme producing bacteria. If an adequate number of catalase enzyme producing bacteria proliferate in colonic region, then might be a chance of decreasing the colon cancer risk by increasing its antioxidant capacity and thereby decreases H_2O_2 levels in colonic region. Through this manner, it is possible to minimize the cancer cell growth and spread in colon. *Lactococcus lactis* is the potential strain which is involved in controlling colon cancer of such activity and experimentally proven in DMH induced murine model [122]. Another aspect of colon carcinogenesis might be due to association of bacterial enzymes like nitroreductase, β-glucuronidase, which are involved in transformation of pro-carcinogens into carcinogen [123]. Results in the experimental studies (a rat model study) show that upon supplementation of probiotic *L. acidophilus,* along with meat diet, which contain 72% beef, it is observed that there is a nearly 50% decrease in activities for faecal enzymes like β-glucuronidase and nitroreductase. Another research on *L. acidophilus* strain, through an experimental animal model study, also demonstrated that consumption of such strain leads to a decrease in faecal enzymatic activities like nitroreductase, azoreductase, and β-glucuronidase [124,125]. According to (Gorbach 2000) the suppression of bacterial enzyme activities like urease, β-glucuronidase, nitroreductase, hydrolase and tryptic activity was noted upon *Lactobacillus GG* administration [126].

It is well known that foodborne genotoxic compounds such as mycotoxins and plant glycosides or genotoxins created during food processing such as heterocyclic amines and polycyclic aromatic hydrocarbons are capable of expressing risk within the gut [127]. Mycotoxins, as an example, are carcinogenic fungal metabolites that contaminate cereals meant for human consumption and feed for animal consumption. Dairy probiotics like Propionibacteria were shown to remove mycotoxins from aqueous solutions in vitro [128,129]. Dairy propionibacterium were also shown to bind cyanotoxins like microcystin-LR and heavy metals like lead and cadmium [130,131]. Therefore ingestion of such probiotic propionibacterium might reduce bioavailability and absorption of these carcinogenic compounds, thus reducing cancer risk. Metabolic degradation of AFB1 by viable *L.rhamnusos GG* has been excluded as a possible binding mechanism,since heat- and acid-killed *L.rhamnusos GG* remove AFB1 even more effectively than viable bacteria [132]. The binding of carcinogenic aflatoxin B1 by *L.rhamnosus GG* has been reported. It has been proposed that components will bound covalently to peptidoglycan or components of cell wall, play a major role in AFB1 binding [133]. Studies have also reported that *Saccharomyces cerevisiae* CECT 1891 and *L. acidophilus* 24 have the capacity to remove Fumonisin (B1) from liquid medium and the removal was reversible and fast. It was confirmed that removal of Fumonisin B1 amount was depended on both microorganism and the toxin concentration and for this purpose viability of cells is not required. Another study revealed that removal of Fumonisin B1 involves structural integrity of the cell wall of microorganism and it doesn't involve any Fuminosin B1 molecule chemical modification process [134].

To understand the mechanism of inhibition of colon carcinogenesis, experiments were conducted by using azoxymethane- a potential carcinogen generally used to induce colon cancer in rodents and the same also prompted aberrant crypt foci in rats. Inhibition of colon carcinogenesis was noted due to a stimulated growth of bifidobacteria in the colon. The growth of bifidobacteria leads to lowering of pH, which is attributed to further inhibit aberrant crypt foci, crypt multiplicity and growth of *E. coli* and clostridia in rats [135,136]. In addition, dietary ingestion of *Bifidobacterium longum* (lyophilised culture) showed that there is a significant suppression in tumor multiplicity as well as a decrease in the size of tumor volume. It also alters the intermediate biomarkers of colon cancer, thereby providing strong anti-tumor activity and in another study, it was noted that expression levels of ras-p21(oncoprotein) and cell proliferation in colonic mucosa cells

was decreased. Upon addition of *B. longum* to the rat diet and thereby providing anti-tumor activity [137,138].

