

Potential Anticarcinogenic Effects of Lactic Acid Bacteria and Probiotics in Detoxification of Process-Induced Food Toxicants

Nasim Khorshidian,¹ Mojtaba Yousefi Asli,¹ Hedayat Hosseini,² Mahdi Shadnoush,^{3,4,*} and Amir Mohammad Mortazavian²

¹Department of Food Science and Technology, Student Research Committee, Faculty of Nutrition Sciences, Food Science and Technology/National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

²Department of Food Science and Technology, Faculty of Nutrition Sciences, Food Science and Technology/National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

³Department of Clinical Nutrition, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

⁴Department of Clinical Nutrition, School of Medicine, Semnan University of Medical Sciences, Semnan, IR Iran

*Corresponding author: Mahdi Shadnoush (PhD), Department of Clinical Nutrition, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran. E-mail: mshadnoush@gmail.com

Received 2016 August 05; Revised 2016 September 22; Accepted 2016 October 04.

Abstract

Context: Nowadays, it has been proved that there is a relation between dietary habits and incidence of different types of cancers. Consumption of processed foods exposes human to a wide range of toxicants such as heterocyclic aromatic amines, polycyclic aromatic hydrocarbons, acrylamide and nitrosamines that have mutagenic and carcinogenic effects on body and especially induce colon cancer. Under such circumstances, search for antimutagenic agents and helpful strategies have gained interest.

Evidence Acquisition: We performed a computerized search of Scopus, Pubmed and google scholar databases with keywords: cancer, food toxicants, lactic acid bacteria, and probiotics.

Results: Natural dietary compounds like lactic acid bacteria and probiotics can be beneficial in decline of detrimental effects associated with toxicants formed during food processing. It has been stated that binding ability of lactic acid bacteria and probiotics via their cell wall have prominent roles in detoxifying these toxicants. Also, this capability is influenced by various factors.

Conclusions: It can be concluded that probiotics can play a vital role in prevention of colon cancer that is induced by food toxicants and their incorporation into food can be helpful in this respect.

Keywords: Anticarcinogenic, Cancer, Food Toxicants, Lactic Acid Bacteria, Probiotics

1. Context

Today, the production of healthy and safe food is a key element in food industry. Unlike the food industry's efforts to produce safe food, it is likely that food can be either contaminated during the process or prepared from contaminated raw materials (1). Therefore, food products consumers can be environmentally exposed to both intentional and unintentional additives and pollutants which can have adverse effects on health over time. Environmental contaminants such as heavy metals, pesticides and mycotoxins can be released to water and food production chain and lead to formation of unwanted harmful chemical compounds during processing, resulting in various adverse health effects and chronic toxicity, especially cancer (1-3). Cancer is a very serious and complicated disease created by out of control and irregular growth of cell (4), whose prevalence is remarkably increasing. Ex-

cept for genetic defects which contribute to 5 to 10% of cancer incidences, the rest (90% to 95%) can be limited by changing lifestyle, increasing physical activity, avoiding smoking and utilizing nutritionally balance diet together with the foods free from contaminants (4, 5). Lung, colon/rectum, breast, and prostate cancers are the most widespread among 100 human cancers (4). It is reported that bowel cancer is the second and third most prevalent type of cancer in Europe and worldwide, respectively (6, 7). Among the different aspects of lifestyle, it is generally approved that diet and nutritional factors have a major role in incident of cancer, particularly gastrointestinal tract-related ones such as colorectal cancer (CRC) (8, 9) and it is observed that 30% - 40% of cancer cases can be possibly prevented by improvement of diet and getting proper nutritional factors (10). Researches have shown that diets rich with fruits and vegetables seem to have a protective effect on CRC development (11) while the risk of CRC is en-

hanced by increasing consumption of red meat and animal fat (9, 12). Heterocyclic aromatic amines (HCA), polycyclic aromatic hydrocarbons (PAH), nitrites and nitrates are related to meat consumption. On the other hand, mycotoxins (aflatoxins) and acrylamide (13) might have important roles in the etiology of CRC (14-16). Although various approaches are utilized as common treatment options for CRC such as surgery, chemotherapy and radiotherapy (13, 17), lactic acid bacteria and probiotics gained a lot of attention as antimutagens and preventive agents in colon carcinogenesis (17-23).

2. Evidence Acquisition

In this article, binding ability of different LABs and probiotics strains to some food toxicants and the underlying mechanisms as well as parameters affecting this ability are reviewed. For the literature review, we have used standard search strategies involving the querying of available online databases (Scopus, Pubmed and google scholar) by using terms including “Anticarcinogenic”, “Cancer”, “Heterocyclic aromatic amines”, “Polycyclic aromatic hydrocarbons”, “Acrylamide”, “Nitrosamine”, “Lactic acid bacteria”, and “Probiotics”. No specific key words have required as inclusion criteria. The reference lists of each article have been reviewed in details to find additional articles. Articles found were categorized according to binding of lactic acid bacteria and probiotics to four types of food toxicants and were reviewed independently in full text.

3. Results

3.1. Lactic Acid Bacteria Probiotics, and Colon Cancer Prevention

‘Lactic acid bacteria (LAB)’ are varied groups of bacteria that produce mainly lactic acid as a consequence of carbohydrate fermentation. LABs are gram-positive, non-motile and cocci or rods which have been widely utilized to produce various foods especially fermented ones (1, 24). *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus* are the major genera of LABs (25). Most of them are presented in the oral cavity, the intestinal tract, and vagina normal flora having different beneficial effects and preservative properties (26, 27). Most of their useful properties are attributed to their ability to adhere to the intestinal mucosa (28). In addition, it is mentioned that LABs have protective effects against different toxic compounds in foods such as mycotoxin, acrylamide, polycyclic aromatic hydrocarbons (PAHs), heterocyclic amines (HCAs) and amino acid pyrolysates (16). ‘Probiotics’ that mainly belong to the type ‘lactobacillus’ or

