

Colon Cancer Prevention through Probiotics: An Overview

Korada Siva Kumar¹, Nagendra Sastry², Himabindu Polaki¹ and Vijendra Mishra^{1*}

¹Department of Basic and Applied Sciences and Food Science and Technology, National Institute of Food Technology Entrepreneurship and Management (NIFTEM), Haryana, India

²Department of Biochemistry/Bioinformatics, School of Life Sciences, Institute of Science, GITAM University, Vishakhapatnam, India

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 microbiota 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 'toxigants', 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 definitions; 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".

***Corresponding author:** Vijendra Mishra, Department of Basic and Applied Sciences, National Institute Of Food Technology Entrepreneurship and Management (NIFTEM), Plot No. 97, Sector 56, HSIIDC Industrial Estate, Kundli, District Sonapat, Haryana-131028, India, Tel: 09034016639; E-mail: vijendramishra.niftem@gmail.com

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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*, *Pedococcus Leuconostoc*, *Enterococcus* 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):

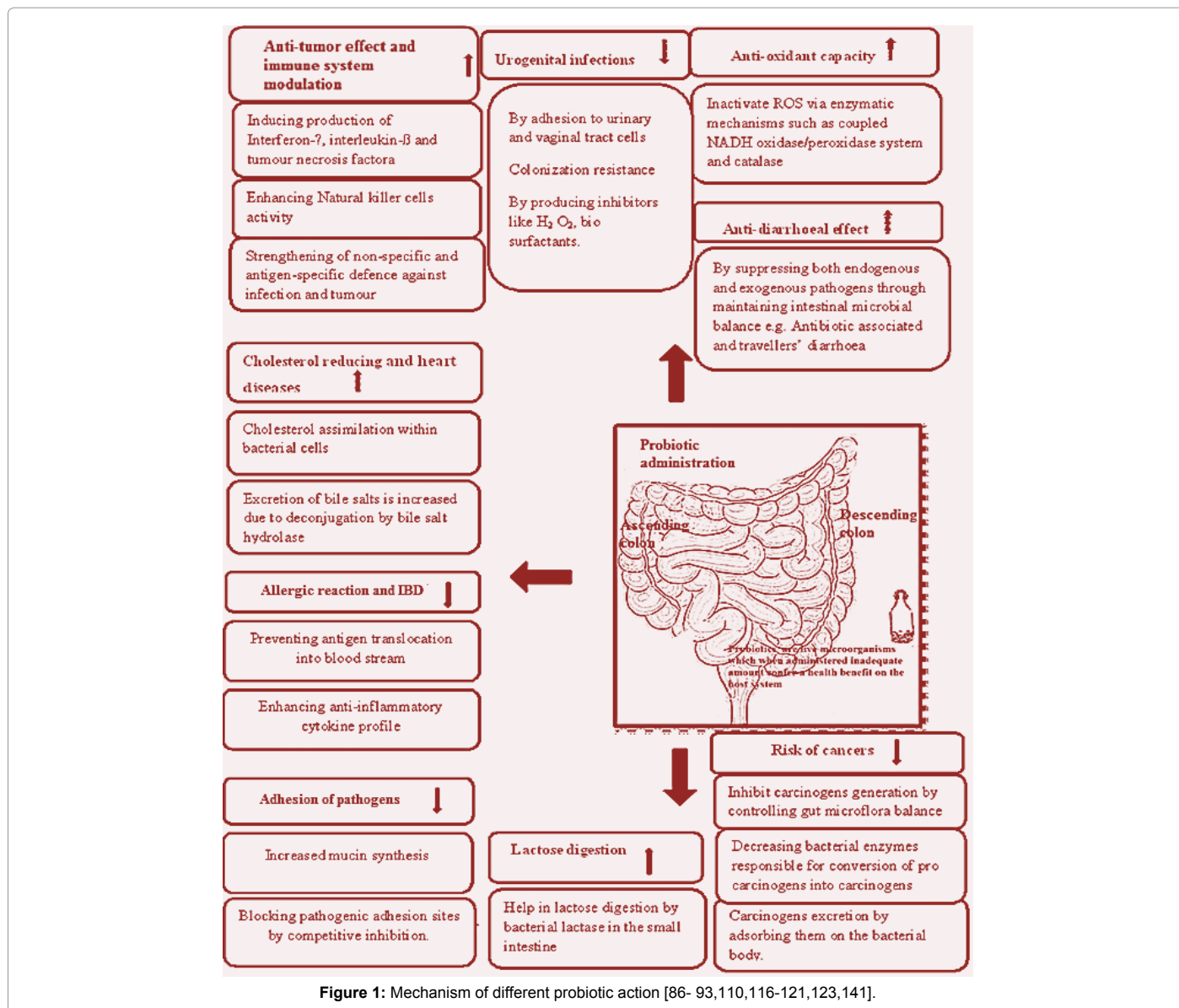


Figure 1: Mechanism of different probiotic action [86- 93,110,116-121,123,141].

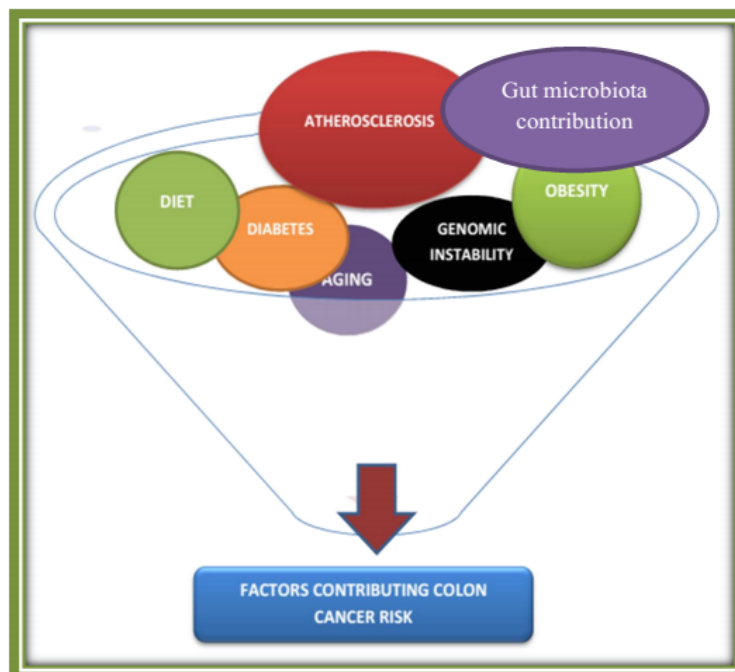


Figure 2: Factors contributing colon cancer risk.

