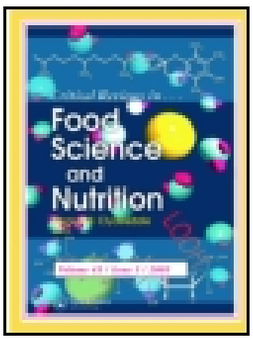

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Detoxification of cancerogenic compounds by lactic acid bacteria strains

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ABSTRACT

Carcinogens in food are an important issue that threat people's health right now. Lactic acid bacteria (LAB) strains as well-known probiotics have shown numerous perspectives in being used as a good food additive to confront cancerogenic compounds in recent years. Some LAB strains can remove cancerogenic compounds from medium environment via direct physical binding and avoid re-pollution of poisonous secondary metabolites which are generated from degradation of cancerogenic compounds. This article presents a whole overview of the physical-binding of LAB strains to such common cancerogenic compounds existed in food and feed environments as mycotoxins, polycyclic aromatic hydrocarbons (PAHs), heterocyclic amines (HAs) and pthalic acid esters (PAEs).In most cases, summaries of these published researches show that the binding of LAB strains to cancerogenic compounds is a physical process. Binding sites generally take place in cell wall, and peptidoglycan from LAB cells is the chief binding site. The adsorption of lactic acid bacteria to cancerogenic compounds is strain-specific. Specially, the strains from the two genera *Lactobacillus* and *Bifidobacterium* show a better potential in binding cancerogenic compounds. Moreover, we firstly used molecular dynamic computer model as a highly potential tool to simulate the binding behavior of peptidoglycan from *Lactobacillus acidophilus* to DBP, one of pthalic acid esters with genetic toxicity. It was seen that the theoretical data were quite consistent with the experimental results in terms of the ability of this bacterium to bind DBP. Also, the toxicity reduction of cancerogenic compounds by LAB strains could be achieved either in gastrointestinal model or animal tests and clinical researches as well. In conclusion, carefully selected LAB strains should be a good solution as one of safety strategies to reduce potential risk of cancerogenic compounds from food-based products.

KEYWORDS

Lactic acid bacteria; physical binding; cancerogenic compounds; mycotoxins; polycyclic aromatic hydrocarbons; pthalic acid esters

List of abbreviations: AMPIP: 2-amino-1-methyl-6-phenyl-imidazo 4, 5-b. pyridine; AMIQ: 2-amino-3-methyl-3H-imidazoquinoline; AMPI: 2-amino-3-methyl-9H-pyrido 3, 3-6. Indole; AαC: 2-amino-9H-pyrido [2, 3-b] indole; AFB1: Aflatoxin B1; AA3: Amino acid in position 3; BCW: Bacterial cell wall; Bap: Benzo[a]pyrene; DEO: 1, 2, 7, 8-diepoxyoctane; DiMeIQx: 2-amino-3-methyl-3Himidazo [4, 5-f] quinolone; DMAB: 3,2′-dimethyl-4-aminobiphenyl; DMBA: 9, 10-dimethyl-1, 2-benz[a]anthracene; DEHP: Bis (2-ethylhexyl) phthalate; DEP: Diethyl phthalate; DMSO: Dimethyl sulfoxide; DBP: Di-n-butyl phthalate; DON: Deoxynivalenol; DG: Lowest binding energies; DON: Vomitoxin; EMS: Ethyl methanesulfonate .; EPS: Exopolysaccharides; FTIR: Fourier transformed infrared spectroscopy; FB1: Fumonisin B1; FB2: Fumonisin B2; GI: Percent genotoxicity inhibition; Glu-P-1: 2-amino-6-methyldipyrido-[1, 2-a: 3, 2-d] imidazole; HAs: Heterocyclic amines; HepG2: Hepatoma cell line; HPLC: High performance liquid chromatography; HCAs: 2-amino-3, 4, 8 -trimethyl-3H-imidazo [4, 5-f] quinoxaline; IQ: 2-amino-3-methyl-3H-imidazo [4, 5-f] quinoline; IARC: International Agency for Research on Cancer; LAB: Lactic acid bacteria; MeIQ: 2-amino-3, 4 -dimethyl-3H-imidazo [4, 5-f] quinolone; MALDI-TOF/TOF MS: Matrix-assisted laser desorption ionization mass spectrometry-time of flight/ time of flight; MCN: Micronucleus assays; MMS: Methyl methanesulfonate; MD: Molecular dynamic; MNNG: N-methyl-N′-nitro-N-nitrosoguanidine; NF: 2-nitroflourene; NPD: 4-nitro-*O*-phenylen-ediamine; NAG: N-acetylglucosamine; NAM: N-acetylmuramicdisaccharide; NMR: Nuclear magnetic resonance; PhIP: 2-amino-3, 8 –dimethylimidazo [4, 5-f] quinoxaline; PG: Peptidoglycan; PBS: Phosphate-buffered saline .; PAHs: Polycyclic aromatic hydrocarbons; PP: Protoplasts; PAEs: Pthalic acid esters; SCGE: 2-amino-1-methyl-6-phenylimidazo [4, 5-b]-pyridine; SP: Spheroplasts; Trp-P-1: 3-amino-1, 4-dimethyl-5H-pyrido [4, 3-b] indole; Trp-P-2: 3-amino-1-methyl-5H-pyrido [4, 3-b] indole; TADB: Teichoic acid-deficient bacteria; TBARS: Thiobarbituric acid-reactive substances; ZEN: Zearalenone; 4-NQO: 4-nitroquinoline-1-oxide; α'-ZOL: α'-Zearalenol

1. Introduction

In recent years, more and more cases referring to the poisoning of cancerogenic compounds in food and feed have been erupted. The involved compounds are a wide range of chemicals including mycotoxins, polycyclic aromatic hydrocarbons (PAHs), heterocyclic amines (HAs), and pthalic acid esters (PAEs) (Hathout and Aly, 2014; Knasmüller et al., 2001;

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Mrozik et al., 2003; Özogul and Hamen, 2017; Silva et al., 2017; Zhang et al., 2005).

Mycotoxins in food and feed mainly cover aflatoxins, fumonisins, ochratoxins, trichothecenes, zearalenones, and ergotalkaloids (Ahlberg et al., 2015). The types of mycotoxins affect their toxic properties. Aflatoxins result in cancer by the cross-link of DNA and guanine (Bren et al., 2007). Fumonisins inhibit the activity of ceramide synthase, catalyze sphingol to D-sphinganine by breaking lipid metabolism of nerve cells, and finally result in cellular damage and variation (Soriano et al., 2005). Ochratoxin A stops the activity of succinode hydrogenase and oxidase of cytochrome C in mitochondrial of liver cell of rats (Wei et al., 1985). Vomitoxin (DON) and T-2 toxin lead to the apoptosis of hematopoietic stem cell and immune cell (Parent-Massin, 2004).

PAHs consist of phenanthrene, naphthalene, fluoranthene, pyrene, and benzo[a]pyrene (Bap). They are often generated from the incomplete combustion of organic raw materials, like gas production, automobile exhaust, timber dispose, and waste incineration (Mrozik et al., 2003, Su Seo et al., 2009). Moreover, improper cooking methods also induce PAHs generation in some food (Kazerouni et al., 2001). PAHs are relative stable in environments, and hard to be decomposed. They can be ingested by human through inhalation, intraoral approach and dermal route, and then change into cancerogenic compounds by the role of cytochrome enzyme P450 (Uno et al., 2001).

High temperature from cooking, like boil, fry, and barbecue, easily leads amino acids in meat and fish to be converted to the chemicals heterocyclic amines (HAs). Generally, HAs cover these chemicals like 3-amino-1, 4-dimethyl-5H-pyrido [4, 3-b] indole (Trp-P-1), 2-amino-6-methyldipyrido- [1, 2-a:3, 2-d] imidazole (Glu-P-1), 2-amino-3-methyl-3H-imidazo [4, 5-f] quinoline (IQ), 2-amino-3,4-dimethyl-3H-imidazo[4,5-f]quinolone (MeIQ), and 3-amino-1-methyl-5H-pyrido [4,3-b] indole (Trp-P-2). HAs are addressed to be the main cause of human colon cancer (Felton et al., 1997).

Pthalic acid esters (PAEs), i.e., diesters of 1, 2-benzenedicarboxylic acid (phthalic acid) whose nickname is plasticizers, are widely used in food, drink, and wine contact materials, medical devices, cosmetics, and childcare articles. Their main types consist of Diethyl phthalate (DEP), Di-n-butyl phthalate (DBP), and Bis (2-ethylhexyl) phthalate (DEHP) (Hernandez-Diaz et al., 2009; Silva et al., 2017). Phthalates are confirmed to be involved in endocrine disruption, particularly alterations of reproductive physiology in different classes of vertebrates, fishes and mammals (Martine et al., 2012). Furthermore, exposure to DBP and DEP causes the inhibition of acetylcholinesterase activity, and changes the transcriptional levels of selected neuron-related genes (Xu et al., 2013).

Numerous physical, chemical, and biological methods have been designed to eliminate cancerogenic compounds. However, most of these methods are expensive, complicate, and low efficiency, and even cause poisonous secondary metabolite to be generated. These methods are often hard to be popularized and large-scale practiced (Bhat et al., 2010; Natale et al., 2009; Mishra and Das, 2003). Thus, ever-increasing requirements of good life quality and health care from consumers strongly desire high efficient and safety approaches for the removal of these mentioned cancerogenic compounds.