‘Bifidobacteria’ are non-toxic and non-pathogenic, show in vivo functional properties and are considered probiotics (1, 29, 30). The term ‘probiotic’ is originated from Greek meaning ‘for life’ and these microorganisms bring health benefits to humans/animals principally by balancing intestinal flora. Apart from *Lactobacillus* and *Bifidobacterium* spp., *Pediococcus acidilactici*, *Enterococcus* spp. and *Saccharomyces boulardii* are also used as probiotics (1, 29, 31). Various health properties such as anti-mutagenic and anti-carcinogenic effects, modulation of the immune system, suppression of allergies, decreasing cholesterol levels, anti-infection properties, relief of lactose intolerance symptoms, and improving the nutritional value have been ascribed to LABs and probiotics (25, 32-35). The anti-carcinogenic effect, especially preventing colorectal cancer, is one of the most important health consequences of LABs and probiotics which are taken into consideration by most researchers (20, 22, 23, 25, 36-38). Although the exact mechanisms of colon cancer prevention have not been identified entirely, there are several possible mechanisms that could explain their anti-cancer properties. LABs and probiotics may inhibit colon cancer by enhancing the host’s immune response, changing the metabolism of intestinal microflora (19), reducing intestinal inflammation such as inflammatory bowel disease (IBD) (39-41), binding/adsorption of carcinogens by cell surface and peptidoglycans (42-46), altering the xenobiotic metabolizing enzyme, preventing the oxidative stress processes, and reducing reactive oxygen species (4, 47, 48). Additionally, it is indicated that production of various free fatty acids, organic acids, and other metabolites as a consequence of non-digestible carbohydrate fermentation in the gut and reducing pH are the other pathways hindering incidence of colon cancer (49). It is expressed that probiotic bacteria participate in detoxification and biotransformation of xenobiotics and converting them to less toxic metabolites as well as slowing down conversion of nontoxic procarcinogens to highly toxic metabolites and hinder tumor formation (50). Figure 1 shows main mechanisms of LABs and probiotics action in prevention of cancer. Also the main factors that have effects on the binding ability of lactic acid bacteria (LAB) and probiotics are shown in Figure 2.

3.2. Binding Ability of LABs and Probiotics to Heterocyclic Aromatic Amines (HAA) and Polycyclic Aromatic Hydrocarbons (PAH)

Heterocyclic aromatic amines are a class of chemical compounds that possess at least one heterocyclic ring and are classified into two major groups. One group, known as ‘pyrolytic HAAs’, are formed as a result of pyrolysis of some amino acids like tryptophan, glutamic acid, phenylalanine, and ornithine at elevated temperatures (> 250°C).

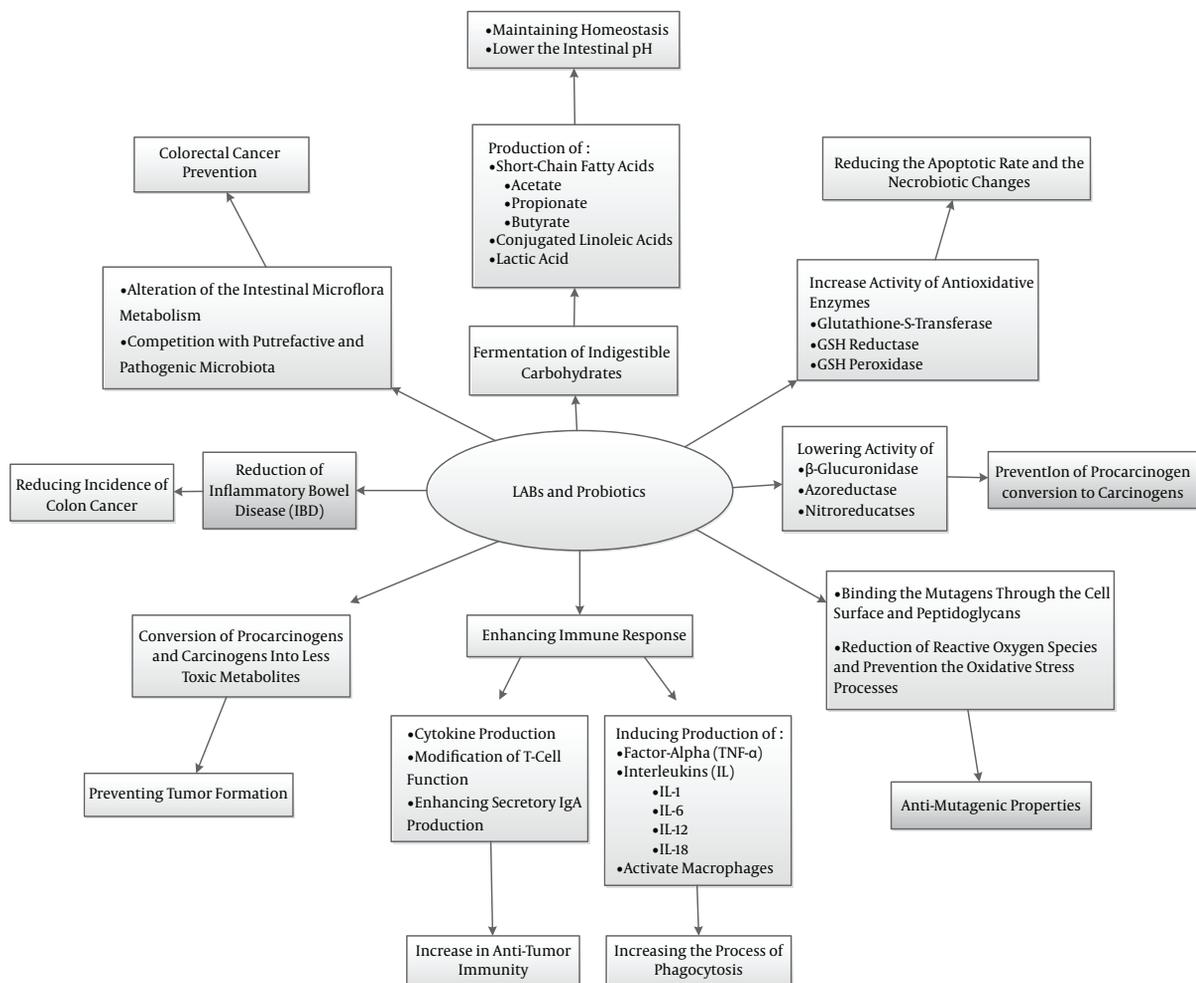


Figure 1. Main Mechanisms of Lactic Acid Bacteria (LABs) and Probiotics Action in Prevention of Cancer

The second group of HAAs are the aminoimidazoarenes (AIAs). These compounds are formed in muscle foods (meat and fish) that are cooked to medium and well-done states at high temperatures (150 - 250°C). The Maillard reaction is assumed to have a significant role in the formation of AIAs (51-53). Epidemiological studies revealed the relation between intakes of HAAs and cancers of various organs like colon, rectum, breast, pancreas, lung, prostate, stomach and esophagus (54). The international agency for research on cancer (IARC) regards some of the HAAs as possible human carcinogens (MeIQ, MeIQx and PhIP, class 2B) and one as a probable human carcinogen (IQ, class 2A) (55). In order to mitigate the risk of cancers driven by HAAs, using lower temperatures and avoiding prolonged cooking and broiling of meat, and direct exposure to a naked flame can be profitable. On the other hand, the other methods

involve inhibition and abrogation of these compounds activities in biological systems (53, 56).