Probiotics like *Bifidobacterium* B12 and *L. plantarum*, have shown significant role in the anti-genotoxicity effect. Experimental evidence indicates that these two probiotics show decreased faecal water associated genotoxicity towards HT-29 cells, thereby proposing that above mentioned probiotics may be to prevent the initial stages of colon cancer [139]. In the context to the discussion, probiotics like *Bifidobacterium spp*, *L.helveticus, L.bulgaricus* and *S.thermophillus* have undergone an assay recently with HT29 colon epithelium cancer cell line and it was noticed that they or certain compounds produced by them interact with colonic epithelial cells directly and thereby growth rate is decreased and differentiation is induced [140]. Some studies observed that upon oral administration of *Lactobacillus salivarius* UCC118 in a placebo-controlled study, incidence of mucosal inflammation and colon cancer activity was decreased in IL-10 knockout mice by changing intestinal microflora, thereby it also decreased coliforms, enterococcus and *Clostridium perfringens* levels in the probiotic fed group [141]. Another possible reason for the onset of gut cancer risk is exposure of diet containing heterocyclic amines. In vitro studies demonstrating that certain strains of LAB are able to decrease food-borne carcinogens like heterocyclic amines (formed during cooking of meat at high temperature and is closely related to onset of colorectal cancer by means of producing byproducts upon fermentation by gut microbiota and thereby it causes DNA damage). Heterocyclic amines are found to have the greatest binding capacity. The extent of binding is dependent on mutagen and bacterial strain used and the binding was mostly due to the cation exchange mechanism. In addition some literature evidence shows that certain lactobacilli can degrade food-borne carcinogens like dimethylnitrosamine and diphenylnitrosamines [82,142,143]. With reference to the food borne carcinogen, whole cells of bifidobacteria have also been found to bind with the mutagen-carcinogen 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole, thus removing it via feces physically and subsequently reducing its absorption in intestinal lumen [144]. It has been noticed that supplementation of fermented milk with *L. acidophilus* is able to decrease the count of faecal putrefactive bacteria like coliforms and increase the count of beneficial microorganisms like lactobacilli in the intestine [145] (Figure 3 and Table 1).

Bottlenecks/Future Challenges in Study

Most of the positive outcomes given by the probiotic treatment are in observed form under experimental conditions. Future challenges are needed in the direction of standradising methodology to study effects. Long term safety studies of probiotics is also required. FAO/WHO jointly had proposed a guideline for recently identified/ less reported strain with no history of safe human use and strains that are not in the category of GRAS. For safety demonstration they have to undergo various in vitro and in vivo assessments and it also include toxicity studies like acute, sub-acute and chronic studies are also suggested for all newly identified strains which when taken in adequate amounts. To study dosage optimisation is required and also study the variability of effects in different category of persons such as aged groups,immunocompromised persons. For clinical studies