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 convert 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 an 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 factors 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 result of age [45,46]. These bacteriological changes have been reported to often coincide with an increase 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 heterozygosity [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-days 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 somehow proportional to the leptin levels and are raised in obese individuals. Leptin is the product of the ob (obese) gene, plays 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].

Arteriosclerosis 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 arteriosclerosis in a number of ways, such as regulating the endothelial cell function (which is to be considered as an initial onset event in arteriosclerosis), vascular inflammation and formation of plaques [75,76]. In addition, in another study it is noted that hypo adiponectinemia is closely concerned with inflammatory arteriosclerosis 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 tumourigenesis [82]. The microflora in intestinal 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 Roberfroind [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 H₂O₂. 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. perfringens* 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. perfringens*. 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 faeces fed with active diet containing synbiotic mixture of dietary inulin (5%w/w) and *Bifidobacterium longum*; and they observed 55% lower fecal β -glucuronidase activity and 30% lower ammonia concentrations, when compared to the control rats [109]. In addition, some strains of Lactobacillus especially *Lactobacillus 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-1 β (Interleukin-1 β) 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 H₂O₂ (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

H₂O₂ 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.rhamnosus GG* has been excluded as a possible binding mechanism, since heat- and acid-killed *L.rhamnosus 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.thermophilus* 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 standardising 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

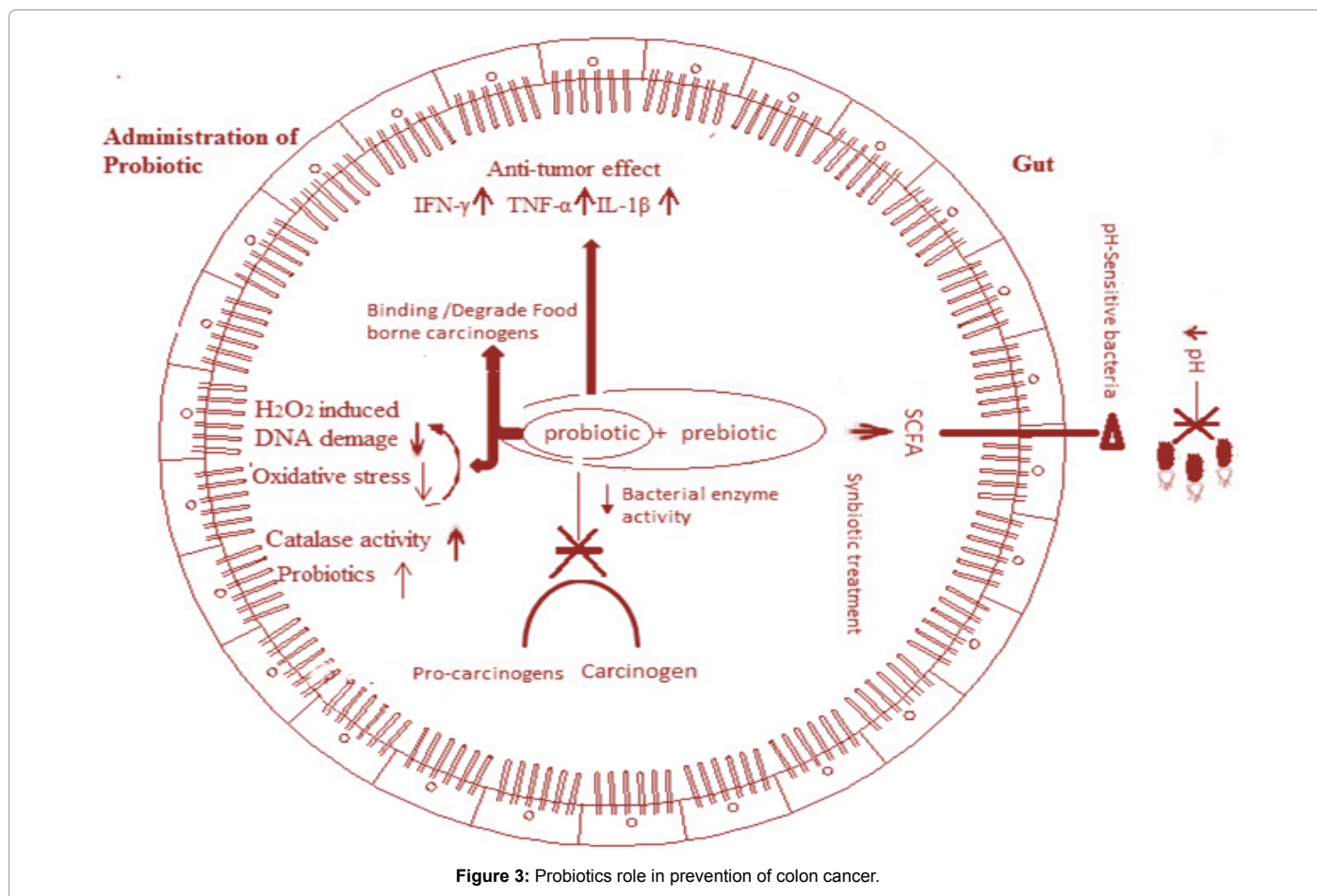


Figure 3: Probiotics role in prevention of colon cancer.