Polycyclic aromatic hydrocarbons (PAH) are a group of organic compounds that contain two or more fused aromatic rings consisting carbon and hydrogen atoms. PAHs are formed as a consequence of incomplete combustion of fossil fuels, and since they are air pollutants, the soil and ground water can be contaminated and therefore, they can enter into the food chain (57). More than 100 different PAHs have been recognized, but the US environmental protection agency (EPA) has listed 16 PAHs as the main contaminants of food sources (58). The most important HAAs and PAHs are listed in Table 1. Different strategies of decontamination processes to reduce PAH levels in fish oils, including solvent extraction (ethanol) and adsorbent extraction (e.g., activated carbon, mussel shell, and wood

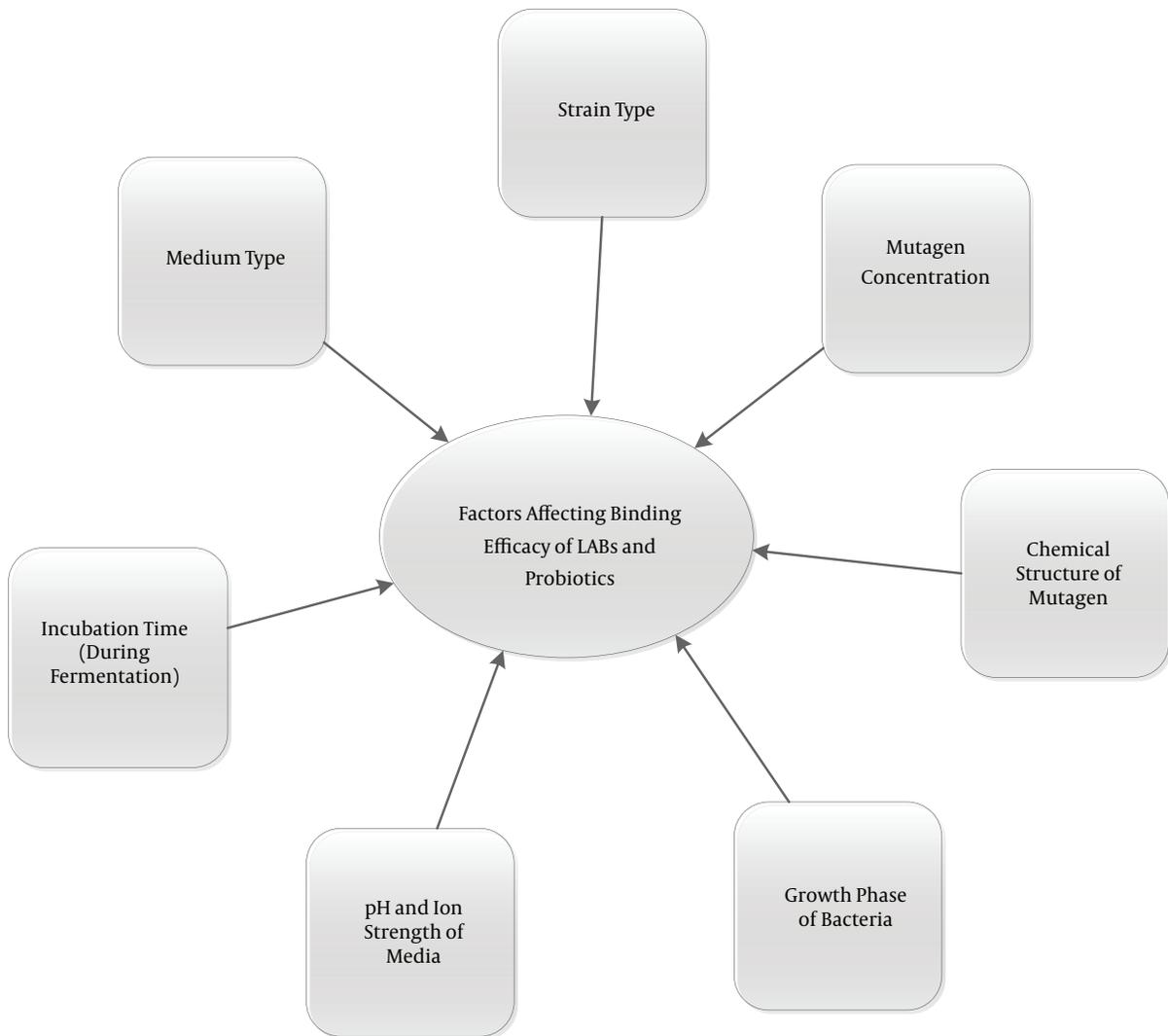


Figure 2. Main Factors Affect Binding Ability of Lactic Acid Bacteria (LAB) and Probiotics

ashes), and in smoked meat products, such as different smoking conditions, type of casing, and different types of wood chips, have been explored (59, 60). Apart from the proposed strategies and methods to diminish the level of formed HAAs and PAHs, some investigation have been performed considering the potential inhibitory activity of probiotics versus mutagenic compounds in foods induced by processing.