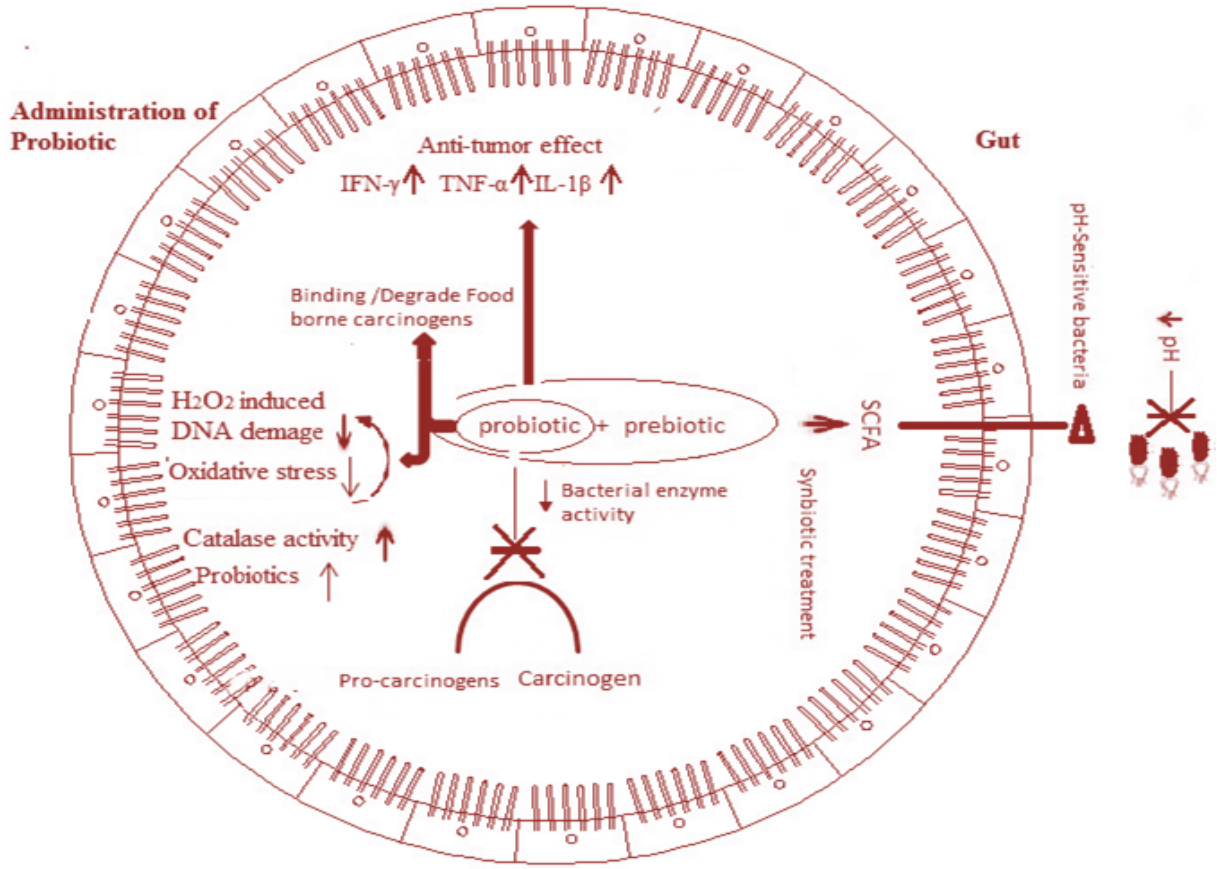

Figure 3: Probiotics role in prevention of colon cancer.