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	<i>L.acidophilus</i> 145 and <i>B. longum</i> 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.	<i>L. rhamnosus</i> LC705 and <i>P. freudenreichii</i> ssp. <i>shermanii</i> 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.	<i>Bifidobacterium lactis</i> 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 <i>Lactobacillus acidophilus</i> and they determined excretion of urinary and fecal mutagenic activity.	<i>L. acidophilus</i>	Upon consumption of <i>Lactobacillus acidophilus</i> decreased mutagenic excretion was observed.	[149]
Conducted a clinical study with 20 healthy subjects and they undergo consumption of probiotic mix product containing both <i>Lactobacillus rhamnosus</i> LC-705 and <i>Propionibacterium fereudenreichii</i> JS and they evaluated aflatoxin B1 level in fecal samples.	<i>L.rhamnosus</i> LC-705 <i>P. fereudenreichii</i> 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	<i>L. acidophilus</i> , <i>B. bifidum</i> , <i>Streptococcus</i> (<i>Lactococcus</i>) <i>lactis</i> , <i>L. lactis</i> subsp. <i>Cremoris</i>	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 <i>Lactobacillus casei</i> , 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.	<i>L.casei</i>	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% <i>Bifidobacterium animalis</i> DN-173010-FM. Group 4: supplemented with 30% <i>Streptococcus thermophilus</i> 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	<i>B.animalis</i> DN-173 010-FM. <i>Streptococcus thermophilus</i> DN-001 158-FM	Aberrant crypts incidence was decreased compared to control diet. -Decreased HAA metabolism was noticed and reduced colonic DNA lesions, fecal 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 \times 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	<i>Bacillus polyfermenticus</i>	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 <i>Lactobacillus rhamnosus</i> IMC501 Group 3: (Positive control), Mice given with PhIP(2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine- a food mutagen)+ physiological saline. Group 4: <i>Lactobacillus rhamnosus</i> IMC501 + PhIP. Afterwards they undergone comet assay for to evaluate DNA damage in colon	<i>L.rhamnosus</i> IMC501	<i>Lactobacillus rhamnosus</i> 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(pioxicam) 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.	<i>L.acidophilus</i> LaVK2, <i>L.plantarum</i> 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 preparations and they analysed for activities of β -glucosidase and β -glucuronidase.	<i>Lactobacillus acidophilus</i> NCFM, <i>Bifidobacterium adolescentis</i> 2204	Probiotic treatment 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	<i>Lactobacillus casei</i>	High number of Bifidobacteria in fecal 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 <i>Lactobacillus reuteri</i> 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 <i>L. reuteri</i> against bile salt cytotoxicity.	<i>Lactobacillus reuteri</i>	It preprecipitate 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).	<i>L. rhamnosus</i> 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 in vivo 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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References

- Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, et al. (2009) Bacterial community variation in human body habitats across space and time. *Science* 326: 1694-1697.
- Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: the unseen majority. *Proc Natl Acad Sci U S A* 95: 6578-6583.
- Moran NA, McCutcheon JP, Nakabachi A (2008) Genomics and evolution of heritable bacterial symbionts. *Annu Rev Genet* 42: 165-190.
- Mason P (2001) Prebiotics and probiotics. *The Pharmaceutical Journal* 266: 118-121.
- Tannock GW (1997) Probiotic properties of lactic-acid bacteria: plenty of scope for fundamental R & D. *Trends Biotechnol* 15: 270-274.
- Brown K, DeCoffee D, Molcan E, Gibson DL (2012) Diet-induced Dysbiosis of

the intestinal Microbiota and the Effects on immunity and Disease. *Nutrients* 4: 1095-1119.

- van Vliet MJ, Tissing WJ, Dun CA, Meessen NE, Kamps WA, et al. (2009) Chemotherapy Treatment in Pediatric Patients with Acute Myeloid Leukemia Receiving Antimicrobial Prophylaxis Leads to a Relative Increase of Colonization with Potentially Pathogenic Bacteria in the Gut. *Clinical Infectious Diseases* 49: 262-270.
- Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, et al. (2011) Linking long-term dietary patterns with gut microbial enterotypes. *Science* 334: 105-108.
- Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, et al. (2012) Gut microbiota composition correlates with diet and health in the elderly. *Nature* 488: 178-184.
- Antonopoulos DA, Huse SM, Morrison HG, Schmidt TM, Sogin ML, et al. (2009) Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation. *Infect Immun* 77: 2367-2375.
- Zhong L, Zhang X, Covasa M (2014) Emerging roles of lactic acid bacteria in protection against colorectal cancer. *World J Gastroenterol* 20: 7878-7886.
- FAO/WHO (2002) Guidelines for the evaluation of probiotics in Food; Report of a joint Food and Agriculture Organisation of the United Nations/World Health Organisation Working Group on Drafting guidelines for the evaluation of probiotics in food:London,Ontario,Canada.
- Salminen S, Ouwehand A, Benno Y, Lee YK (1999) Probiotics: how are they defined. *Trends in Food Science & Technology* 10: 107-110.
- Sivieri K, Bedani R, Cavallini DCU, Rossi EA (2013) Probiotics and Intestinal Microbiota: Implications in Colon Cancer Prevention. *Lactic Acid Bacteria - R & D for Food, Health and Livestock Purposes*, Dr. J. Marcelino Kongo (Ed.), ISBN: 978-953-51-0955-6, InTech.
- Kroustis G (1979) *Omnia opera Hippokratris-Apanta ta toulppokratouV.* (in Ancient Greek and Latin) (ed) 1979, University of Athens, Athens, Greece.
- Marini F, Radin S, Tenchini P, Mangiante G, Manganelli F, et al. (1989) [Reinterpretation of the hepatic abscess, a new dimension in abdominal digestive surgery]. *Chir Ital* 41: 79-116.
- Metchnikoff E (1908) *Optimistic studies* New York: Putman's Sons, pp: 161-183.
- Colin H, Francisco G, Gregor R, Glenn RG, Daniel JM, et al. (2014) Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 11: 506-514.