The binding capacity of either whole cells, cell wall skeleton (CWS), or any component of CWS of *Lactobacillus acidophilus* IFO 13951 and *Bifidobacterium bifidum* IFO 14252 to six HCAs (Trp-P1, Glu-P-1, Phe-P-1, MeIQ, IQ, and MeIQx) were examined. The binding efficacy was variable between the mentioned strains according to the mutagen com-

pound. The binding of Trp-P-1 and Trp-P-2 were the highest, but the binding of Glu-P-1, Phe-P-1, and IQ were lower by the two bacteria. Treating whole cells and CWS by lysozyme and α -amylase, decreased the binding of Trp-P-1 and Trp-P-2 by about 30%. The authors pointed out that the main component responsible for binding activity of these bacteria was peptidoglycan of CWS (45). In another study, binding ability of mutagens Trp-P1, PhIP, IQ, and MeIQx to eight human intestinal or LAB strains (*L. acidophilus* NCFB 174, *Lactobacillus fermentum* KLD and *Bifidobacterium longum* BB 5368, *Clostridium perfringens* ATCC 1314, *Bacteroides fragilis* NCTC 9343, *Escherichia coli* ATCC 25922, *Lactococcus lactis* ssp. *lactis* NCFB 604 and *Lactococcus lactis* ssp. *cremoris* NCFB 607) were reported. The results exhibited that all

Table 1. The Most Important PAHs and HAA Compounds Formed in Food

Polycyclic Aromatic Hydrocarbons	Heterocyclic Aromatic Amine
Naphthalene	2-amino-3-methylimidazo [4,5-f] quinoline (IQ)
Acenaphthylene,	2-Amino-3,4-dimethyl-3H-imidazo [4,5-f] quinoline (MeIQ)
Acenaphthene	2-Amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP)
Fluorene	2-amino-6-methyldiprido [1,2-a:3',2'-d] imidazole (Glu-P-1)
Anthracene	3-Amino-1,4-dimethyl-5H-pyrido [4,3-b] indole (Trp-P-1)
Phenanthrene	3-amino-1-methyl-5H-pyrido [4,3-b] indole (Trp-P-2)
Fluoranthene	2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline (MeIQx)
Pyrene	
Chrysene	
Benz[a]anthracene	
Benzo[b]fluoranthene	
B[a]P	
Benzo[k]fluoranthene,	
Indeno[1,2,3-cd]pyrene	
Benzo[g,h,i]perylene	
Dibenz[a,h]anthracene	

the tested strains were able to bind Trp-P1 and by using 20 μg or more of lyophilized cell, 90% - 96% binding of the mutagen was recorded by LAB strains. In contrast, *E. coli*, *B. fragilis* and *Cl. perfringens* were less effective at these high levels (61). In a work carried out by Sreekumar and Hosono, 28 strains of *Lactobacillus gasseri* and 2 strains of *B. Longum* were verified regarding their binding properties to amino acid pyrolysates (Trp-P-1, Trp-P-2, MeIQ, IQ and Glu-P-1) and antimutagenic properties with Trp-P1. Among the inspected strains, the greatest percentage of antimutagenicity and binding were rendered by four strains of *L. acidophilus* (SBT0274, SBT1703, SBT10239, and SBT10241) and 1 strain of *B. longum* (SBT 2928) which were selected for subsequent studies on the effect of cell concentration, pH, incubation time and mutagen concentration. It was illustrated that in all of these selected strains, cell concentration of 2 mg caused a binding degree of 88% - 95% to 0.2 mg Trp-P1 during 30 minutes incubation at pH 7. It was also implied that purified cell wall of the strains were more impressive compared to crude extracts, peptidoglycan, or cell extracts in mutagen binding. Treating cell walls with meta-

periodate or trichloroacetic acid that oxidize OH groups in the cis position to aldehydes and carbon acid groups, and degrade carbohydrate or remove polymer from the structure, reduced binding capability whereas enzymic treatment with trypsin or proteinase K had no effect. Thus, it could be concluded that the bacterial binding receptors lie in the bacterial cell wall polysaccharides, and the intact glucose molecules have a significant role in binding (42). In contrast to this study, it was displayed that cells and peptidoglycan of *Lactobacillus plantarum* mutant strain had no binding ability while capsular cells of the mutant (cell and EPS attached to the cell surface) showed binding ability (62).

The growing and survivability of four *Lactobacillus* strains (*L. casei* LOCK 0900, *L. casei* LOCK 0908, *L. paracasei* LOCK 0919 and *L. plantarum* LOCK 0945) were examined in the presence of three heterocyclic aromatic amines (IQ, MeIQx, or PhIP). In order to examine the growing ability of bacteria, they were incubated with HAA compounds at concentrations of 5 and 25 $\mu\text{g}/\text{mL}$ for 24 hours in MRS broth and survival of lactobacilli was monitored by incubation in phosphate buffer for maximum 120 hours. It was demonstrated that the growth of the strains was not influenced by the presence of IQ, MeIQx, or PhIP at two levels, except in the case of *L. casei* 0900 where the number of the living cell decreased slightly at the level of 25 $\mu\text{g}/\text{mL}$ PhIP. HCA compounds at concentrations of 5 $\mu\text{g}/\text{mL}$ had no impact on survival of bacteria in the phosphate buffer, but at 25 $\mu\text{g}/\text{mL}$, various results were obtained depending on the type of strain. Three of four strains were not influenced by PhIP and IQ until the period of 120 hours. The most resistant strain was *L. plantarum* 0945, while *L. casei* 0908 and *L. paracasei* 0919 were the most sensitive to MeIQx and *L. casei* 0900 to IQ. Totally, it was implicated that probiotic bacteria are able to bind HCAs in human body and are removed in feces (63). In a study carried out by Stidl et al. 12 strains from eight different LAB species (*B. longum*, *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. helveticus*, *L. kefir*, *L. plantarum* and *S. thermophilus*) either contained in fermented foods or in the human gastrointestinal tract were explored regarding their binding capacities to five HCA including A α C, PhIP, IQ, MeIQx and DiMeIQx. Among the tested eight species of *Lactobacillus*, *L. helveticus* and *S. thermophilus* were seven to eight times more effective than *L. Kefir* and *L. plantarum* strains in detoxification. The results also revealed that the detoxification of mutagens was as follows: A α C > DiMeIQx > MeIQx > IQ > PhIP (22). Faridnia et al. (64) assessed the binding ability of *Bifidobacterium pseudocatenulatum* G4, *B. longum* BB536, and *E. coli* ATCC 25922 to heterocyclic aromatic amines, including Trp-p-2, IQ, MeIQx, 7,8DiMeIQx and PhIP at pH 5.6 and 6.8.

The effect of bacterial cell concentration on binding

ability was also monitored. Results indicated that *B. pseudocatenulatum* G4 was the most effective strain in binding to HCA compounds followed by *B. longum*, and *E. coli*. It was concluded that gram-positive bacterium, due to its cell wall structure, was more efficient compared with gram-negative strains. Binding to mutagens was pH dependent and the maximum binding ability were observed at pH 6.8 in all bacteria, but the two bifidobacteria showed higher activity than *E. coli*. The interaction between HCA compounds and various concentrations of *B. pseudocatenulatum* G4 (106, 108 and 1010) was evaluated which showed that the highest reduction in HCA amount occurred at a level of 10^{10} cfu/ mL (64).