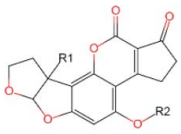
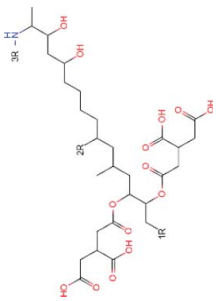
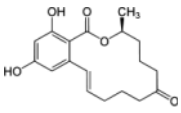
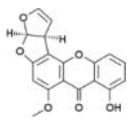
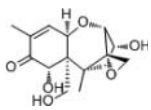
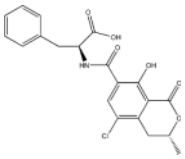
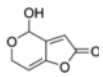
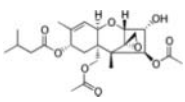
Lactic acid bacteria (LAB), a generic term for sets of microorganisms which can ferment lactose to lactate, have been attributed to have health-promoting effects on the host over the past decades, especially reducing the risk of carcinogenic and mutagenic compounds, and preventing colon cancer (Clements and Carding, 2016; Sanders et al., 2014; Rafter, 2002; Gibson et al., 2010). The benefits of LAB can be realized by the consumption of probiotics-related food products (Bordoni et al., 2017). Thus, LAB strains are widely used in food and feed processing, aquaculture industry, medical and health treatment.

Over the past 30 years, hundreds of literatures have involved in the researches on the detoxification of cancerogenic compounds by LAB strains. Information linked to the ability of LAB strains to remove cancerogenic compounds in food and feed was presented in Table 1. The data of Table 1 covering the types, cancer hazard ranking and limitation level of these chemicals result from International Agency for Research on Cancer (IARC), Food and Drug Administration (FDA), and China national standard (GB). Regarding that mycotoxin, PAHs, HAs, and PAEs commonly have multiple-ring molecular structure, a similar removal approach to these carcinogens by LAB strains might be take place. Therefore, this Article was designed to present a whole overview of the de-toxication and anti-mutagenicity by LAB strains via their binding to carcinogens. It documented the removal capacity of various LAB strains to carcinogens, and dealt with these factors affecting the ability of LAB strains to bind cancerogenic chemicals. Especially, it firstly used the binding of *Lactobacillus* strains to DBP as a case to highlight the possible mechanism of LAB in removing cancerogenic compounds via molecular dynamic (MD) simulation.

2. Detoxification mechanism

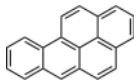
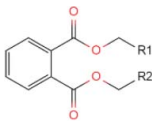
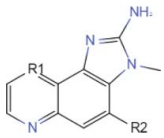
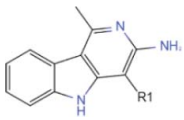
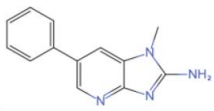
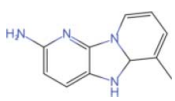
Update, 2 proposals have been given to explain how LAB strains detoxicate carcinogens. One is on a physical absorption between LAB strains and cancerogenic chemicals. Another is that LAB strains can mitigate the risk of carcinogens via their metabolism. However, most scholars favor the first finding, because they found that the heat dead pellets of LAB strains had the same capacity to remove AFB1 as their viable pellets did (El-Nezami et al., 1998a). Topcu et al. confirmed that the binding percentages of *Enterococcus faecium* strain M74 to AFB1 and patulin were 30.5% and 39.9%, respectively; whereas those of the heat treatment pellets from the same strain subjected to 121°C for 20 min were 27.1% for AFB1 and 35.3% for patulin (Topcu et al., 2010). No significant difference between the live cells and dead cells of this bacterium to detoxicate the two mycotoxins was observed. *L. rhamnosus* GG was reported to have capacity to bind AFB1 after heat or acid treatment. The binding percentage of this bacterium to AFB1 was 83% for heat-treated cells and 84% for acid-treated cells, compared to that of its viable strains (76%) (Haskard et al., 2000). El-Nezami et al. tested the binding ability of *L. rhamnosus* GG and *L. rhamnosus* LC705 to zearalenone (ZEN) and ά-zearalenol (ά-ZOL). The binding percentage of

Table 1. The variety, cancer hazard ranking (CHR) and limitation in food and feed of cancerogenic compounds referring to LAB strains.

Chemicals	General structure	molecular formula	CAS number	CHR	Limitation μg/kg	Reference
Mycotoxins	 Aflatoxins (AFs)	$C_{17}H_{12}O_6$ (AFB_1)	1162-65-8	1	0.5–20 μg/kg	GB2761-2011
		$C_{17}H_{14}O_6$ (AFB_2)	7220-81-7	1	AFB_1:0.1–12 μg/kg; Sum of AFB1, AFB2, AFG1 and AFG2: 4–15 μg/kg	(EC) No 1881/2006
		$C_{17}H_{12}O_7$ (AFG_1)	1165-39-5	1		
		$C_{17}H_{14}O_7$ (AFG_2)	7241-98-7	1		
		$C_{17}H_{12}O_7$ (AFM_1)	6795-23-9	2B	0.5 μg/kg	GB2761-2011
		$C_{17}H_{14}O_7$ (AFM_2)	6885-57-0	—	—	—
	 Fumonisins	$C_{36}H_{61}NO_{16}$ (FA_1)	117415-48-2	—	—	—
		$C_{36}H_{61}NO_{15}$ (FA_2)	117415-47-1	—	—	—
		$C_{34}H_{59}NO_{15}$ (FB_1)	116355-83-0	2B	(FB1 + FB2 + FB3) ppm (mg/kg) 2–4 (FDA-1998-N-0050); Maximum levels (μg/kg) Sum of FB1 and FB2: 200–4000 (EC) No 1881/2006.	
		$C_{34}H_{59}NO_{14}$ (FB_2)	116355-84-1	—		
	 Zearalenone	C18H22O5 (ZEN)	17924-92-4	—	60 μg/kg	GB2761-2011
	 Sterigmatocystin	$C_{18}H_{12}O_6$ (ST)	10048-13-2	2B	—	—
	 Vomitoxin	$C_{15}H_{20}O_6$ (VT/DON)	51481-10-8	—	1000 μg/kg	GB2761-2011
	 Ochratoxin A	$C_{20}H_{18}ClNO_6$ (OTA)	303-47-9	2B	5 μg/kg	GB2761-2011
	 (Patulin)	$C_7H_6O_4$ (Patulin)	149-29-1	3	50 μg/kg	GB2761-2011
	 T-2 toxin	$C_{24}H_{34}O_9$ (T-2)	21259-20-1	3	—	—

(*Continued on next page*)

Table 1. (*Continued*)

Chemicals	General structure	molecular formula	CAS number	CHR	Limitation μg/kg	Reference
Polycyclic aromatic hydrocarbons (PAHs)		$C_{20}H_{12}$ (BaP)	50-32-8	1	1.0–6.0 (μg/kg)	(EC) No 1881/2006
Pthalic acid esters (PAEs)		$C_{24}H_{38}O_4$ (DEHP)	117-81-7	2B	0.006 mg/L 1.5 mg/kg	FDA-2012-D-0316 Chinese Ministry of Health-No-2011-551
		$C_{16}H_{22}O_4$ (DBP)	84-74-2	—	0.3 mg/kg	Chinese Ministry of Health-No-2011-551
		$C_{12}H_{14}O_4$ (DEP)	84-66-2	—	—	—
Heterocyclic amines (HAs)		$C_{11}H_{10}N_4$ (IQ)	76180-96-6	2A	—	—
		$C_{12}H_{12}N_4$ (MeIQ)	77094-11-2	2B	—	—
		$C_{11}H_{11}N_5$ (MeIQx)	77500-04-0	2B	—	—
		$C_{13}H_{13}N_3$ (Trp-P-1)	62450-06-0	2B	—	—
		$C_{12}H_{11}N_3$ (Trp-P-2)	62450-07-1	2B	—	—
		$C_{13}H_{12}N_4$ (PhIP)	105650-23-5	2B	—	—
		$C_{11}H_{10}N_4$ (Glu-P-1)	67730-11-4	2B	—	—

Note: The Cancer Hazard Ranking according to agents classified by the International Agency for research on cancer (IARC) monographs, volumes 1–116: Group 1: Carcinogenic to humans; Group 2A: Probably carcinogenic to humans; Group 2B: Possibly carcinogenic to humans; Group 3: Not classifiable as to its carcinogenicity to humans; Group 4: Probably not carcinogenic to humans. FDA: Food and Drug Administration; GB: China national standard; EC: European Commission. R1 and R2 represent different chemical groups for one class of compounds in the general structure.

L. rhamnosus GG after heat treated was 70% for ZEN and 68% for ά-ZOL, or 69% for ZEN and 68% for ά-ZOL after acid treated, compared to the control groups with a 52% for ZEN and 50% for ά-ZOL (El-Nezami et al., 2002). Wang et al. (2015b) reported the binding of *L. plantarum* B7 and *L. pentosus* X8 to fumonisin B1 (FB1) and fumonisin B2 (FB2). The adsorption of the two lactobacilli strains to fumonisins did not depend on the strain's viability either in the heat or acid treatment. It is well-known that heat and acid treatments denature protein, and break down polysaccharide glycosidic bond and protein ammonium sulfate bond to monosaccharide and peptide fragment, leading peptidoglycan to loose and releasing more binding sites. Thus, the removal of cancerogenic compounds by LAB strains is proved to be a physical absorption instead of metabolism.