19. Zubillaga M, Weill R, Postaire E, Goldman C, Caro R, et al. (2001) Effect of probiotics and functional foods and their use in different diseases. *Nutr Res* 21: 569-579.
20. Joint FAO/WHO (2002) Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food London, Ontario, Canada.
21. Shah NP (2004) Probiotics and prebiotics. *Agro-Food Ind.Hi-tech* 15: 13-16.
22. Fonden R, Mogensen G, Tanaka R, Salminen S (2000) Effect of culture containing dairy products on intestinal micro flora, human nutrition and health-current knowledge and future perspectives. *IDF Bull* 352: 4-30.
23. Bhatia SJ, Kochar N, Abraham P, Nair NG, Mehta AP (1989) Lactobacillus acidophilus inhibits growth of Campylobacter pylori in vitro. *J Clin Microbiol* 27: 2328-2330.
24. Reddy NR, Roth SM, Eigel WN, Pierson MD (1998) Foods and food ingredients for prevention of diarrhoea diseases in children in developing countries. *J Food Prot* 51: 66-75.
25. Agerbaek M, Gerdes LU, Richelsen B (1995) Hypocholesterolaemic effect of a new fermented milk product in healthy middle-aged men. *Eur J Clin Nutr* 49: 346-352.
26. Reid G, Bruce AW, Fraser N, Heinemann C, Owen J, et al. (2001) Oral probiotics can resolve urogenital infections. *FEMS Immunol Med Microbiol* 30: 49-52.
27. Niedzielin K, Kordecki H, Birkenfeld B (2001) A controlled, double-blind, randomized study on the efficacy of Lactobacillus plantarum 299V in patients with irritable bowel syndrome. *Eur J Gastroenterol Hepatol* 13: 1143-1147.
28. Famularo G, De Simone C, Pandey V, Sahu AR, Minisola G (2005) Probiotic lactobacilli: an innovative tool to correct the malabsorption syndrome of vegetarians? *Med Hypotheses* 65: 1132-1135.
29. Kimura K, McCartney AL, McConnell MA, Tannock GW (1997) Analysis of fecal populations of bifidobacteria and lactobacilli and investigation of the immunological responses of their human hosts to the predominant strains. *Appl Environ Microbiol* 63: 3394-3398.
30. Fuller R, Gibson GR (1997) Modification of the intestinal microflora using probiotics and prebiotics. *Scand J Gastroenterol Suppl* 222: 28-31.
31. Huerta S, Goulet EJ, Livingston EH (2006) Colon cancer and apoptosis. *Am J Surg* 191: 517-526.
32. INCA – Instituto nacional de câncer (2008) Ações de prevenção primária e secundária no controle do câncer. Rio de Janeiro: Inca 2008: 628.
33. Stein K, Borowicki A, Scharlau D, Schettler A, Scheu K, et al. (2012) Effects of synbiotic fermentation products on primary chemoprevention in human colon cells. *J Nutr Biochem* 23: 777-784.
34. Platz EA, Willett WC, Colditz GA, Rimm EB, Spiegelman D, et al. (2000) Proportion of colon cancer risk that might be preventable in a cohort of middle-aged US men. *Cancer Causes Control* 11: 579-588.
35. Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, et al. (2008) Cancer is a preventable disease that requires major lifestyle changes. *Pharm Res* 25: 2097-2116.
36. Felton JS, Knize MG (1991) Occurrence, identification, and bacterial mutagenicity of heterocyclic amines in cooked food. *Mutat Res* 259: 205-217.
37. Marshall JR (2008) Prevention of colorectal cancer: diet, chemoprevention, and lifestyle. *Gastroenterol Clin North Am* 37: 73-82.
38. Bruce WR, Wolever TM, Giacca A (2000) Mechanisms linking diet and colorectal cancer: the possible role of insulin resistance. *Nutr Cancer* 37: 19-26.
39. Bruce WR (1987) Recent hypotheses for the origin of colon cancer. *Cancer Res* 47: 4237-4242.
40. Deschner EE, Cohen BI, Raicht RF (1981) Acute and chronic effect of dietary cholic acid on colonic epithelial cell proliferation. *Digestion* 21: 290-296.
41. McGarr SE, Ridlon JM, Hylemon PB (2005) Diet, anaerobic bacterial metabolism, and colon cancer: a review of the literature. *J Clin Gastroenterol* 39: 98-109.
42. Majumdar APN, Basson MD (2006) Effect of Aging on the Gastrointestinal Tract. New York, NY: Academic 2006: 405-433.
43. Hopkins MJ, Sharp R, Macfarlane GT (2001) Age and disease related changes in intestinal bacterial populations assessed by cell culture, 16S rRNA abundance, and community cellular fatty acid profiles. *Gut* 48: 198-205.
44. Hopkins MJ, Macfarlane GT (2002) Changes in predominant bacterial populations in human faeces with age and with Clostridium difficile infection. *J Med Microbiol* 51: 448-454.
45. Woodmansey EJ, McMurdo ME, Macfarlane GT, Macfarlane S (2004) Comparison of compositions and metabolic activities of fecal microbiotas in young adults and in antibiotic-treated and non-antibiotic-treated elderly subjects. *Appl Environ Microbiol* 70: 6113-6122.
46. Bartosch S, Fite A, Macfarlane GT, McMurdo ME (2004) Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Applied and Environmental Microbiology* 70: 3575-3581.
47. Saunier K, Doré J (2002) Gastrointestinal tract and the elderly: functional foods, gut microflora and healthy ageing. *Dig Liver Dis* 34 Suppl 2: S19-24.
48. Fearon ER, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. *Cell* 61: 759-767.
49. Rabeneck L, Davila JA, El-Serag HB (2003) Is there a true "shift" to the right colon in the incidence of colorectal cancer? *Am J Gastroenterol* 98: 1400-1409.
50. Hope ME, Hold GL, Kain R, El-Omar EM (2005) Sporadic colorectal cancer-role of the commensal microbiota. *FEMS Microbiol Lett* 244: 1-7.
51. Polaki P (2007) The many ways of Wnt in cancer. *Curr Opin Genet Dev* 17: 45-51.
52. Sanders LM, Henderson CE, Hong MY, Barhoumi R, Burghardt RC, et al. (2004) An increase in reactive oxygen species by dietary fish oil coupled with the attenuation of antioxidant defenses by dietary pectin enhances rat colonocyte apoptosis. *J Nutr* 134: 3233-3238.
53. Smith K, Bui TD, Poulson R, Kaklamanis L, Williams G, et al. (1999) Up-regulation of macrophage wnt gene expression in adenoma-carcinoma progression of human colorectal cancer. *Br J Cancer* 81: 496-502.
54. Zeng H, Lazarova DL (2012) Obesity-related colon cancer: dietary factors and their mechanisms of anticancer action. *Clin Exp Pharmacol Physiol* 39: 161-167.
55. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ (2003) Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 348: 1625-1638.
56. Sauter ER, Garofalo C, Hewett J, Hewett JE, Morelli C, et al. (2004) Leptin expression in breast nipple aspirate fluid (NAF) and serum is influenced by body mass index (BMI) but not by the presence of breast cancer. *Horm Metab Res* 36: 336-340.
57. Attele AS, Shi ZQ, Yuan CS (2002) Leptin, gut, and food intake. *Biochem Pharmacol* 63: 1579-1583.
58. Garofalo C, Surmacz E (2006) Leptin and cancer. *J Cell Physiol* 207: 12-22.
59. Sulkowska M, Golaszewska J, Wincewicz A, Koda M, Baltaziak M, et al. (2006) Leptin—from regulation of fat metabolism to stimulation of breast cancer growth. *Pathol Oncol Res* 12: 69-72.
60. Sierra-Honigmann MR, Nath AK, Murakami C, García-Cardeña G, Papapetropoulos A, et al. (1998) Biological action of leptin as an angiogenic factor. *Science* 281: 1683-1686.
61. Rouet-Benzineb P, Aparicio T, Guilmeau S, Pouzet C, Descatoire V, et al. (2004) Leptin counteracts sodium butyrate-induced apoptosis in human colon cancer HT-29 cells via NF-kappaB signaling. *J Biol Chem* 279: 16495-16502.
62. Attoub S, Noe V, Pirolo L, Bruyneel E, Chastre E, et al. (2000) Leptin promotes invasiveness of kidney and colonic epithelial cells via phosphoinositide 3-kinase-, rho-, and rac-dependent signaling pathways. *FASEB J* 14: 2329-2338.
63. Liu Z, Uesaka T, Watanabe H, Kato N (2001) High fat diet enhances colonic cell proliferation and carcinogenesis in rats by elevating serum leptin. *Int J Oncol* 19: 1009-1014.
64. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, et al. (2008) Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 57: 1470-1481.
65. Björntorp P (1991) Metabolic implications of body fat distribution. *Diabetes Care* 14: 1132-1143.