The impact of pH on binding capacity has been illustrated in previous studies (38, 45, 65, 66). In rats, absorption of HAAs in stomach occurred at pH above 4 (61). Binding of HAAs to *L. acidophilus* and *B. longum* cell walls was 80% at pH 5 (67). Contrary to these results, Tsuda et al. reported a maximum binding of Trp-P-1 to *L. plantarum* mutant strain at pH 8 (62).

Detoxifying effect of *L. casei* DN 114001 in MRS broth and modified MRS broth in the presence of 5 - 25 $\mu\text{g/mL}$ of IQ, MeIQx or PhIP were studied. It was implied that none of the HCA compounds affected the growth and survival of *L. casei* DN 114001 during 24 hours and 168 hours incubation in MRS broth and modified MRS broth, respectively. After 24 hours cultivation in MRS broth, the amount of IQ and PhIP were reduced significantly (98% - 99%) and in the case of MeIQx, the degree of reduction was 27%. In modified MRS broth, decreasing HCA concentration was lower as a result of lower cell density and was dependent on the growth phase of bacteria. IQ decreased by 49% - 54% in the stationary phase of growth (after 24 hours of cultivation), while the MeIQx amount was reduced by 11.2% in the logarithmic (till 24 hours), stationary and early death phase of growth which imply that dead cells have the additional ability to absorb carcinogens (66). The antigenotoxic capability of the probiotic *L. rhamnosus* IMC501 was investigated in mice treated with PhIP. 10 days before administration of PhIP, mice were fed with the suspension of lactobacilli and abundance of lactobacilli in feces, effects on fecal enzymatic activity and DNA damage in the colon and liver cells were determined. It was observed that after 5 days of probiotic administration, the number of lactobacilli increased in the feces and activity of β -glucuronidase and β -N-acetylglucosaminidase (high activity in patients with colorectal cancer) decreased 63% and 26%, respectively. It was also reported that the extent of DNA damage in colon cells significantly decreased, whereas no genotoxic effect was recognized in liver cells (23). On approval of this study, genotoxicity of fecal water (FW) and the activity of two enzymes (β -glucuronidase and β -glucosidase) in human feces in

three age groups (children, adults and elderly) after incubation with 50 $\mu\text{g/mL}$ IQ and three probiotic strains including (*L. casei* LOCK 0900, *L. casei* LOCK 0908, and *L. paracasei* LOCK0919) demonstrated reduction in β -glucuronidase activity in feces and the reduced amount was 64% in the case of *L. casei* 0900, 76% for *L. paracasei* 0908 in children and in the elderly group, it was alleviated by 58% for *L. casei* 0900 to 82% for *L. paracasei* 0919. The same pattern was observed in the decline of β -glucosidase activity. In the elderly group, the decrease range was from 83% (*L. casei* 0900) to 90% (*L. paracasei* 0919), and in the children group from 37% (*L. casei* 0900) to 55% (*L. casei* 0908) and in the adult group, there was no significant change in the activity of the enzymes. The greatest inhibition extent (64.5%) of fecal water genotoxicity by lactobacilli was achieved in the adult group (63). It is assumed that probiotics can adhere to colonocytes and restrict absorption of mutagens to the intestine (45) or decrease their bioavailability (68).

In another study by the aforementioned author, the impact of the same probiotic strains was evaluated on the fecal enzyme and genotoxic activity in human fecal water (children, adults and the elderly) in the presence of PhIP. It was reported that the mean β -glucuronidase activity was 64% lower among children feces after incubation with *L. casei* 0900, 75% lower for *L. casei* 0908 and 65% lower for *L. paracasei* 0919 and in elderly group, the most remarkable decline was observed as 82% for *L. casei* 0900, 83% for *L. casei* 0908 and 90% for *L. paracasei* 0919. In terms of β -glucosidase activity, probiotic strains induced a slight change in children and adults. In elderly, the enzyme activity was 87% lower for *L. casei* 0900, 79% for *L. casei* 0908 and 92% for *L. paracasei* 0919. *L. casei* 0908 and *L. paracasei* 0919 were the most effective in decreasing genotoxicity in children and adults. In the elderly group, *L. casei* 0900 was the most efficient strains (63). In another study by Klewicka et al. the protective effect of feeding beetroot juice fermented with *Lactobacillus brevis* 0944 and *L. paracasei* 0920 against aberrant crypt foci (ACF) formation in rat colon in the presence of PhIP. PhIP was used as a carcinogen at a dose of 10 $\mu\text{g/day}$. Lactofermented beetroot juice decreased the number of ACF from 59 to 26, malondialdehyde in the liver and cytotoxic and genotoxic effects of fecal water in PhIP-treated rats (69).

In a study by Tavan et al. a mixture of three HCA- IQ, MeIQ and PhIP were given to male rats for a 7 week period with a cumulative dose of 250 mg of the HCA per kg body weight. The impact of four different diets including supplemented with 20% water, 30% non-fermented milk, 30% *Bifidobacterium animalis* DN-173010 fermented milk, 30% *S. thermophilus* DN-001 fermented milk on aberrant crypt foci (ACF) induction initiated by HCA compounds were determined. It was stated that consumption of milk, espe-