As the decomposition of cancerogenic compounds by LAB strains was concerned, early in 1984 Goldin and Gorbach demonstrated that oral administration of fermented milk with *L. acidophilus* (2×10^6 CFU/mL) reduced 2 to 4-folds quantity of β-glucuronidase, nitroreductase and azoreductase in human feces. These three enzymes catalyzed cancerogenic precursor to cancerogenic compounds. These enzymes' quantity would return to original level in feces after the consumption of the fermented milk was stopped (Goldin and Gorbach, 1984). Lankaputhra et al. reported the removal of 6 *L. acidophilus* strains and 9 bifidobacteria strains to *N*-methyl, *N*X-nitro, *N*-nitrosoguanidine (MNNG), 2-nitroflourene NF, 4-nitro-*O*-phenylen-ediamine (NPD), 4-nitroquinoline-*N*-oxide (NQO), aflatoxin-B (AFB), 2-amino-3-methyl-3H-im-idazoquinoline (AMIQ), 2-amino-

1-methyl-6-phenyl-imidazo 4, 5-b. pyridine (AMPIP), and 2-amino-3-methyl-9H-pyrido 3, 3–6. indole (AMPI). It was noted that the anti-mutagenicity of the viable strains was better than the dead strains did. Meanwhile, such metabolites from LAB strains as acetic acid, butyrate, lactic acid and pyruvic acid could inhibit mutation to a varying extent (Lankaputhra and Shah, 1998). These LAB strains contained in fermented milk was thought to play a certain role in anti-mutagenicity due to their metabolism. Nybom et al. evaluated the capacity of 5 LAB strains from *L. rhamnosus* strains GG and LC-705, *B. longum* 46, *B. lactis* strains 420 and Bb12 to remove microcystin-LR. The ability of 5 LAB strains to detoxicate microcystin-LR were reduced significantly after their live cells were treated with 1 mol/L HCl at 90°C for 30 min. LAB strains were thought to directly metabolize microcystin-LR or generate several enzymes to degrade microcystin-LR, but not via their physical absorption (Nybom et al., 2007). Conclusively, all up-dated studies have proved the ability of selected LAB strains to remove carcinogens and mutagens, but their detoxification mechanism still needs to be investigated.

3. Binding sites

3.1. Peptidoglycan as main binding site

Various studies have noted that the components of cell wall from LAB bind carcinogenic compounds differently, and peptidoglycan from cell wall is considered as the main binding site. Haskard et al. treated viable *L. rhamnosus* GG with periodate, pronase E, and lipase, and measured its binding percentage to AFB1. The treatment of periodate easily oxidized *cis* OH groups to aldehydes and carbon acid groups, leading to the largest decrease of this bacterium to AFB1. Use of lipase to treat *L. rhamnosus* GG cells did not significantly decrease the ability of this bacterium to bind AFB1. It means that the carbohydrate components from cell wall involve in the binding of *L. rhamnosus* GG to AFB1, but not involvement of lipoteichoic acid in binding to AFB1 (Haskard et al., 2000). Lahtinen et al. further stated that peptidoglycan might be the main component from *L. rhamnosus* GG cell wall for this strain to bind AFB1, rather than exopolysaccharides (EPS) and S-protein (Lahtinen et al., 2004). In a similar case, Zhao et al. chose 2 lactobacilli strains for Bap-binding. *L. plantarum* CICC 22135 had a percentage of 66.76% Bap-binding, and *L. pentosus* CICC 23163 had 64.31%. Different components extracted from the two bacterial cell walls showed difference in binding Bap. Among all components of cell wall, peptidoglycan was observed to be main site to adsorb Bap. For strain CICC 22135, 76.06% Bap was bound by its peptidoglycan. The peptidoglycan from strain CICC 23163 bound 78.71% Bap. Moreover, the integrity of peptidoglycan was very important for Bap-binding. A great decrease in binding Bap, about 44.13% reduction for strain CICC 22135 and 39.75% for strain CICC 23163 was observed after intact peptidoglycans were disintegrated by ultrasonic vibrations (Zhao et al., 2013). In Niderkorn et al.'s study, *L. paraplantarum* CNRZ 1885, *Streptococcus thermophilus* RAR1, *S. thermophilus* CNRZ 1066 and the non-capsular, non-exopolysaccharide (EPS)-producing mutant *S. thermophilus* JIM 8752, and *Lactococcus lactis* subsp. *cremoris* MG1363 and its mutants in which the synthesis of certain cell wall components and adhesion properties are stopped, were used to bind FB1 and FB2.The results obtained with mutant strains were in agreement with those of bacteria treated with physicochemical and enzymatic techniques, and proved that peptidoglycan was the main binding site instead of other components from cell wall. Use of commercially peptidoglycans purified from the Gram-positive bacteria *Micrococcus luteus* and *Bacillus subtilis* at different concentration levels to bind FB showed that these polymers bound the mycotoxin in an analogue dependent manner. Data from these studies confirmed that peptidoglycan played a key role in adsorbing carcinogenic compounds (Niderkorn et al., 2009).

The peptidoglycan scaffold of LAB bacterial cell wall is a repeating unit which consists of *N*-acetylglucosamine and (NAG)-*N*-acetylmuramic disaccharide (NAM) [NAG-(β-1,4)-NAM] having a peptide attached to the D-lactyl moiety of each NAM (Meroueh et al., 2006). Data from HPLC, NMR, FT-IR discovery, MALDI-TOF/TOF MS and amino acid analysis have already been able to describe the accurate structure of peptidoglycan from LAB cells. For instance, the molecular scaffold of peptidoglycan from *L. acidophilus* consists of NAG-(β-1,4)-NAM-L-Ala-D-Glu-L-Lys-D-Ala (Wu et al., 2013), although the peptide chain L-Lys-D-Asp may be linked to its peptidoglycan (Schleifer and Kandler, 1972). The disaccharide peptide monomer, bisdisaccharide peptide dimer and trisdisaccharide peptide trimer were isolated from *L. acidophilus* cell walls, but the bisdisaccharide peptide dimer represented the maximum fraction of this bacterium peptidoglycan (Coyette and Ghuysen, 1970). Thus, it is feasible to visualize a peptidoglycan scaffold of LAB cell wall with bisdisaccharide peptide dimer.

Molecular dynamic stimulation, as an emerging and versatile tool, is accurately used to calculate diffusion-related data of small molecule in membrane at the molecular level (Srinophakun and Martkumchan, 2012; Sudibjo and Spearot, 2011; Yang and Achenie, 2012; Zhang et al., 2009). In our laboratory, MD stimulation was explored to examine the binding behavior of peptidoglycan from *L. acidophilus* cell wall to DBP, a carcinogen with genetic toxicity. The molecular dynamic of the peptidoglycan binding to DBP was performed using the software Materials studio 7.0 (Ling et al., 2012). The modules "Visualizer", "Amorphous Cell" and "Forcite" from Materials Studio7.0 were used to work out MD simulation in this study (Accelrys Software Inc., San Diego, CA), and the force field were COMPASS II and DREIDING. Firstly, the module "Visualizer" was used to establish the three-dimensional structures of peptidoglycan plus DBP. Next, a packing model composed of DBP and peptidoglycan repeat unit were constructed in the module "Amorphous Cells" with an initial density of 1 g/cm^3. Third, the packing model was subsequently optimized in order that an annealing procedure was fixed up. The annealing procedure were heated from 300 K to 500 K at an interval of 30 K, and subsequently cooled back. For each temperature interval, 5 ps NPT dynamics were designed to maintain the equilibrium status of the simulation system. Afterwards, a further 20 ps NPT and 20 ps NVT molecular dynamic simulation was

Table 2. Interaction energies between DBP and peptidoglycan.

Energy type (kcal/mol)		E_{PG}	E_{DBP}	E_{total}	ΔE
Valence energy 0	Bond	252.360	3881.087	4133.446	0
	Angle	241.084	2378.125	2619.209	0
	Torsion	100.887	1657.836	1758.723	0
	Inversion	45.283	175.782	221.065	0
Non-bond energy 176.53	Hydrogen bond	−15.412	0.000	−30.717	15.31
	Van der Waals	366.668	2874.292	3153.844	87.12
	Long range correction	−0.298	−76.010	−85.820	9.51
	Electrostatic	16.651	1955.540	1907.592	64.60

performed at 298 K, and the atomic trajectory was recorded for the subsequent analysis. Consequently, the interaction energy designated by ΔE (kcal/mol) between DBP and peptidoglycan could be calculated according to thermodynamic equation 1.

$$\Delta E = (E_{PG} + E_{DBP}) - E_{total} \quad (1)$$

where E_{total} stands for the energy of system containing DBP molecules and PG molecule; E_{PG} indicates the energy of the isolated PG molecule; and E_{DBP} represents the energy of DBP molecules, respectively. Interaction energies between DBP and peptidoglycan were calculated by DREIDING force field, and the results were shown in Table 2 and Fig. 1.