66. Kissebah AH, Vydellingum N, Murray R, Evans DJ, Hartz AJ, et al. (1982) Relation of body fat distribution to metabolic complications of obesity. *J Clin Endocrinol Metab* 54: 254-260.
67. Krotkiewski M, Björntorp P, Sjöström L, Smith U (1983) Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. *J Clin Invest* 72: 1150-1162.
68. Donahue RP, Abbott RD (1987) Central obesity and coronary heart disease in men. *Lancet* 1: 821-824.
69. Powell DR, Suwanichkul A, Cabbage ML, DePaolis LA, Snuggs MB, et al. (1991) Insulin inhibits transcription of the human gene for insulin-like growth factor-binding protein-1. *J Biol Chem* 266: 18868-18876.
70. LeRoith D, Baserga R, Helman L, Roberts CT Jr (1995) Insulin-like growth factors and cancer. *Ann Intern Med* 122: 54-59.
71. Baserga R, Hongo A, Rubini M, Prisco M, Valentini B (1997) The IGF-I receptor in cell growth, transformation and apoptosis. *Biochim Biophys Acta* 1332: F105-126.
72. Singh P, Rubin R (1993) Insulin-like growth factors and binding proteins in colon cancer. *Gastroenterology* 105: 1218-1237.
73. MacDougald OA, Burant CF (2007) The rapidly expanding family of adipokines. *Cell Metab* 6: 159-161.
74. Cao H, Gerhold K, Mayers JR, Wiest MM, Watkins SM, et al. (2008) Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. *Cell* 134: 933-944.
75. Zhang H, Cui J, Zhang C (2010) Emerging role of adipokines as mediators in atherosclerosis. *World J Cardiol* 2: 370-376.
76. Jardé T, Caldefie-Chézet F, Goncalves-Mendes N, Mishellany F, Buechler C, et al. (2009) Involvement of adiponectin and leptin in breast cancer: clinical and in vitro studies. *Endocr Relat Cancer* 16: 1197-1210.
77. Lago F, Dieguez C, Gómez-Reino J, Gualillo O (2007) The emerging role of adipokines as mediators of inflammation and immune responses. *Cytokine Growth Factor Rev* 18: 313-325.
78. Kelesidis I, Kelesidis T, Mantzoros CS (2006) Adiponectin and cancer: a systematic review. *Br J Cancer* 94: 1221-1225.
79. Sugiyama M, Takahashi H, Hosono K, Endo H, Kato S, et al. (2009) Adiponectin inhibits colorectal cancer cell growth through the AMPK/mTOR pathway. *Int J Oncol* 34: 339-344.
80. Saxena A, Chumanevich A, Fletcher E, Larsen B, Lattwein K, et al. (2012) Adiponectin deficiency: Role in chronic inflammation induced colon cancer. *Biochimica et Biophysica Acta* 1822: 527-536.
81. Sobhani I, Amiot A, Le Baleur Y, Levy M, Auriault ML, et al. (2013) Microbial dysbiosis and colon carcinogenesis: could colon cancer be considered a bacteria-related disease?, *Ther Adv Gastroenterol*. 6: 215-229.
82. Huycke MM, Gaskins HR (2004) Commensal bacteria, redox stress, and colorectal cancer: mechanisms and models. *Exp Biol Med (Maywood)* 229: 586-597.
83. Rolfe RD1 (2000) The role of probiotic cultures in the control of gastrointestinal health. *J Nutr* 130: 396S-402S.
84. Anuradha S, Rajeshwari K (2005) Probiotics in health and disease. *J Ind Acad Clin Med* 6: 67-72.
85. Rafter J1 (2002) Lactic acid bacteria and cancer: mechanistic perspective. *Br J Nutr* 88 Suppl 1: S89-94.
86. De Preter V, Geboes K, Verbrugghe K, De Vuyst L, Vanhoutte T, et al. (2004) The in vivo use of the stable isotope-labelled biomarkers lactose-[15N]ureide and [2H4]tyrosine to assess the effects of pro- and prebiotics on the intestinal flora of healthy human volunteers. *Br J Nutr* 92: 439-446.
87. Hayatsu H, Hayatsu T (1993) Suppressing effect of *Lactobacillus casei* administration on the urinary mutagenicity arising from ingestion of fried ground beef in the human. *Cancer Lett* 73: 173-179.
88. Mack DR, Ahrne S, Hyde L, Wei S, Hollingsworth MA (2003) Extracellular MUC3 mucin secretion follows adherence of *Lactobacillus* strains to intestinal epithelial cells in vitro. *Gut* 52: 827-833.
89. Conway PL, Gorbach SL, Goldin BR (1987) Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. *J Dairy Sci* 70: 1-12.
90. Sanders ME, Huis in't Veld J (1999) Bringing a probiotic-containing functional food to the market: microbiological, product, regulatory and labeling issues. *Antonie Van Leeuwenhoek* 76: 293-315.
91. Takeda K, Suzuki T, Shimada SI, Shida K, Nanno M, et al. (2006) Interleukin-12 is involved in the enhancement of human natural killer cell activity by *Lactobacillus casei* Shirota. *Clin Exp Immunol* 146: 109-115.
92. Pessi T, Sütas Y, Saxelin M, Kallioinen H, Isolauri E (1999) Antiproliferative effects of homogenates derived from five strains of candidate probiotic bacteria. *Appl Environ Microbiol* 65: 4725-4728.
93. Saarela M, Lähteenmäki L, Crittenden R, Salminen S, Mattila-Sandholm T (2002) Gut bacteria and health foods--the European perspective. *Int J Food Microbiol* 78: 99-117.
94. Zhu Y, Michelle Luo T, Jobin C, Young HA (2011) Gut microbiota and probiotics in colon tumorigenesis. *Cancer Lett* 309: 119-127.
95. Chambers WM, Warren BF, Jewell DP, Mortensen NJ (2005) Cancer surveillance in ulcerative colitis. *Br J Surg* 92: 928-936.
96. Roberfroid M1 (2007) Prebiotics: the concept revisited. *J Nutr* 137: 830S-7S.
97. Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* 125: 1401-1412.
98. Glenn RG, Karen PS, Robert AR, Kieran MT, Arland H, et al. (2011) Dietary prebiotics: current status and new definition. *IFIS Functional Foods Bulletin* 7: 1-19.
99. Wollowski I, Reckemmer G, Pool-Zobel BL (2001) Protective role of probiotics and prebiotics in colon cancer. *Am J Clin Nutr* 73: 451S-455S.
100. Moreau MC, Gaboriau-Routhiau V (2000) Influence of resident intestinal microflora on the development and functions of the intestinal-associated lymphoid tissue. In R. Fuller & G. Perdigón (Eds.), *Probiotics 3 Immunomodulation by the gut flora and probiotics 2000*: 69-114.
101. Cherrington CA, Hinton M, Pearson GR, Chopra I (1991) Short-chain organic acids at pH 5.0 kill *Escherichia coli* and *Salmonella* spp. without causing membrane perturbation. *J Appl Bacteriol* 70: 161-165.
102. Rosignoli P, Fabiani R, De Bartolomeo A, Spinozzi F, Agea E, et al. (2001) Protective activity of butyrate on hydrogen peroxide-induced DNA damage in isolated human colonocytes and HT29 tumour cells. *Carcinogenesis* 22: 1675-1680.
103. Hague A, Elder DJ, Hicks DJ, Paraskeva C (1995) Apoptosis in colorectal tumour cells: induction by the short chain fatty acids butyrate, propionate and acetate and by the bile salt deoxycholate. *Int J Cancer* 60: 400-406.
104. Marchetti C, Migliorati G, Moraca R, Riccardi C, Nicoletti I, et al. (1997) Deoxycholic acid and SCFA-induced apoptosis in the human tumor cell-line HT-29 and possible mechanisms. *Cancer Lett* 114: 97-99.
105. Hass R, Busche R, Luciano L, Reale E, Engelhardt WV (1997) Lack of butyrate is associated with induction of Bax and subsequent apoptosis in the proximal colon of guinea pig. *Gastroenterology* 112: 875-881.
106. Thirabunyanon M, Hongwittayakorn P (2013) Potential probiotic lactic acid bacteria of human origin induce antiproliferation of colon cancer cells via synergic actions in adhesion to cancer cells and short-chain fatty acid bioproduction. *Appl Biochem Biotechnol* 169: 511-525.
107. Rafter J, Bennett M, Caderni G, Clune Y, Hughes R, et al. (2007) Dietary synbiotics reduce cancer risk factors in polypectomized and colon cancer patients. *Am J Clin Nutr* 85: 488-496.
108. Borneo FR (1994) Undigestible sugars in food products. *Am J Clin Nutr* 59: 763S-769S.
109. Lee JW, Shin JG, Kim EH, Kang HE, Yim IB, et al. (2004) Immunomodulatory and antitumor effects in vivo by the cytoplasmic fraction of *Lactobacillus casei* and *Bifidobacterium longum*. *J Vet Sci* 5: 41-48.
110. Matsuzaki T (1998) Immunomodulation by treatment with *Lactobacillus casei* strain Shirota. *Int J Food Microbiol* 41: 133-140.
111. Luu HH, Zhang R, Haydon RC, Rayburn E, Kang Q, et al. (2004) Wnt/beta-catenin signaling pathway as a novel cancer drug target. *Curr Cancer Drug Targets* 4: 653-671.
112. Malhotra P, Anwar M, Nanda N, Kochhar R, Wig JD, et al. (2013) Alterations in K-ras, APC and p53-multiple genetic pathway in colorectal cancer among Indians. *Tumour Biol* 34: 1901-1911.