Study	Probiotic used for study	Conclusion	Ref
***In vivo* human studies**			
Undertook 9 healthy volunteers and they undergo intervention with standard yoghurt or probiotic yoghurt and then they were incubated with HT-29 clone19A human colon tumor cells from their collected fecal water after dietary intervention. Then they underwent DNA damage studies in colon cells	*L.acidophilus 145* and *B. longum 913*	Probiotic yoghurt intervention decreased DNA damage in colon cells,in comparison to standard yoghurt	[146]
Conducted on 38 healthy male subjects who undergo double-blind, randomized, two period crossover, placebo controlled study and they supplemented probiotic bacteria on daily dose for 4 weeks then they evaluated levels of harmful carcinogenic bacterial enzymes.	*L. rhamnosus LC705* and *P. freudenreichii ssp. shermanii JS*	Probiotic administration significantly decreases the β-glucosidase activity with increased counts of Propionibacteria.	[147]
Performed cross over,placebo controlled study with7 healthy subjects (1male and 6 females).Each volunteer is supplemented with 100g/day of LKM512 yogurt or placebo for 2 weeks.Then they evaluated for faecal mutagenicity.	*Bifidobacterium lactis LKM512*	Probiotic yoghurt consumption significantly reduces the faecal mutagenicity in all 7 healthy subjects when compared to the placebo treatment	[148]
Undertook 11 subjects for their study and as a part of their diet, they're given with fried beef patties for 3 days daily twice. In phase 1 they provided with ordinary Lactococcus fermented milk and thereafter in phase 2 they supplemented fermented milk containing *Lactobacillus acidophilus* and they determined excretion of urinary and fecal mutagenic activity.	*L. acidophilus*	Upon consumption of *Lactobacillus acidophilus* decreased mutagenic excretion was observed.	[149]
Conducted a clinical study with 20 healthy subjects and they undergo consumption of probiotic mix product containing both *Lactobacillus rhamnosus LC-705* and *Propionibacterium fereudenreichii JS* and they evaluated aflatoxin B1 level in fecal samples.	*L.rhamnosus LC-705 P. fereudenreichii JS*	Probiotic mixture successfully decreased the aflatoxin levels in faecal samples.	[150]
Undergone 3 week of study with nine healthy volunteers and they supplemented with fermented dairy product before, during, after containing a probiotic mix of 4 cultures Finally, they assessed for fecal concentration of azoreductase, nitroreductase, β-glucuronidase. which are involved in colon risk	*L. acidophilus, B. bifidum, Streptococcus (Lactococcus) lactis, L. lactis subsp. Cremoris*	After 3 weeks of fermented dairy product consumption, there is a decreased nitroreductase activity is noted, However no changes is observed in others during experiment	[151]
Undertook 398 subjects and they divided randomly into 4 groups, then administered with *Lactobacillus casei*, wheat bran,both or neither.At the end of 4 years they undergo a process of colonoscopy for the presence or absence of new colonic polyps.	*L.casei*	Among all treatments,probiotic treatment was found to significantly decreases atypical colonic polyps.	[152]
***In vivo* animal model studies**			
Undertook F344 male rats and divided into 4 groups, then supplemented with: Group 1: 20% water. Group 2: supplemented with 30% non-fermented skim milk. Group 3: supplemented with 30% *Bifidobacterium animalis* DN-173010-FM. Group 4: supplemented with 30% *Streptococcus thermophilus* DN-001 158-FM After that, during 1 week they were provided experimental diet followed by HAA (heterocyclic aromatic amines) consumption for 7-8 weeks and then they evaluated aberrant crypt assessment, measuring HAA metabolism by enzymatic dosages, fecal mutagenicity by using 3d test and colonic lesion damage by comet assay	*B.animalis DN-173 010-FM. Streptococcus thermophilus DN-001 158-FM*	Aberrant crypts incidence was decreased compared to control diet. -Decreased HAA metabolism was noticed and reduced colonic DNA lesions, feacal mutagenicity was also noticed.	[153]
Undertook F344 male rats and they divided into 3 groups Group 1: Fed with low fiber and high fat diet Group 2:Fed with low fiber and high fat diet+DMH (1, 2-dimethylhydrazine dihydrochloride) treated Group 3: Fed with low fiber and high fat diet+DMH+probiotic (3×10^8 cfu/1.3g). Then throughout the experiment they maintain the diet and then they analyzed for a count of aberrant crypt foci and antioxidant system	*Bacillus polyfermenticus*	Upon probiotic treatment aberrant crypt foci number was significantly decreased when compared to DMH treated group and also exhibited a protective effect on colon carcinogenesis process and on antioxidative system	[154]
Undertook 20 mice and they randomly divided into 4 groups Group 1: (Negativecontrol), Mice given normal physiological saline (0.9% NaCl). Group 2: Mice supplemented with the *Lactobacillus rhamnosus IMC501* Group 3: (Posititve control), Mice given with PhIP(2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine- a food mutagen)+ physiological saline. Group 4*: Lactobacillus rhamnosus* IMC501 + PhIP. Afterwards they undergone comet assay for to evaluate DNA demage in colon	*L.rhamnosus IMC501*	*Lactobacillus rhamnosus IMC501* exhibit protective effect on PhIP-induced DNA damage in colon cells	[155]
Undertook 24 male wistar rats for a 32 week study and divided them randomly into 5 groups. Group1: (control group) supplemented with buffalo milk(BM) Group2: (DMH control group)Injected with DMH. Group3: Administered with BM + PXC(piroxicam) in DMH. Group4:Supplemented with probiotic dahi+DMH Group5:Supplemented with probiotic dahi+PXC+DMH. Afterwards rats were sacrificed at 8,16 and 32 week and then evaluated for Thiobarbituric acid reactive substances (TBARS)+ glutathione-S-transferase activity.	*L.acidophilus LaVK2, L.plantarum Lp9.*	Probiotic Dahi that is administered individually or in combination with PXC to experimental rats possesses a potent protective effect against DMH-induced colorectal carcinogenesis by lowering the levels of TBARS, faecal β-glucuronidase and by enhancing the activity GST in liver and colorectal tissues	[156]