Fig. 1 describes the active sites of peptidoglycan which binds DBP. DBP is embedded in the nets structure of the peptidoglycan. As calculated by Materials Studio 7.0, the non-bonding interaction energies between DBP and peptidoglycan were 176.53 kcal/mol, including hydrogen bond, Vander Waals and electrostatic energies (Table 2). Additionally, seven hydrogen bonds were formed between the hydrogen atoms of peptidoglycan and the oxygen atoms of DBP. Obvious changes in these groups N-H, C-O, C-N, and O-H would take place in the stimulation of peptidoglycan to bind DBP. These results were further proved by FTIR data from the binding of *L. acidophilus* to DBP (Fig. 2). Fig. 2 describes the spectra of the binding of *L. acidophilus* NCFM to DBP. The absorption peaks which represent these groups N-H, C-O, C-N, and O-H mainly appeared in the ranges of 3300 to 2850 cm^{-1} and 1750 to 900 cm^{-1} (Fig. 2A). Compared to the control, these representative peaks in FTIR spectra really showed some changes when the bacterial cells were used to bind 5.0 mg/L DBP, and they changed strongly if the concentration of DBP were raised from 5.0 mg/L to 100.0 mg/L (Fig. 2B). This further indicates that only these groups N-H, C-O, C-N, and O-H seem to be involved in the adsorption of peptidoglycan to DBP. In addition, it is because the formation of intermolecular hydrogen bonding between DBP and the O-H and N-H group of cell wall components leaded to the increase in the bound length of O-H and N-H, causing a significant peak shifting from 3287 to 3182 cm^{-1} (red shift) (Mielcarek and Dołęga, 2016). Of course, the shifting of peaks from 1235 to 1243 cm^{-1} or 1077 to 1101 cm^{-1} revealed the strong intermolecular interaction between DBP and the C-N or C-O groups of the bacterial cell wall, respectively (Korkmaz et al., 2013). In a word, data from MD stimulation and FTIR spectra confirm that O-H, N-H, C-N and C-O as the major groups of peptidoglycan from LAB cells play a key role in adsorbing DBP. And, no chemical reaction energies involve in the binding process. Our findings also were supported by the evidences from Tripathi et al. (2012) and Wang et al. (2015a).

3.2. Bacterial cell wall as binding site

Some scholars hold this point that not only peptidoglycan is involved in the adsorption to carcinogenic compounds, but the multiply components from bacterial cell wall may work together for binding to these chemicals as well. Zhang et al. found that polysaccharides and peptidoglycan from lactic acid bacteria as the major components showed a good ability to bind 3-amino-1, 4-dimethyl-5H-pyrido [4, 3-b] indole (Trp-P-1). (Zhang and Ohta, 1991 and 1993a).Hernandez-Mendoza et al. tested the binding of different cell components from *L. casei* Shirota and *L. reuteri*, including EPS, bacterial cell wall (BCW), protoplasts (PP), spheroplasts (SP), teichoic acid-deficient bacteria (TADB) to AFB1. Their results showed that besides peptidoglycan, teichoic acid might take part in the binding process too (Hernandez-Mendoza et al., 2008). In El-Nezami et al.'s study, polysaccharides from the cell wall of *L. rhamnosus* GG were thought as the main component responsible for binding zearalenone (El-Nezami et al., 2004). Because purified peptidoglycan showed a lower Trp-P-1-binding than intact cell wall did (about less than 70%), Sreekumar and Hosono (1998a and 1998b) indicated that the carcinogenic chemical was mainly removed by the multiple components from bacterial cell wall. However, Delcour et al. (1999) stressed that polysaccharides from the cell wall of lactic acid bacteria strains mainly consisted of such forms as EPS, peptidoglycan, and wall teichoic and lipoteichoic acids. Thus, it is believable that the peptidoglycan from LAB cells should play a major role in binding carcinogenic compounds.

4. Factors affecting binding capacity

4.1. LAB strains diversity

Hernandez-Mendoza et al. compared the ability of 5 LAB strains, i.e. *L. reuteri* NRRL14171, *Bifidobacterium bifidum* NCFB2715, *L. casei* Shirota, *L. johnsonii* NCC 533, and *L. casei defensis*DN-114-0015 to bind AFB1. The binding percentage of these probiotic strains to AFB1 varied from 15% for *L. casei* Shirota to 58% for *L. reuteri* NRRL14171 (Hernandez-Mendoza et al., 2008). In their other study, 8 *L. casei* strains were screened for the removal of AFB1 via binding. It was seen that the binding percentage of these 8 strains to AFB1 ranged between 14% for *L. casei* ATCC 334 and 49% for *L. casei* L30

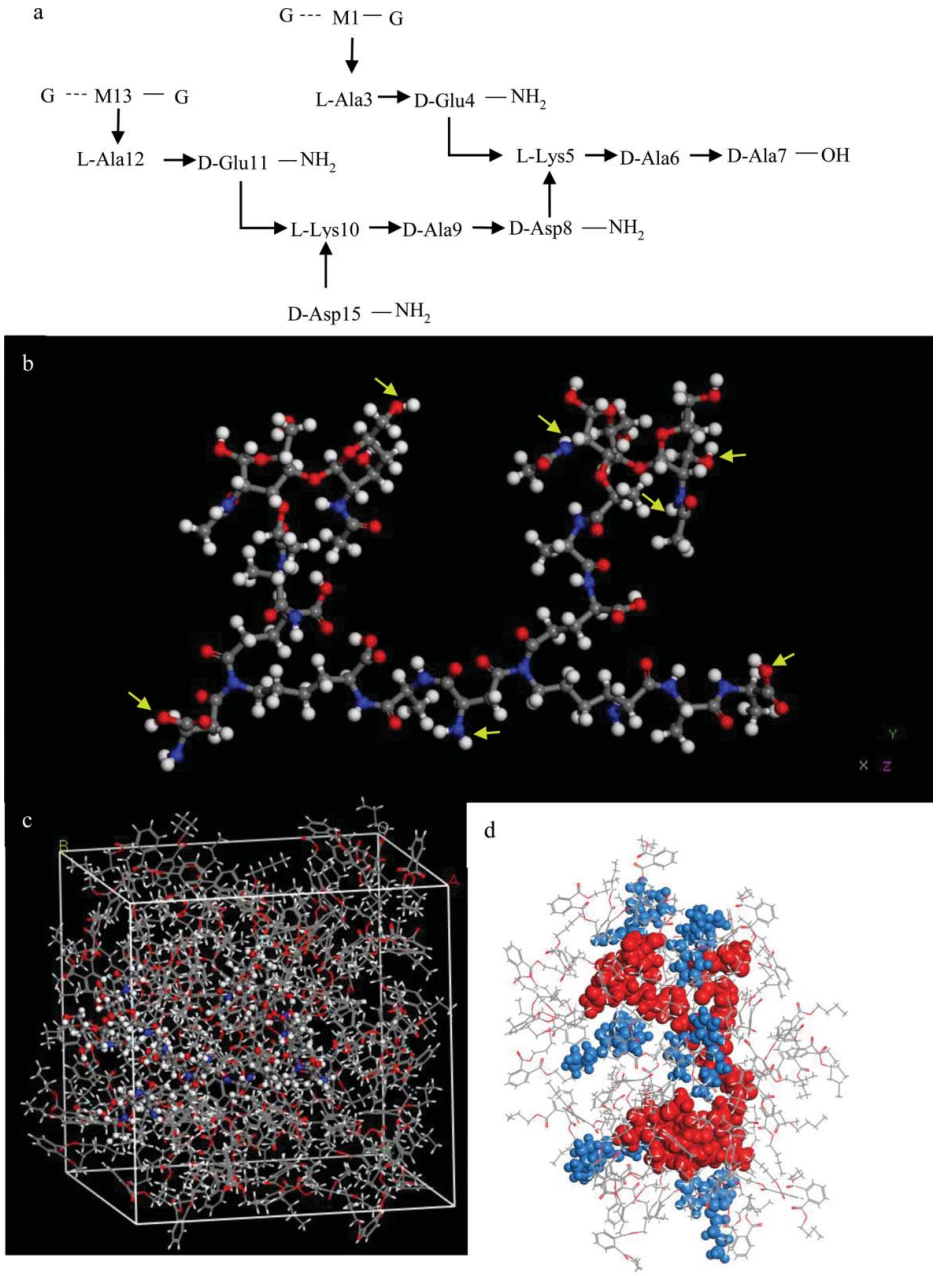


Figure 1. a. Peptidoglycan (PG) repeat unit of *Lactobacillus acidophilus* reported by Coyette and Ghuysen (1970), Schleifer and Kandler (1972), and Wu et al. (2013). b. Peptidoglycan (PG) model of *Lactobacillus acidophilus* was built by "Visualizer modules" of Material studio7.0. c. and d. Binding interactions of PG to DBP. Binding of DBP in the PG active sites is indicated, wherein it interacts closely with key residues of the active sites. Hydrogen bonds are observed between DBP: O and NAM_1: HN, NAG_2: HO, NAG_2: HN, D-Ala_7: HO, D-Asp_8: HN, NAG_{14}: HO, and D-Asp_{15}: HO.

(Hernandez-Mendoza et al., 2009). Clearly, AFB1-absorbing by lactic acid bacteria was strain-specific.