113. Urbanska AM, Bhatena J, Martoni C, Prakash S (2009) Estimation of the potential antitumor activity of microencapsulated *Lactobacillus acidophilus* yogurt formulation in the attenuation of tumorigenesis in *Apc(Min/+)* mice. *Dis Dis Sci* 54: 264-273.
114. Escamilla J, Lane MA, Maitin V (2012) Cell-free supernatants from probiotic *Lactobacillus casei* and *Lactobacillus rhamnosus* GG decrease colon cancer cell invasion in vitro. *Nutr Cancer* 64: 871-878.
115. Guz J, Foksinski M, Siomek A, Gackowski D, Rozalski R, et al. (2008) The relationship between 8-oxo-7,8-dihydro-2'-deoxyguanosine level and extent of cytosine methylation in leukocytes DNA of healthy subjects and in patients with colon adenomas and carcinomas. *Mutation Research* 640: 170-173.
116. Foksinski M, Rozalski R, Guz J, Ruzskowska B, Sztukowska P, et al. (2004) Urinary excretion of DNA repair products correlates with metabolic rates as well as with maximum life spans of different mammalian species. *Free Radic Biol Med* 37: 1449-1454.
117. Amanatidou A, Bennik MH, Gorris LG, Smid EJ (2001) Superoxide dismutase plays an important role in the survival of *Lactobacillus* sake upon exposure to elevated oxygen. *Arch Microbiol* 176: 79-88.
118. Bruno-Bárcena JM, Andrus JM, Libby SL, Klaenhammer TR, Hassan HM (2004) Expression of a heterologous manganese superoxide dismutase gene in intestinal lactobacilli provides protection against hydrogen peroxide toxicity. *Appl Environ Microbiol* 70: 4702-4710.
119. Kullisaar T, Songisepp E, Mikelsaar M, Zilmer K, Vihalemm T, et al. (2003) Antioxidative probiotic fermented goats' milk decreases oxidative stress-mediated atherogenicity in human subjects. *Br J Nutr* 90: 449-456.
120. Kullisaar T, Zilmer M, Mikelsaar M, Vihalemm T, Annuk H, et al. (2002) Two antioxidative lactobacilli strains as promising probiotics. *Int J Food Microbiol* 72: 215-224.
121. Lee J, Hwang KT, Heo MS, Lee JH, Park KY (2005) Resistance of *Lactobacillus plantarum* KCTC 3099 from Kimchi to oxidative stress. *J Med Food* 8: 299-304.
122. de Moreno de LeBlanc A, LeBlanc JG, Perdigón G, Miyoshi A, Langella P, et al. (2008) Oral administration of a catalase-producing *Lactococcus lactis* can prevent a chemically induced colon cancer in mice. *J Med Microbiol* 57: 100-105.
123. Goldin BR (1990) Intestinal microflora: metabolism of drugs and carcinogens. *Ann Med* 22: 43-48.
124. Goldin BR, Gorbach SL (1976) The relationship between diet and rat fecal bacterial enzymes implicated in colon cancer. *J Natl Cancer Inst* 57: 371-375.
125. Goldin BR, Gorbach SL (1980) Effect of *Lactobacillus acidophilus* dietary supplements on ,2-dimethylhydrazine dihydrochloride-induced intestinal cancer in rats. *J Natl Cancer Inst* 64: 263-265.
126. Gorbach SL (2000) Probiotics and gastrointestinal health. *Am J Gastroenterol* 95: S2-4.
127. Sugimura T, Wakabayashi K, Nakagama H, Nagao M (2004) Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. *Cancer Sci* 95: 290-299.
128. El-Nezami HS, Chrevatidis A, Auriola S, Salminen S, Mykkänen H (2002) Removal of common *Fusarium* toxins in vitro by strains of *Lactobacillus* and *Propionibacterium*. *Food Addit Contam* 19: 680-686.
129. Niderkorn V, Boudra H, Morgavi DP (2006) Binding of *Fusarium* mycotoxins by fermentative bacteria in vitro. *J Appl Microbiol* 101: 849-856.
130. Ibrahim F, Halttunen T, Tahvonen R, Salminen S (2006) Probiotic bacteria as potential detoxification tools: assessing their heavy metal binding isotherms. *Can J Microbiol* 52: 877-885.
131. Halttunen T, Collado MC, El-Nezami H, Meriluoto J, Salminen S (2008) Combining strains of lactic acid bacteria may reduce their toxin and heavy metal removal efficiency from aqueous solution. *Lett Appl Microbiol* 46: 160-165.
132. el-Nezami H, Kankaanpää P, Salminen S, Ahokas J (1998) Physicochemical alterations enhance the ability of dairy strains of lactic acid bacteria to remove aflatoxin from contaminated media. *J Food Prot* 61: 466-468.
133. Lahtinen SJ, Haskard CA, Ouwehand AC, Salminen SJ, Ahokas JT (2004) Binding of aflatoxin B1 to cell wall components of *Lactobacillus rhamnosus* strain GG. *Food Addit Contam* 21: 158-164.