Gnotobiotic studies Gnotobiotic animals is an animal which contain only known strains of bacteria and other certain microorganisms. This term also includes germ- free animals because of the known status of their microbial communities. Gnotobiotic animals are born in sterile conditions which may include delivery from the mother through caesarean method and then transferred the new-born to an environment where everything is sterile such as air, water and food in a microbiologically controlled sterile environment. Such animals are used by the researchers' and expose solely to microorganism interest of their study [157,158]			
Undertook Germ free Lister Hooded rats associated with human faecal flora(gnotobiotic) and they supplemented with probiotic preperations and they analysed for activities of β-glucosidase and β-glucuronidase.	*Lactobacillus acidophilus* NCFM, *Bifidobacteriurn adolescentis 2204*	Probiotic treatement successfully suppressed the activities of β-glucosidase and β-glucuronidase in colon.	[159]
Undertook 32 Germ free male fischer rats inoculated with human intestinal microflora(gnotobiotic) and they supplemented milk fermented with probiotic afterwards they determine composition and metabolic activities(beta-glucuronidase)of intestinal microbiota	*Lactobacillus casei*	High number of Bifidobacteria in feacal content and low activity of beta-glucuronidase is observed.	[160]
Simulator of the Human Intestinal Microbial Ecosystem studies (SHIME) Simulator of the Human Intestinal Microbial Ecosystem (SHIME) is a computerized controlled device used as a scientifically validated model to study nutrition studies, enzymatic studies and microbial parameters in the GI tract and also it is useful to analyse the intestinal microbial community composition [161-164].			
Undertaken microbial suspension of *Lactobacillus reuteri* in a batch culture mode with the help of SHIME(Simulator of the Human Intestinal Microbial Ecosystem) to asses the protective effect of the bile salt hydrolase-active L. reuteri against bile salt cytotoxicity.	*Lactobacillus reuteri*	It prepeciptate the deconjugated bile salts and adsorbed on the surface of bacterium by physical binding and makes the harmful bile salts less bioavailability, Hence protective in nature against colon cancer risk	[165]
Cell line based study			
Performed experiment by using probiotics on two different cancer cell lines-Colon (HI-29) and PANC-1(Pancreas).	*L. rhamnosus* ATCC 9595	Probiotic treatment decreases the cancer growth successfully in cell lines	[166]

Table 1: Various scientific based and clinical based evidences for role of probiotics in colon cancer prevention.

and invivo studies validated clinical outcome measures are required and the clinical effects,safety and data of one probiotic strain cannot be extrapolated to another probiotic strain even though it is a closely related strain.

Conclusion

Gut microbiota play a central role in maintaining the healthy bowel and any microbial imbalance may show upsetting effects on host system. Probiotics are the live beneficial microorganism and these microbes can set a healthy environment for gut system. Various mechanisms elucidate the preventive role of probiotics in colon cancer risks. All of above scientific evidences and various in vitro and in vivo based studies indicate that use of probiotics may prevent the risk of colon cancer. But most of the studies related to prevention of colon cancer by using probiotics are unclear, further confirmation studies are needed and the observed effects cannot be generalised. Future research needs in terms of the underlying mechanism of action involved in each of the observed effects.

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