Corsetti et al. isolated 25 *L. casei* strains from ewe cheeses from Abruzzo region, central Italy. They investigated the anti-genotoxicity of these *L. casei* strains towards 4-nitroquinoline-1-oxide (4-NQO) and N-methyl-N′-nitro-N-nitroso-guanidine (MNNG) *in vitro*. The percentage of genotoxicity inhibition (GI) was calculated from residual activity (SOS induction factor) evaluated on supernatants in relation to that of a positive control. In the test for 4-NQO, 12 strains showed good antigenotoxicity (GI > 75%), and 9 strains had moderate antigenotoxicity (GI from 25% to 75%). The other strains have poor antigenotoxicity (GI < 25%). But for MNNG, only one strain showed good antigenotoxicity, and 11 strains did not have any antigenotoxicity. A study also confirmed the findings of Cenci et al. (Cenci et al., 2002). It means that the antigenotoxic activity of lactic acid bacteria is strain-dependent (Corsetti et al., 2008).

Stidl et al. examined the removal of cooked-food mutagens (AaC, PhIP, IQ, MeIQx, DiMeIQx) by 8 LAB species including *L. acidophilus, L. bulgaricus, L. casei, L. helveticus, L. plantarum, S. thermophilus, L. kefir* and *B. longum*. Summary of the total binding capacities of each species towards all HAs showed that *L. helveticus* and *S. thermophiles* were the most effective species in inactivating these mutagens. *L. plantarum* and and *L. kefir* exhibited a poor activity in binding these Has (only 4 to 13% HAs were bound). Statistically, the binding capacity of the

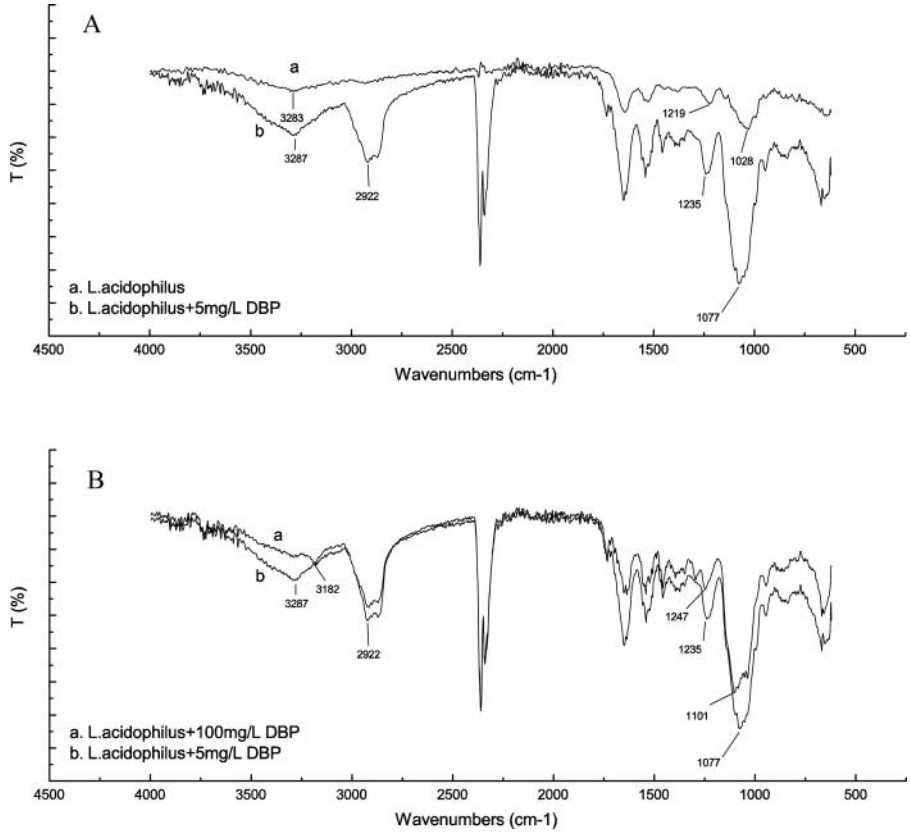


Figure 2. Spectrogram of Fourier transformed infrared spectroscopy (FTIR) of *Lactobacillus acidophilus* NCFM and *Lactobacillus acidophilus* NCFM+DBP.

8 species to HAs differed significantly from each other (Stidl et al., 2008).

The removals of carcinogens by LAB strains from published literatures over the past years were summarized in Fig. 3. Accumulated data from these figures strongly indicate that the capacity of lactic acid bacteria to remove carcinogens diversify significantly, depending on their strains and species. Regarding the diversity of carcinogenic compounds originated from various environments, the screening of high-efficient LAB strains with ability to bind carcinogens is necessary and meaningful for future work (Zhao et al., 2017).

4.2. pH value

The pH value may be an important factor in affecting LAB strains to bind carcinogenic compounds. Bolognani et al. dealt with the relationship between the binding ability of *B. longum* and *L. acidophilus* strains to BaP and AFB_1 and pH ranges. The two bacteria absorbed the carcinogenic compounds the most at pH 5.0. Significant decrease in their Bap- and AFB1-binding was observed when pH was down or up (Bolognani et al., 1997). Topcu et al. addressed that *E. faecium* M74 showed the best binding ability to AFB1 (28.6%) and PAT (43.9%) when pH was 7.0 and 4.0, respectively (Topcu et al., 2010). Hatab et al. investigated the ability of several LAB strains, including *B. bifidum* 6071, *B. animalis* 6165, *L. rhamnosus* 6149, *L. delbrueckii* ssp. *lactis*22170, and *L. delbrueckii* ssp. *lactis* 22165 to PAT. The optimal removal of these strains towards PAT took place at a low pH range of 3.0 to 4.0. Especially, strains 6071 and 6149 adsorbed PAT more than 50% (Hatab et al., 2012). Haskard et al. thought that an increase in pH range from 2.5 to 8.5 did not affected the ability of *L. rhamnosus* GG to bind AFB1, but significantly influenced this bacterium to adsorb AFB_{2a} (Haskard et al., 2000). However, study from El-Nezami et al. indicated that a change in pH range from 3.5 to 8.5 did not significantly change the binding effect of *L. rhamnosus* GG to ZEN (El-Nezami et al., 2004). Clearly, the optimal pH for each LAB strain is quite different in binding different cancerogenic compounds. Summary of the mentioned information reminds us that like the careful selection of strains, pH as a factor has to be considered when the investigation of LAB strains with an ability to bind carcinogenic compounds is carried out.

4.3. Incubation time

How an incubation time as a factor affects the binding process of LAB strains to carcinogenic compounds has not been discussed in details yet. Observation from El-Nezami et al. showed that the binding of LAB strains, including *L. rhamnosus* GG, *L. rhamnosus* LC-705, *Propionibacterium freudenreichii* spp. *shermanii* JS, *L. acidophilus* ATCC 4356, *L. gasseri* ATCC 33323, and *L. casei* Shirota YIT 9018 to AFB1 was a rapid process. No significant differences in the percentage of residual AFB1 were detected between 0 h and 72 h among these strains, except for *L. casei* Shirota. Even for strains GG and LC705, Their best binding capacity to AFB1 was at 0 h. Approximately 80% AFB1 was removed by the two strains at this moment (El-Nezami et al., 1998b). Similarly, Peltonen et al. assessed the binding ability of 3 LAB strains from *L. amylovorus* CSCC 5160, *L. amylovorus* CSCC 5197, and