134. Pizzolitto RP, Salvano MA, Dalcero AM (2012) Analysis of fumonisin B1 removal by microorganisms in co-occurrence with aflatoxin B1 and the nature of the binding process. *Int J Food Microbiol* 156: 214-221.
135. Lijinsky W, Saavedra JE, Reuber MD (1985) Organ-specific carcinogenesis in rats by methyl- and ethylazoxymethanes. *Cancer Res* 45: 76-79.
136. Reddy BS, Hamid R, Rao CV (1997) Effect of dietary oligofructose and inulin on colonic preneoplastic aberrant crypt foci inhibition. *Carcinogenesis* 18: 1371-1374.
137. Singh J, Rivenson A, Tomita M, Shimamura S, Ishibashi N, et al. (1997) *Bifidobacterium longum*, a lactic acid-producing intestinal bacterium inhibits colon cancer and modulates the intermediate biomarkers of colon carcinogenesis. *Carcinogenesis* 18: 833-841.
138. Reddy BS (1998) Prevention of colon cancer by pre- and probiotics: evidence from laboratory studies. *Br J Nutr* 80: S219-223.
139. Burns AJ, Rowland IR (2004) Antigenotoxicity of probiotics and prebiotics on faecal water-induced DNA damage in human colon adenocarcinoma cells. *Mutat Res* 551: 233-243.
140. Uccello M, Malaguarnera G, Basile F, D'agata V, Malaguarnera M, et al. (2012) Potential role of probiotics on colorectal cancer prevention. *BMC Surg* 12 Suppl 1: S35.
141. O'Mahony L, Feeney M, O'Halloran S, Murphy L, Kiely B, et al. (2001) Probiotic impact on microbial flora, inflammation and tumour development in IL-10 knockout mice. *Aliment Pharmacol Ther* 15: 1219-1225.
142. Morotomi M, Mutai M (1986) In vitro binding of potent mutagenic pyrolysates to intestinal bacteria. *J Natl Cancer Inst* 77: 195-201.
143. Rowland IR, Grasso P (1975) Degradation of N-nitrosamines by intestinal bacteria. *Appl Microbiol* 29: 7-12.
144. Zhang XB, Ohta Y (1993) Microorganisms in the gastrointestinal tract of the rat prevent absorption of the mutagen-carcinogen 3-amino-4-dimethyl-5H-pyrido(4,3-b)indole. *Can J Microbiol* 39: 841-845.
145. Ayebo AD, Angelo IA, Shahani KM (1980) Effect of ingesting *Lactobacillus acidophilus* milk upon faecal flora and enzyme activity in humans. *Milch Wissenschaft* 35: 730-733.
146. Oberreuther-Moschner DL, Jahreis G, Rechkemmer G, Pool-Zobel BL (2004) Dietary intervention with the probiotics *Lactobacillus acidophilus* 145 and *Bifidobacterium longum* 913 modulates the potential of human faecal water to induce damage in HT29clone19A cells. *Br J Nutr* 91: 925-932.
147. Hatakka K, Holma R, El-Nezami H, Suomalainen T, Kuisma M, et al. (2008) The influence of *Lactobacillus rhamnosus* LC705 together with *Propionibacterium freudenreichii* ssp. *shermanii* JS on potentially carcinogenic bacterial activity in human colon. *Int J Food Microbiol* 128: 406-410.
148. Matsumoto M, Benno Y (2004) Consumption of *Bifidobacterium lactis* LKM512 yogurt reduces gut mutagenicity by increasing gut polyamine contents in healthy adult subjects. *Mutat Res* 568: 147-153.
149. Lidbeck A, Övervik E, Rafter J, Nord CE, Gustafsson J (1992) Effect of *Lactobacillus acidophilus* supplements on mutagen excretion in faeces and urine in humans. *Microbial Ecol Health Dis* 5: 59-67.
150. El-Nezami HS, Mykkänen H, Kankaanpää P, Suomalainen T, Salminen S, et al. (2000) The ability of a mixture of *Lactobacillus* and *Propionibacterium* to influence the faecal recovery of aflatoxins in healthy Egyptian volunteers: A pilot clinical study. *Biosci Microflora* 19: 41-45.
151. Marteau P, Pochart P, Flourié B, Pellier P, Santos L, et al. (1990) Effect of chronic ingestion of a fermented dairy product containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on metabolic activities of the colonic flora in humans. *Am J Clin Nutr* 52: 685-688.
152. Ishikawa H, Akedo I, Otani T, Suzuki T, Nakamura T, et al. (2005) Randomized trial of dietary fiber and *Lactobacillus casei* administration for prevention of colorectal tumors. *Int J Cancer* 116: 762-767.
153. Tavan E, Cayuela C, Antoine JM, Trugnan G, Chaugier C, et al. (2002) Effects of dairy products on heterocyclic aromatic amine-induced rat colon carcinogenesis. *Carcinogenesis* 23: 477-483.
154. Park E, Jeon GI, Park JS, Paik HD (2007) A probiotic strain of *Bacillus polyfermenticus* reduces DMH induced precancerous lesions in F344 male rat. *Biol Pharm Bull* 30: 569-574.