cially fermented milk significantly abolished the number of ACFs in rats. The inhibition degree was 66% in milk-supplemented diet, 96% with *B. animalis* fermented milk and 93% in *S. thermophilus* fermented milk. Additionally, HCA metabolism, fecal mutagenicity and colon DNA lesions were declined and there was no difference between two strains in terms of protective effect (70). In a related work by Duangjitcharoen et al. degrading of two HCA compound PhIP and IQ ($50 \mu\text{g}/\text{mL}^{-1}$) by three strains of *L. plantarum* were determined. At the beginning of the test (0 minute), sudden binding of all strains to mutagen compounds was observed. The highest binding percentage to PhIP (46.32% after 24 hours) and IQ (85.34% after 144 hours) occurred in the strain *L. plantarum* CM4. It was deduced that this new strain could be utilized for human consumption as a protective agent against undesirable compounds in food and reactive metabolites in the gastrointestinal tract (71). Degradation of 16 different PAH compounds ($0.25 \mu\text{g}$ of each compound /ml media) by three lactic acid bacteria (*B. bifidum*, *S. thermophilus* and *L. bulgaricus*) was studied in MRS medium during different incubation periods (2, 4, 6, 8, 10, 12, 24, 48 and 72 hours) at 37°C . Furthermore, yogurt by a mixture of buffalo and cow's milk inoculated with yogurt starter (a mixture of *S. thermophilus* and *L. bulgaricus*) was prepared. $0.02 \mu\text{g}/\text{mL}$ of each PAH was added and degradation of PAH compounds was also determined during 3 hours. It was claimed that PAH decline was related to bacterial species and incubation period. During the incubation periods (2-72 hours), the reduction (%) relative to the initial concentration of PAHs ($4 \mu\text{g}/\text{mL}$) ranged from (46.6 to 92.9), (51.8 to 94.9) and (77.7 to 92.4), by *B. bifidum*, *S. thermophilus* and *L. bulgaricus*, respectively. It is noteworthy that the maximum reduction of PAHs by *B. bifidum* and *S. thermophilus* was observed after incubation for 10 and 12 hours, and was found to be 92.6 and 96.0 %, respectively. However, the highest reduction by *L. bulgaricus* was recorded after 48 hours and was found to be 92.4%. At the end of incubation time (72 hours), the PAHs reducing capability was as follows: *L. bulgaricus* (91.5%), *S. thermophilus* (87.7%) and *B. bifidum* (46.6%). The results of PAHs biodegradation in yogurt showed an inconsiderable reduction within incubation periods (1, 2 and 3 hours) and the reduction percentage was 3.46 in the final product. It was assumed that the existence of PAHs depend on a number of factors such as the type of microorganism, the interaction between microorganisms, the microbial concentration, the composition of the medium, and the microbial growth conditions of temperature and pH (36).

The binding ability of lactic acid bacteria isolated from rice and wheat miso to eight different HCA was investigated. The experiment illustrated that all of the bacterial isolates could bind to Trp-P-1, Trp-P-2, MeAaC, and PhIP ef-

ficiently. Considering the mutagen type, it was expressed that except one isolate, others bounded to Trp-P-1 and Trp-P-2 more than 85%. In the case of MeAaC, the extent of binding was more than 90% whilst Glu-P-1, IQ, and MeIQ were bound relatively low. Hence, bacterial and mutagen types were the factors affecting the binding proportion (37). It was assumed that Van der Waals (hydrophobic) interactions were important factors in the binding of mutagens. It was stated that more hydrophobic compounds like A α C and DiMeIQx are bound more efficiently than IQ and PhIP. Furthermore, the tryptophan pyrolysates are more hydrophobic than the quinolines, quinoxalines and PhIP and are removed better than other HAA compounds (22). For subsequent studies, two isolates were selected which were distinguished as *Pediococcus. acidilactici* and named as *P. acidilactici* 1 and *P. acidilactici* 2. In the next step, cell wall fractions, heat-treated cells, and cytoplasmic contents were evaluated for their binding ability to HCA compounds. It was shown that except cytoplasmic content, pure cell wall and peptidoglycan fraction in both strains possessed more binding capability in comparison to bacterial cells. Heat treatment of lyophilized cells of both strains did not modify binding capacity and therefore, binding of the mutagens by cells is not the mechanism involved. Also, enzymic treatment with various enzymes had no impact on binding except a decrease in enzyme activity.

The authors inferred that binding activity of the cell walls of bacteria and cells as a whole were not influenced by the damage; therefore, extracellular substances or structures had no function in this procedure. When HCA compounds were acetylated, none of the two strains were able to bind the mutagens which were attributed to substitution of the amino group by the acetyl group and indicating the role of the amino group in the binding property. The proposed mechanism of binding activity was the reaction of peptidoglycan with amino group of mutagen compounds (37).

In a study by Zsivkovits et al. the effect of four Lactobacillus strains consisting of *L. bulgaricus* 291, *S. thermophilus* F4, *S. thermophilus* V3 and *B. longum* BB536 on DNA damage induced by HCAs, which are generally found in fried beef (beef mix) and chicken mix, in the liver and colon of female rats were examined. Lactic acid bacteria were either administered simultaneously or at different time intervals before giving HCA. It was indicated that all strains prevented damage caused by beef mix after giving of 1×10^{10} cells/animal while in the case of chicken mix, the effect was not significant. It was also found that the impact was considerable at 1×10^7 cells/animal and even when was given 12 hours before beef mix. Hence, consumption of probiotic dairy products several hours before cooked and fried meats would be beneficial considering the reduc-

tion of DNA damage (72). Terahara et al. surveyed absorption of Trp-P-1 and MeIQx by *L. delbrueckii* ssp. *bulgaricus* 2038 and *S. thermophilus* 1131 in distilled water, buffer solutions and intestine. They pointed out that the amount of bound Trp-P-1 and MeIQx in strain 2038 was 94.1% and 60.8% respectively, as well as 83.2% and 32.2% in strain 1131. In addition to these findings, it was specified that the absorption of the mutagens was pH dependent. The highest binding of strain 1131 to Trp-P-1 happened at the range of 4-8 but strain 2038 bounded to Trp-P-1 and MeIQx at pH 7. The results of HCA absorption in the small intestine of rats by loop test showed that strain 1131 was effective in the binding reduction of Trp-P-1 than strain 2038. This was pertained to the similarity of the pH of absorption of Trp-P-1 by strain 1131 is similar and the small intestine (6, 7, 38). It was proved that incorporation of lyophilized cultures of *B. longum* BB536 (0.5%) in male and female rats diet during 56 weeks restrained colon, liver and mammary carcinogenesis induced by IQ (73). In a study potential binding ability of the goat probiotics (*L. reuteri* DDL 19, *L. alimentarius* DDL 48, *Enterococcus faecium* DDE 39 and *B. bifidum* DDBA) at cell concentrations of 1×10^6 , 1×10^8 and 1×10^{11} cfu/mL against B[a]P and sodium azide was reported. A higher antimutagenicity (74%) was recognized in the mixture of goat probiotics than any individual strains at the same cell concentration. Also, the B[a]P-probiotic complex was stable after washing with DMSO (74).