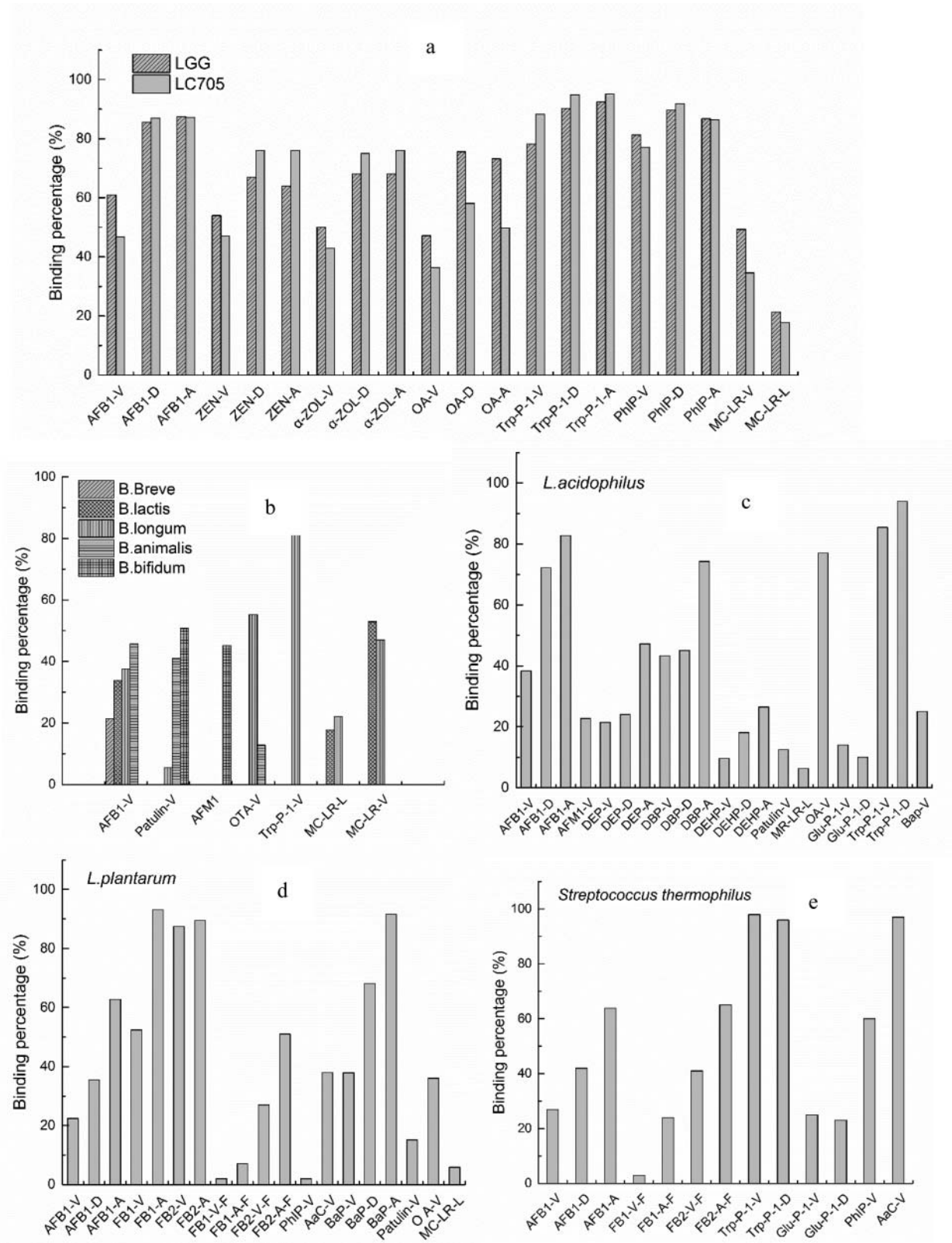


Figure 3. The binding percentage of LAB strains to carcinogens. a. The binding percentage of *L. rhamnosus* strains GG and LC705 to carcinogens (El-Nezami et al., 1998b, Haskard et al., 2000, Haskard et al., 2001, El-Nezami et al., 2002, El-Nezami et al., 2004, Turbic et al., 2002, Lahtinen et al., 2004, Nybom et al., 2007, Gratz et al., 2007, Halttunen et al., 2008) b. The binding percentage of *Bifidobacterium* species to carcinogens. (Zhang and Ohta, 1993a, Sreekumar and Hosono, 1998a, Peltonen et al., 2001, Nybom et al., 2007, Halttunen et al., 2008, Fuchs et al., 2008, Hatab et al., 2012, Serrano-Nino et al., 2013). c. The binding percentage of *L. acidophilus* to carcinogens. (Zhang and Ohta, 1993a, El-Nezami et al., 1998b, Haskard et al., 2001, Peltonen et al., 2001, Nybom et al., 2007, Fuchs et al., 2008, Qi et al., 2011, Zhao et al., 2013, Serrano-Nino et al., 2013, Zhao et al., 2015). d. The binding percentage of *L. plantarum* to carcinogens. (Haskard et al., 2001, Peltonen et al., 2001, Zinedine et al., 2005, Nybom et al., 2007, Fuchs et al., 2008, Stidl et al., 2008, Niderkorn et al., 2009, Qi et al., 2011, Zhao et al., 2013, Wang et al., 2015b). e. The binding percentage of *Streptococcus thermophilus* to carcinogens. (Zhang and Ohta, 1993a, Haskard et al., 2001, Stidl et al., 2008, Niderkorn et al., 2009). Abbreviations in figures: Viable bacteria cells (V). Dead bacteria cells (D). Acid treated bacteria cells (A). Lyophilized bacteria cells (L). In Foodstuff (F).

L. rhamnosus Lc 1/3 to AFB1. AFB1 in solution by strains CSCC 5160, CSCC 5197 and Lc 1/3 were bound rapidly, with AFB1-binding percentage of 52.6%, 66.5%, and 76.9% at 0 h, respectively. During the whole periods of 72 h incubation time, strain CSCC 5160 bound 73.2% AFB1 at 72 h; strain CSCC 5197 absorbed 72.4% AFB1 at 48 h; and strain Lc 1/3 adsorbed 76.9% AFB1 at 0 h. The difference in incubation time for the bind of LAB strains to AFB1 was probably attributed to the different constitution of bacterial cell wall structures (Peltonen et al., 2001). Gratz et al. utilized Caco-2 cells line model to investigate the modulation of AFB1 metabolism and intestinal cell toxicity in the presence of *L. rhamnosus* GG. Strain GG (5×10^{10} CFU/mL) bound 61.0% AFB1 during 1 h-incubation time. However, the total AFB1 bound by strain GG only was decreased to 40.9% during 24 h-incubation time. It also indicates that strain GG does not bind AFB1 covalently (Gratz et al., 2007). Serrano-Niño et al. described the removal of AFM1 by several LAB strains at 0 h, 4 h, and 12 h intervals of incubation time. These strains were *L. acidophilus* NRRL B-4495, *L. reuteri* NRRL B-14171 and *L. rhamnosus* NRRL B-442, *L. johnsonii* NRRL B-2178 and *B. bifidum* NRRL B-41410. It was seen that the AFM1-binding percentages of these strains differed from each other at different incubation intervals. Strains NRRLB-442 and NRRLB-4495 showed the highest AFM1 binding, with a binding percentage of 23.88% and 24.09% at 4 h, respectively. Strain NRRLB-14171 showed the highest AFM1 binding after 12 h. No difference in incubation time was observed for the AFM1 binding of strains NRRLB-41410 and NRRLB-2178 (Serrano-Niño et al., 2013). In a word, it is clear that the optimal incubation time for LAB to bind carcinogenic compounds varies largely from strain to strain, but mostly it should be a rapid process between 0 h and 4 h. Extended incubation time does not enhance LAB strains to bind more carcinogenic compounds significantly.

4.4. Bacterial cell concentration

How many concentrations are required for LAB cells to adsorb carcinogenic compounds? It should be a topic worthy of addressing. El-Nezami et al. studied the relationship between the cell concentrations of two strains, i.e. *L. rhamnosus* GG and *L. rhamnosus* LC-705 and the removal of AFB1 in liquid media. The removing effect of AFB1 was not observed until their bacterial cell concentrations were promoted to 2×10^9 dramatically (El-Nezami et al., 1998b). Zsivkovits et al. investigated the absorption performance of several LAB strains to HAs extracted from deep-fried meats, including 2-amino-1-methyl-6-phenylimidazo [4, 5-b] -pyridine (SCGE), 2-amino-3,8-dimethylimidazo [4, 5-f] quinoxaline (PhIP), 2-amino-3, 4, 8-trimethyl-3H-imidazo[4, 5-f] quinoxaline (HCAs), 2-amino-9H-pyrido [2, 3-b] indole and 2-amino-3-methyl-3Himidazo [4, 5-f] quinolone (DiMeIQx). They feed rats by gavage of the strains from *L. bulgaricus* 291, *S. thermophilus* F4, *S. thermophiles* V3, and *B. longum* BB536 which bind HAs compounds. The damaged extent of DNA was measured in colon and liver cells by single cell gel electrophoresis (SCGE) tests. The inhibition of HAs induced DNA damage was dose dependent, and was significant when 1×10^7 cells/animal were administered (Zsivkovits et al., 2003). Gratz's used two cell concentration levels of *L. rhamnosus* GG, i.e. 1×10^{10} CFU/mL and 5×10^{10} CFU/mL to bind AFB1. The AFB1-binding percentage by this probiotic bacterium varied between 40.1% and 61.0% (Gratz et al., 2007). Therefore, there is no clear answer about how many bacterial cells are required for carcinogens binding, but the binding effect of LAB strains should be dosage-dependent. A high cell concentration will increase the removal quantity of carcinogenic compounds to some extent in a certain chemical solution.

4.5. Types of carcinogenic compounds

There a diversity in carcinogenic compounds' types, although they have a multiple-ring molecular structure. Orrhage et al. investigated the binding capacity of 7 LAB strains and *E. coli* to HAs formed in the cooking of protein-rich food. Minor differences were observed in the binding capacity of the tested strains, but the mutagenic compounds were bound with markedly different efficiencies. Trp-P-2 was almost completely bound, and about 50% PhIP was adsorbed. However, little IQ and MeIQx were bound (Orrhage et al., 1994). Sreekumar et al. evaluated *in vitro* the ability of 4 *L. acidophilus* strains (SBT0274, SBT1703, SBT10239, and SBT10241) and *B. longum* (SBT 2928) with good antimutagenicity to bind HAs, including Glu-P-1, IQ, MeIQ, Trp-P-1, and Trp-P-2. The percentages of these HAs compounds bound were significant different among these strains, and even among strains from the same species. For instance, *L. acidophilus* SBT 2928 was able to bind 88.8% Trp-P-1, 74.2% Trp-P-2, 39.4% MeIQ, 43.9% IQ, and 12.3% Glu-P-1, respectively. *L. acidophilus* SBT0274 was likely to adsorb 92.4% Trp-P-1, 80.3% Trp-P-2, 56.6% MeIQ, 39.1% IQ, and 11.3% Glu-P-1, respectively (Sreekumar and Hosono 1998a). It was noted that the binding ability of *L. rhamnosus* GG to four aflatoxins occurred in an order of AFB1>AFB2>AFG1>AFG2. The reduction in the binding percentage of this strain was due to the decreasing of the adsorbed compound's polarity (EI-Nezami et al., 1996). A recent work from our laboratory showed that the adsorption of *L. acidophilus* NCFM to DEP, DBP, and DEHP was significantly difference. Its binding percentage was 21.48% for DEP, 43.32% for DBP and 9.62% for DEHP, respectively (Zhao et al., 2015).