155. Luca Dominici, Milena Villarini, Francesca Trotta, Ermanno Federici, Giovanni Cenci, et al. (2014) Protective Effects of Probiotic *Lactobacillus rhamnosus* IMC501 in Mice Treated with PhIP. *J Microbiol Biotechnol* 24: 371-378.
156. Dheeraj M, Vinod KK, Renu S, Dilip S (2013) Anticarcinogenic Effect of Probiotic Dahi and Piroxicam on DMH-induced Colorectal Carcinogenesis in Wistar Rats. *American Journals of Cancer Therapy and Pharmacology* 1: 17.
157. Reyniers JA (1959) Germfree Vertebrates: Present Status. *Annals of the New York Academy of Sciences* 78 : 3.
158. Foster JW, Slonczewski JL (2009) *Microbiology, An Evolving Science*. W.W.Norton: 871.
159. Cole CB, Fuller R, Carter SM (1989) Effect of Probiotic Supplements of *Lactobacillus acidophilus* and *Bifidobacterium adolescentis* 2204 on P-glucosidase and P-glucuronidase Activity in the Lower Gut of Rats Associated with a Human Faecal Flora. *MICROBIAL ECOLOGY IN HEALTH AND DISEASE* 2: 223-225 (1989).
160. Djouzi Z, Andrieux C, Degivry MC, Bouley C, Szylit O (1997) The association of yogurt starters with *Lactobacillus casei* DN 114.001 in fermented milk alters the composition and metabolism of intestinal microflora in germ-free rats and in human flora-associated rats. *J Nutr* 127: 2260-2266.
161. Possemiers S, Verthé K, Uyttendaele S, Verstraete W (2004) PCR-DGGE-based quantification of stability of the microbial community in a simulator of the human intestinal microbial ecosystem. *FEMS Microbiol Ecol* 49: 495-507.
162. de Wiele TV, Boon N, Possemiers S, Jacobs H, Verstraete W (2004) Prebiotic effects of chicory inulin in the simulator of the human intestinal microbial ecosystem. *FEMS Microbiol Ecol* 51: 143-153.
163. De Boever P, Deplancke B, Verstraete W (2000) Fermentation by gut microbiota cultured in a simulator of the human intestinal microbial ecosystem is improved by supplementing a soygerm powder. *J Nutr* 130: 2599-2606.
164. Massimo M, Iris P, Pieter Van Da, Tom Van Dw, Sam P (2012) An in vitro technology platform to assess host-microbiota interactions in the gastrointestinal tract. *Dietary Fibres & Pre/Probiotics* 23: 8-11.
165. De Boever P, Wouters R, Verschaeve L, Berckmans P, Schoeters G, et al. (2000) Protective effect of the bile salt hydrolase-active *Lactobacillus reuteri* against bile salt cytotoxicity. *Appl Microbiol Biotechnol* 53: 709-714.
166. Kim JU, Kim Y, Han K, Sejong OH, Whang KY, et al. (2006) The function of cell bound and cell released Exopolysaccharides produced by *Lactobacillus rhamnosus* ATCC 9595. *J Microbiol Biotech* 16: 939-945.