3.3. Binding Ability of LABs and Probiotics to Acrylamide

In April 2002, the Swedish Food Administration found a remarkable amount of acrylamide in various heat treated carbohydrate-rich foods such as potato chips and crisps, coffee and bread (75) and thereafter it was classified as a probable human carcinogen by the International agency for research on cancer (76). Acrylamide is an electrophile molecule and thus can react to nucleophilic groups such as amines, carboxylates, and those that are commonly found in biological molecules such as DNA. Exposure to acrylamide causes DNA damage and at high doses, neurotoxic and reproductive effects have been observed while exposure to low, but prolonged doses, results in peripheral neuropathy with the presence or absence of central nervous system complications (76, 77). Because of the undesirable impacts of acrylamide on human health, many strategies have been investigated in order to alleviate the amount of acrylamide in foods. These approaches include reduction of precursors in raw materials (78-80), changing the process parameters such as temperature, pH and addition of amino acid and salts (81-84) and post processing approaches like chromatography, evaporation, polymerization (85-87). Recently, application of specific strains of lactic acid bacteria has been explored owing to their ability

to reduce the acrylamide content in foods. Serrano-Nino et al. evaluated the potential ability of 14 lactic acid strains (*L. casei* Shirota (SHI), *L. reuteri* northern regional research laboratory (NRRL) 14171 (LR), *L. johnsonii* (ATCC) 3200 (JH), *L. acidophilus* ATCC 4796 (AC), *L. fermentum* ATCC 11976 (FER), *L. rhamnosus* ATCC 13075 (RHA), *L. helveticus* ATCC 27558 (HL), *L. casei* ATCC 334 (L334), *L. casei* L9 (L9), *L. casei* L30 (L30), *L. casei* 12A (12A), *L. casei* 21/1 (L21/1), *L. casei* 7R1 (7R1), *L. casei* (DPC) 3968) to remove acrylamide (5 and 10 $\mu\text{g/mL}$) *in vitro* after 0, 4 and 12 hours incubation at 37°C and different pH (3, 5 and 8). Stability of bacterial-acrylamide complex was also determined. It was implicated that the acrylamide binding abilities were pH, concentration, and strain dependent. Binding to acrylamide varied with respect to incubation time. At 0 hour, the amount of bound acrylamide was from 11.89 to 29.13%, depending on the strains and the maximum binding was observed in strains AC and SHI. It was proposed that binding was a rapid process and occurred passively on the bacterial surface (88). This is in accordance with the findings by Hernandez-Mendoza et al that observed aflatoxin B1 bound 15 to 58% at 0 hour (89). After 4 hours, L334 had the best binding ability (29.13%) and after 12 hours, the maximum binding was demonstrated by SHI and LR (24.95 and 24.01%, respectively). Generally, Strains JH, FER and L334 displayed the weakest ability to bind AA, whereas LR strain was the best one at any of the incubation period time. By increasing the time from 0 to 4 and 12 hours, the amount of AA bound by strains JH, SHI, L30, 12A, DCP and 7R1 enhanced notably. For other strains including HL, RHA, L9, L334 and L21/1, the amount of acrylamide bound to bacteria after 12 hours was less compared to 0 and 4 h. By enhancement of acrylamide concentration from 5 to 10 $\mu\text{g/mL}$, the binding ability of strains was decreased considerably and in this case, LR showed the highest binding ability among the examined strains. The binding ability of strains was investigated at different pH levels (3, 5 and 8). The maximum binding at pH 8 was observed in the strain L334 while at pH 3, a substantial reduction in binding ability occurred (88). Similarly, Zhang et al. (45) reported a more binding of pyrolyzed mutagen at pH 6-7 as well as Hernandez-Mendoza et al. that implied, *L. reuteri* NRRL 14171 and *L. casei* Shirota bound more effectively at pH 7 than pH 8 (89). It was announced that the probable mechanism of pH influence on binding ability was due to competition between toxic compounds and protons to attach to the negatively charged binding sites (90). The Bacterial-toxin complex was not degraded after three washes with PBS solution which demonstrated that acrylamide binds to the strains irreversibly.

Finally, it was concluded that all the strains had the ability to bind acrylamide at different incubation periods and can be a new detoxification tool for improving the

amount of acrylamide. In another attempt by Serrano-Niño et al, the interaction of acrylamide and aflatoxin B1 with teichoic acids (TA) in the cell wall of the aforementioned lactic acid bacteria was studied. TA was extracted from the cell wall and in order to analyze its components, it was subjected to acid hydrolysis. TA was composed of ribitol, glycerol, glucose, D-alanine and phosphate. The results of binding assay (at 0, 4 and 12 hours incubation at 37°C) revealed that binding of acrylamide was relevant to strain type and incubation time. The maximum binding at 0, 4 and 12 h was detected in the strain L.21/1 (> 15%), L334 (28%) and LR, SHI, 12A and DCP (about 50-65%), respectively. As it was illustrated before, the bacterial-toxin complex was stable and no detectable amount of acrylamide was liberated after three washes. The mechanism of physical binding of toxins to lactic acid bacteria was explicated. It was proposed that there was a relation between components of TA and percentage of bound acrylamide. The presence of the lower amount of glucose, D-alanine or teichoic acid caused more binding of acrylamide to the cell wall of bacteria. H-bonds may develop between carbonyl oxygen and the amino group between adjacent acrylamide and D-Alanine directly attached to position D-4 (L-2) of ribitol (88). Furthermore, the amine group of D-alanine might react with acrylamide units by means of a Michael addition reaction (91). Also, hydrogen bonds may occur between carbonyl (C=O) oxygens of both AFBI and acrylamide, and the hydroxyl groups of either glucose residues or glycerol phosphate substituent attached to the poly (ribitol phosphate) chain.

3.4. Binding Ability of LABs and Probiotics to Nitrosamine

Concerns about the occurrence of N-nitroso compounds (NOCs) in foods is growing, since these compounds can induce tumor growth in human and IARC has classified a number of nitrosamines as probably (Group 2A) or possibly (Group 2B) carcinogenic to humans (92). They are formed by the interaction of secondary or tertiary amines with proper nitrosating species and their existence in food is a consequence of various processes during production, storage, cooking and in some circumstances through migration from packaging materials (93). The ways to mitigate nitrosamine formation in food include reducing nitrite level in curing salt, application of nitrite substituent, using ascorbic acid as an inhibitor agent, utilization of lower temperatures and indirect heating (94). There are also few studies concerning the inhibitory effects of probiotics which can bind to these compounds.