Tanabe et al. indicated that the binding of a strain might depend upon a hydrophobic interaction between LAB cells and carcinogenic compounds (Tanabe et al., 1994). Stidl et al. dealt with the total removal abilities of 8 tested LAB species, according to the accumulation of binding percentages of 96 strains, to the cooked food mutagens like AaC, PhIP, IQ, MeIQx, and DiMeIQx. The binding capacities of the 8 LAB species to these easy-to-formed mutagens declined in the order AaC>DiMeIQx>MeIQx>IQ>PhIP. The pKa values and the logarithms of theoctanol/water partition coefficients from the 5 HA were also analyzed. It was seen that the most hydrophobic compounds like AaC and DiMeIQx were efficiently removed by these bacteria, while IQ and PhIP which are less hydrophobic were bound to a smaller extent. The least hydrophobic HA PhIP almost was not bound by these LAB cells. Thus, pKa values did not play a major impact on the binding process. The hydrophobic property of these mutagens

may be important in affecting the binding ability of a strain (Stidl et al., 2008). Difference in the types of cancerogenic compounds affects the binding effect of LAB strains (Fig. 3). The higher the hydrophobicity of a cancerogenic compound, the more it is absorbed by LAB strains.

5. Binding stability

Numerous studies have evaluated the binding stability of LAB strains to cancerogenic compounds. Haskard et al. evaluated the binding stability of AFB1 by 12 LAB strains, and the more efficient AFB1 binding strains were *L. rhamnosus* GG and LC-705. Consequentially, the complexes formed between AFB1 and strains GG or LC-705 were more stable than those formed with the other strains tested. Heat and acid treatments strengthened the complexes retention for most of tested strains, because more new sites might be released due to the broke-down of the chemical bond of polysaccharide and peptidoglycan in cell wall. Only 6 to 11% bound AFB1 was released from these complexes in water at pH 2, 7, and 10 (Haskard et al., 2001). Lee et al. indicated a linear relationship between the natural log value of the concentration of AFB1 adsorbed and the number of washes for *L. rhamnosus* GG, *L. rhamnosus* LC-705, and *P. freudenreichii* ssp. *shermanii* JS strains. The more AFB1 a bacterial cell has adsorbed, the stronger the AFB1 molecules would remain adsorbed on the cell surface (Lee et al., 2003). Hernandez-Mendoza et al. washed the complex of bacteria and AFB1 with PBS for four times. The residual of AFB1 bound to the bacterial cells of *L. casei* Shirota, *L. casei defensis* and *Bb. Bifidum* was variable. Nearly 80% AFB1 remained bound to *L. casei* Shirota after washing, while washing caused the other species to release about 30% of the AFB1 back to solution. The AFB1 was attached to the bacteria by a weak, noncovalent interaction. The stability of the aflatoxins-bacteria complex seemed to be dependent on the binding ability of a particular strain (Hernandez-Mendoza et al., 2008). According to Topcu et al.'s observation, AFB1 and PAT bound by *E. faecium* was high stability after washing the cells three times with PBS. Only 17 to 23% bound AFB1 and 19 to 25% bound PAT were released from the bacteria-AF complex after washing. Statistically, the binding stability between the viable and nonviable cells of *E. faecium* was not significant difference (Topcu et al., 2010). However, organic solvents strongly destroyed the binding stability between LAB cells and bound cancerogenic compounds. About 83 to 99% of bound AFB1 was released when the aflatoxin-bacteria complex was suspended in methanol, acetonitrile, chloroform, or benzene solvents. Chloroform, followed by benzene, acetonitrile and methanol, had a strong destruction towards the stability of the AFB1-bacteria complex, because the hydrophobicity of the AFB1 molecule most closely matches that of chloroform (Haskard et al., 2001). El-Nezami et al. added NaCl and $CaCl_2$ to ZEN-bacteria complex, and found that the percentage of ZEN bound was promoted although ZEN concentration was increased (EI-Nezami et al., 2004). Thus, the binding between carcinogenic compounds-LAB cell complexes generally is stable, but the stability for one particular carcinogen will be destroyed by organic solvents used in media.

6. Test models *in vitro* and *in vivo*

6.1. Gastrointestinal digestion model

To date, various models, *in vitro* and *in vivo*, have been selected to evaluate the role of LAB strains in removing carcinogenic compounds via binding. Gratz et al. collected intestinal mucus from three porcine colon samples, and then used *L. rhamnosus* GG and a mixture of *L. rhamnosus* LC-705 plus *P. freudenreichii* ssp. *shermanii* JS to bind AFB1 in mucus. It was seen that a pre-incubation between two probiotics and AFB1 reduced the binding of mucus to the mycotoxin. Furthermore, mucus pre-incubation significantly reduced AFB1 binding by 23.8% in the presence of strain GG or 61.2% in the presence of strains LC-705 plus JS (Gratz et al., 2004). A research from our laboratory showed that the Bap-binding percentages of *L.plantarum*121 and *L. pentosus* ML32 were increased more than70% in the environments of salt bile and gastric acid (Qi et al., 2011). To reduce AFM1 toxicity in artificially contaminated milk, Serrano-Niño et al. (2013) used an *in vitro* digestive model to assess the ability of 5 LAB strains to bind the mycotoxin. In the digestive assay, *B. bifidum* NRRL B-41410 was able to reduce the relative toxicity of AFM1 to 45.17%, and 32.20% relative toxicity of AFM1 was decreased by *L. acidophilus* NRRL B-4495. Some reports indicated that LAB strains might increase their binding capacity to AFB1 in the stimulation of gastrointestinal digestion environments, because the exposure of LAB cells to bile results in the alterations of proteins and phospholipids in the cell envelope and thus improvement of the binding percentage (El-Nezami et al., 1998a, Haskard et al., 2001, Begley et al., 2005). Clearly, gastrointestinal digestion model as an *in vitro* tool can be applied to discuss the role of LAB strains in reducing the toxicity of carcinogenic compounds.

6.2. Cell lines model

Various cell lines may be explored to investigate the reduction of carcinogenic compounds bound by LAB strains *in vitro*. Kankaanpää et al verified that removal of AFB1 by *L. rhamnosus* GG reduced the adhesion capability of this strain from 30% to 5% in a Caco-2 adhesion model (Kankaanpää et al., 2000). Some reports indicated that LAB strains not only reduced the uptake of cancerogenic compounds upon membrane, but also relieved DNA damage and enzymes activity in the cell lines model. Gratz et al. used Caco-2 cells model to investigate the potential of *L. rhamnosus* GG to reduce AFB1 availability *in vitro*. Strain GG bound 40.1% and 61.0% AFB1 after 1 h-incubation when the cell concentration of this bacterium was 1×10^{10} CFU/mL and 5×10^{10} CFU/mL, respectively. In addition, DNA fragmentation was apparent in cells treated only with AFB1 but not in cells co-incubated with either 1×10^{10} CFU/mL or 5×10^{10} CFU/mL strain GG. It was concluded that strain GG not only reduced AFB1 uptake but protected both membrane and DNA from damage as well (Gratz et al., 2007). Turner et al. applied *L. rhamnosus* GG to restrict the bioavailability of DON via this strain bind to this toxin in Caco-2 cell model. DON caused a significant reduction in ALP activity of the cell lines (1598 ± 137 U/mg protein), compared to untreated cells (2502 ± 80 U/mg). However, a dose dependent restoration of ALP activity was observed when DON treated

cells were co-incubated with heat inactivated strain GG which has a cell concentration of 1×10^4, 1×10^7, and 1×10^{10} CFU/mL. When viable strain GG were co-incubated with DON a similar restoration of ALP activity was observed, as seen for heat inactivated strain GG (Turner et al., 2008). Micronucleus (MCN) assays were conducted by Fuchs et al. with a human derived hepatoma cell line (HepG2) to prove that LAB strain decrease mycotoxins in liquid media. A substantial decrease of MCN formation (about 39–59%), induced by OTA and PAT, was observed in the presences of *L. acidophilus* VM20 and *B. animalis* VM12. The inhibition of the cell division rates by the toxins was significantly reduced (Fuchs et al., 2008). Compared to the gastrointestinal digestion model, utilization of cell lines model may help us to understand the functions of LAB strains in reducing the risk of carcinogenic compounds better.