Antimutagenic activity of some lactic acid bacteria from fermented milk was assessed against N-nitrosodiethylamine (NDEA), N-nitroso-dimethylamine (NDMA), N-nitroso-piperidine (NPIP) and N-nitroso-pyrrolidine

(NPYR). Among the tested bacteria, the highest inhibitory activity was observed in genus *Leuconostoc*. The strains consisting *Streptococcus lactis* ssp. *diacetylactis* R-63, *Streptococcus cremoris* R-48, and *Leuconostoc paramesenteroides* R-62 and R-8 depicted the most inhibitory activity versus the mutagenicity of NDEA. Therefore, these four strains were selected to evaluate their impact on mutagenicities of NDMA, NPYR, and NPIP. Mutagenicity of NDMA was partly inhibited by the lactic acid bacteria tested and in the case of NPYR and NPIP, these four strains were not so effective. The inhibitory effect of filtrates of cell suspension of lactic acid bacteria on NDEA mutagenicity was also investigated and a strong antimutagenic activity was observed (95). In a study by Grill et al, the influence of NDMA, NPIP and NPYR on growth of six bifidobacteria strain (*B. breve* ATCC 15698, *B. infantis* ATCC 25962, *B. longum* ATCC 15707, *B. longum* ATCC 15708, *B. longum* BB536 and *B. animalis* ATCC 25527) during 24 hours in TYP medium was studied. It was noted that in the concentration range of 2 - 200 µg/mL, the nitrosamines had no effect on the growth of bifidobacteria and only *B. longum* BB536 was able to metabolize nitrosamines. At the level of 2 µg/mL, 20% degradation for NPYR, 16% for NDMA and 10% for NPIP were detected. At 20 µg/mL, 0.5% - 1% decrease and in the case of 200 µg/mL no antimutagenic activity were observed. The inhibitory effect of bifidobacteria was attributed to an intracellular enzymic activity (96). Nowak et al studied binding and degrading ability of five probiotic *Lactobacillus* strains (*L. rhamnosus* LOCK 0900, *L. rhamnosus* LOCK 0908, *L. casei* LOCK0919, *L. casei* DN114001 and *L. brevis* 0945) versus N-nitrosodimethylamine (NDMA) under different culture conditions (24 hours in MRS, 168 hours in modified MRS N, and 168 hours in phosphate buffer). They also investigated the growth and survival of the strains during 24 hours in the presence of NDMA. It was stated that the highest growth was obtained for strain *L. casei* DN 114001 (1×10^{10} CFU/ mL) and the lowest for *Lb. rhamnosus* 0908 (1×10^9 CFU/mL). The morphology of the bacteria was not affected by NDMA even at high concentration of 100 µg/mL and NDMA at different levels was not toxic for lactobacilli. NDMA (2-100 µg/mL) did not influence the survival of the probiotic strains during 168-h incubation in phosphate buffer. In the case of decreasing the amount of NDMA, all strains had the capability of decreasing NDMA concentration in MRS from 2 µg/mL to 0.40 - 0.92 µg/mL after 24 hours cultivation while at the concentration of 20 µg/mL, the NDMA was reduced to 6 µg/mL by only two strains including *L. rhamnosus* 0908 and *L. casei* DN 114001 and at the initial level of 100 µg/mL no change was observed. The ability of bacteria to reduce NDMA amount in MRS N after 168 hours was weak and related to the growth phase and strain of bacteria. During

logarithmic phase, decline of NDMA level was about 0.3 - 0.8 $\mu\text{g}/\text{mL}$ that amplified in stationary phase to the initial level of 10 $\mu\text{g}/\text{mL}$ and again in death phase, 0.6 - 0.9 $\mu\text{g}/\text{mL}$ reduction was found in the case of *L. rhamnosus* 0900, *L. casei* DN 114001 and *L. brevis* 0945 whereas for *L. rhamnosus* 0908 and *L. casei* 0919, NDMA level remained constant. In phosphate buffer, *L. rhamnosus* 0900 lowered the NDMA level from 2 $\mu\text{g}/\text{mL}$ to 1.45 $\mu\text{g}/\text{mL}$. At the concentration of 20 $\mu\text{g}/\text{mL}$, three strains were able to decrease NDMA, but at the level of 100 $\mu\text{g}/\text{mL}$ none of the tested lactobacilli were capable of reducing the NDMA. The lower decrease in NDMA level in MRS N than MRS was attributed to lower numbers of bacteria and higher pH level. It was also announced that the most efficient strain in lowering the concentration and genotoxicity of NDMA was *Lb. brevis* 0945.

As a final note, the decrease of NDMA was dependent on the medium, incubation time, phase of growth, strain type, pH, and NDMA concentration (63). Degrading of two nitroso compounds, including diphenylnitrosamine (DPN) and 1-nitrosopyrrolidine (NPR) by three strains of *L. plantarum* was determined. Among the tested bacteria, *L. plantarum* CM4 which is a new probiotic of non-human origin strain demonstrated the utmost degrading ability of DPN (1 - 100 $\mu\text{g}/\text{mL}$) in a dose-response manner and the highest degradation activity was seen at the concentration of 100 $\mu\text{g}/\text{mL}$ that yielded 11.10 $\mu\text{g}/\text{mol}$ nitrite per mL during 20 hours of incubation time. The breakdown of NPR by all strains was slower than DPN and was not dose-response relationship activity (71).

4. Conclusions

This article reviewed the potential application of different lactic acid bacteria and probiotics in detoxification of various toxicants that are formed during food processing. Reports demonstrated that lactic acid bacteria and particularly probiotics can decline mutagenicity and genotoxicity of these toxicants remarkably by physical binding or enzymic degrading mechanisms. The efficacy of probiotic protective activity depends on several factors such as strain type, medium type, incubation time, pH, growth phase, chemical structure of mutagen, mutagen concentration, and probably existence of different binding sites on the cell wall of bacteria. Thereby, considering the findings in various studies, it can be concluded that probiotics can play a vital role in prevention of colon cancer that is induced by food toxicants. However, most of these studies have been carried out in vitro and further in vivo and clinical trials are still required to support the obtained results and specify the real effects of probiotic in human lumen and elucidate the underlying mechanisms.

Acknowledgments

This research has been supported by student research committee of Shahid Beheshti University of Medical Sciences.

Footnotes

Conflict of Interest: The authors declare that there are no conflicts of interest in this article.

Author Contribution: Hedayat Hosseini, Mahdi Shadnough and Amir Mohammad Mortazavian designed the study. Nasim Khorshidian and Mojtaba Yousefi Asli reviewed the literature and wrote the paper. Amir Mohammad Mortazavian contributed to writing-up and revision of the manuscript. All authors read and approved the final manuscript.

Funding/Support: None declared.

Financial Disclosure: None declared.

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