6.3. Animal experiments

Early in 1980, Goldin et al. found that feeding rats with *L. acidophilus* reduced the incidence of colon cancer induced by 1, 2-dimethylhydrazine dihydrochloride (DMH) (40% vs. 77% in controls) (Goldin and Gorbach, 1980). Pool-Zobel et al. studied the potential of *L. acidophilus* from a commercially available yogurt to prevent the induction of DNA damage by N-methy-N -nitro-N-nitrosoguanidine (MNNG) in colon cells of rats. Rats were feed by a single dose of 10^{10} viable cells/kg body. The viable *L. acidophilus* cells could prevent MNNG-induced DNA damage in colon cells of the rats. Unlike the non-antigenotoxic activity of cell fractions (cytoplasm, cell wall skeleton and cell wall), the peptidoglycan fraction and whole freeze-dried cells of *L. acidophilus* showed antigenotoxic activity (Pool-Zobel et al., 1996). A similar test was done by Zhang and Ohta (1993a). The rats were feed with the mixture of Trp-P-1 plus freeze-dried cells of LAB strains. The content of Trp-P-1 in blood of the rats consuming LAB cells was 40.4 ~ 64.7% lower than that of the rats feed with Trp-P-1 only after 1 h (Zhang and Ohta, 1993b). Studies on the elimination of mytoxins by LAB strains have been done using animal model. The rats firstly were feed with an oral dose of AFB1 (1.5 mg or 4.8 μmol/kg of body weight), and then administrated with probiotic suspension (5×10^{10} CFU *L. rhamnosus* GG/0.5 ml PBS) by oral gavage daily for 3 days. In AFB1-only treated rats, fecal excretion of both AFB1 and AFM1 were 15.6±9.8 nmol/24 h feces, and 27.1± 22.5 nmol/24 h feces, respectively. Probiotic treatment significantly increased fecal excretion of AFB1 by 122% (35.3 ± 21.3 nmol/24 h feces) and of AFM1 by 152% (68.1 ± 26.1 nmol/24 h feces) within 24 h. LAB strains treatment prevents weight loss and reduces the hepatotoxic effects caused by AFB1 by increasing the excretion of orally dosed aflatoxin via the fecal route (Gratz et al., 2006). The main absorption site *in vivo* of LAB strains to carcinogenic compounds was reported in small intestine. Terahra et al. investigated the absorption of heterocyclic amines by the different position of rats' intestine in the presence of *L. delbrueckii* subsp. *bulgericus* 2038 and *S. thermophilus* 1131, using an *in situ* loop technique. Strain 1131 could significantly bind Trp-p-1 at the same pH as that of small intestine (pH 6–7) compared with other pH. And strain 1131 also inhibited Trp-P-1 absorption from the small intestine *in situ* (Terahra et al., 1998).

Some LAB strains bind carcinogenic compounds *in vitro*, but might not always work *in vivo*. Bolognani et al. indicated that Bap, Trp-P-2, MelQ, PhlP and IQ were bound efficiently by the two LAB strains *B. longum* and *L. acidophilus in vitro*, but the oral consumption of the two probiotic bacterial cell suspensions did not lead to a reduction in induced mutagenicity by MelQ, MelQx or Trp-P-2 in the liver of treated mice (Bolognani et al., 1997). In a 35-day piglet experiment, Dänicke and Döll examined the effects of feeding DON-contaminated diet in presence of a probiotic additive (2.3×10^6 CFU/g diet of a one-to-one ratio of *Bacillus subtilis* and *B. licheniformis*) on the animal. DON concentrations in blood of the piglets supplemented with the probiotics were not significantly different those with DON-fed group. It was speculated that the fed probiotic bacteria neither bound nor degraded DON prior to absorption (Dänicke and Döll, 2010). In a word, some animal experiments have positively supported the roles of LAB strains or their fermented foods in decreasing the poisonousness of carcinogenic chemicals *in vivo* via binding mechanism. However, some negative results also have been reported. Thus, more data from *in vivo* tests have to be accumulated to elucidate the possible mechanism of selected LAB strains in removing carcinogenic compounds from damage to the host in the future.

6.4. Clinical research

To date, only a very few cases on the removal of carcinogenic compounds by LAB strains from clinical research have been reported. EI-Nezami et al. carried out a pilot clinical trial in Egypt to investigate the effect of probiotic preparation containing both *L. rhamnosus* strain LC-705 and *P. freudenreichii* spp. *shermanii* JS on the levels of AFB1 in human faecal samples. 20 normal healthy volunteers were selected in this study, and the faecal samples from 11 of the recruited 20 volunteers were positive for AFB1 removal (1.8 ~ 6 μg AFB1/kg faeces). After the second week of the trial, a significant reduction in the level of AFB1 for volunteers who were administered the probiotic preparation was observed, and continued reduction during the follow up period (EI-Nezami et al., 2000). To determine whether the administration of probiotic bacteria could block the intestinal absorption of AFB1 and thereby lead to reduced urinary excretion of aflatoxin B1-N7-guanine (AFB-N7-guanine) (a marker for a biologically effective dose of AFB1 exposure), a ninety-healthy young men from Guangzhou, China were randomly assigned to 2 groups. One group received a mixture of *L. rhamnosus* LC705 and *P. freudenreichii* subsp. *shermanii* strains, and the other group received a placebo preparation. Statistically, significant decrease (36% at week 3 and 55% at week 5) in the concentration of urinary AFB-N7-guanine was observed in the probiotic group (El-Nezami et al., 2006). LAB strains are suggested to be potent and safe means to reduce absorption and increase excretion of dietaryAFB1 from the body. The above studies indicate a positive result due to the increased excretion of AFB1 from human body by LAB strains' bind to AFB1. However, it is hard to evaluate the anti-cancerogenic effect of LAB strains because well-designed clinical researches linked to the removal of carcinogenic compounds are still scarce.

7. Binding of carcinogens by LAB strains in food and feed

Several reports have exhibited the binding ability of LAB strains to carcinogens in food and feed. Turbic et al. used *L. rhamnosus* strain GG and LC-705 to eliminate both dietary mutagens like AFB1, OTA, Trp-P-1, PhIP and such aromatic dietary as caffeine, vitamin B12, folic acid substrates from solution. AaflatoxinB1, Trp-P-1 and PhIP were removed in high a mounts (77–95%) and OTA was removed in moderate amounts (36–76%). These two strains showed usefulness for dietary detoxification (Turbic et al., 2002). Mateo et al. investigated the changes of OTA by *Oenococcus oeni* performed in synthetic medium supplemented with ethanol and OTA. The highest toxin removal percentage (about 29%) was observed in cultures containing 5% ethanol and 2 μg OTA/L. OTA reduction percentages in cultures containing 0% or 5% ethanol (5 μg OTA/L) ranged between 20% and 22%. No changes in OTA levels were observed in *O. oeni* cultures supplemented with 15% ethanol (Mateo et al., 2010). Corassin et al. evaluated the ability of a *Saccharomyces cerevisiae* strain and a mixture of three LAB strains (*L. rhamnosus, L. delbrueckii* spp. *bulgaricus* and *B. lactis*) to bind AFM1 in UHT –treated skim milk which contained 0.5 ng AFM1 mL/L. The AFM1-bound percentage (100.0%) was significantly increased during 60 min when *S. cerevisiae* + LAB pool were used (Corassin et al., 2013). The heat-killed *L. rhamnosus* GG could bind 18.8% and 26.0% AFM1 in recovery skim milk and full scream milk (Pierides et al., 2000). Some components in food and feed were reported to improve the binding capacity of LAB strains to carcinogenic compounds. He et al. studied the performances of *L. acidophilus* NCFM, *L. plantarum*121 and *L. pentosus* ML32 in removing BaP in simulated starch conditions. All of the three *Lactobacillus* strains performed good ability to bind BaP in the presences of starch. Their BaP-removing ability would be improved with an increased starch concentration, and the gelatinization of starch as well as the supplements of glucose and maltose. If the content of glucose or maltose was increased from 0% to 0.5%, the BaP-binding percentage was increased from 20% to 30%. However, the added carbohydrate did not influence the binding ability of dead *Lactobacillus* strains (He et al., 2016).

8. Conclusions and outlooks

Numerous studies have confirmed the anticarcinogenicity and antimutagenicity of LAB strains *in vitro* and *in vivo*. They are from these genera of *Lactobacillus, Streptococcus* and *Bifidobacterium, Lactococcus* and *Enterococcus*. All these LAB strains have been documented to have a good capacity to bind carcinogenic compounds, and the binding capacity is strain-dependent. The binding sites of LAB strains to cancerogenic compounds are in the cell wall. Except of protein, carbohydrates are the main binding sites. There are three types of carbohydrates in the LAB strains cell wall, but peptidoglycan is the chief type besides teichoic acid and exopolysaccharides. The hydrophobic interaction plays an important role in binding, and the binding between molecules is released mainly via hydrogen bond. Vander Waals and electrostatic interaction are also involved in the binding process. The binding is a rapid process. pH affects the ability of LAB strains to bind various cancerogenic compounds. The detoxication effect depends on the cell concentration, and types of carcinogenic compounds. The stability of LAB cell-carcinogenic compound complex more easily is destroyed by the washing of organic solvent than water or PBS buffer.

Currently, theoretical researches have showed that LAB strains are a good potential tool to remove carcinogenic chemicals. For future, the screening of LAB strains with good anticarcinogenicity should be more focused. Evaluation system of cell model and animal tests should be strengthened. To evaluate the binding effect, such peptidoglycans-analogue compounds as purified from different strains should be developed to verify the roles of lactic acid bacteria in removing various carcinogens in the real host system. Omics should be used to study the mechanisms of LAB strains-carcinogens-cell lines or animal models. The binding conditions of LAB strains to carcinogenic compounds should be optimized to effectively eliminate the mutagenic compounds from food and feed-related environments. Some genetic modifications of LAB strains might be required for the improvement of anticarcinogenicity and antimutagenicity.

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