

How fermentation by lactic acid bacteria can address safety issues in legumes food products?

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Hélène Licandro, Phu Ha Ho, Thi Kim Chi Nguyen, Awanwee Petchkongkaew, Hai Van Nguyen, et al.. How fermentation by lactic acid bacteria can address safety issues in legumes food products?. Food Control, 2020, 110, pp.106957. 10.1016/j.foodcont.2019.106957. hal-02394820

HAL Id: hal-02394820 https://institut-agro-dijon.hal.science/hal-02394820

Submitted on 20 Jul 2022

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How fermentation by lactic acid bacteria can address safety issues in legumes food 1 2 products? 3 Hélène Licandro^{1,2}, Phu Ha Ho^{1,3,5}, Thi Kim Chi Nguyen^{1,2}, Awanwee Petchkongkaew^{1,4}, Hai 4 Van Nguyen^{1,3,5}, Son Chu-Ky^{1,3,5}, Thi Viet Anh Nguyen^{1,6}, Da Lorn ^{1,2,7}, Yves Waché^{1,2} 5 ¹Tropical Fermentation Network 6 7 ²International Joint Laboratory « Tropical Bioresources & Biotechnology » (Univ. Bourgogne Franche-Comté, AgroSup Dijon, PAM UMR A 02.102 and School of Biotechnology and 8 Food Technology, Hanoi University of Science and Technology), Dijon, France 9 ³School of Biotechnology and Food Technology (SBFT), Hanoi University of Science and 10 Technology (HUST), Hanoi, Vietnam⁴School of Food Science and Technology, Faculty of 11 Science and Technology, Thammasat University, Thailand 12 ⁵International Joint Laboratory « Tropical Bioresources & Biotechnology » (Univ. Bourgogne 13 Franche-Comté, AgroSup Dijon, PAM UMR A 02.102 and School of Biotechnology and 14 Food Technology, Hanoi University of Science and Technology), Hanoi, Vietnam 15 ⁶Food Industries Research Institute (FIRI), Hanoi, Vietnam 16 ⁷Food Technology and Nutrition Research Unit, Research and Innovation Center, Institute of 17 Technology of Cambodia, Phnom Penh, Cambodia 18 19 20 21 22 23 24 Abstract

Fermented Asian foods have recently gained popularity, crossing from Asian communities to mainstream markets, in many western countries. However, less attention has been paid to the safety of these foods. In South-East Asia fermented products are still produced following traditional methods. Therefore, consumers are not confident in their microbial safety. The challenges awaiting fermentation in South-East Asia are thus to improve safety and quality in a sustainable system producing tasty and typical fermented products. A possible solution could be the use of starter cultures able to increase the safety of food stuffs. Starters could also help to standardize the production process and reduce ripening times. The current review addresses the role of lactic acid bacteria on the microbiological and chemical safety of Asian legume based fermented products. In particular, their role in the reduction of anti nutritional compounds (e.g. phytates) and protein allergenicity is discussed. Moreover, starters can inhibit the development of amino acid decarboxylating microbes preventing the accumulation of biogenic amines, they can also be useful to reduce the accumulation of mycotoxins and inhibit pathogens' development. Finally, their role in the degradation of pesticides is analyzed.

Key words: legumes, lactic acid bacteria, phytates, biogenic amines, mycotoxins, food safety

1. Introduction

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The food system is facing a crisis as consumers are confused by ambivalent information arising all along the food chain. Plant products bring on the one hand vitamins and micronutrients, they have a positive impact on the environment but on the other hand, they might contain non-neglectable amounts of antinutritional factors (ANFs), toxic chemicals and toxins. For their culture, agroindustry promotes the facilitated culture of GMO while scientific studies reveal that the use of pesticide-resisting varieties results in many risks, including the increase in the level of pesticide in culture, in the soil and, ultimately, in the food (Bonny 2016; Tsatsakis, Nawaz et al. 2017)). Some of these issues might find solutions in the future by the selection of low ANF-varieties, by improving cultural techniques, decreasing the use of chemicals, developing storage techniques avoiding mold contamination etc. but this will take time, especially in South-East Asia where farmers are often not enough trained and have economic limitations to implement the required changes in culture practices. Microorganisms functionalities in fermented foods can bring several advantages to consumers (Tamang et al., 2016). As an efficient, rapid and low cost solution to nutritional and food safety concerns, the use of bio-remediation starters can be added to these benefits and this will be discussed in the present work with a special focus on legumes.

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2. Fermented leguminous in South-East Asia

Legumes such as soybeans, black beans, green beans, peanuts... are common ingredients in South-East Asia for food production. They are popular for various reasons. In one hand, when grown, they have the possibility to supply nitrogen to the soil through a mycorrhizal association that makes this family of plant an actor of rotations in organic agriculture. On the other hand, their protein content is also very high making them good nutritional candidates to replace meat or milk, which production is an important source of greenhouse gas, and a big

consumer of plant and water resources especially in developing countries (Tubiello, 2018). In particular, soybeans are most used for the production of traditional fermentation products such as fermented soybean paste (Ban Tuong, Cu Da Tuong – Vn, Nam Dan Tuong – Vn, Sieng – Kh), fermented soybean curd (Chao - Vn), Tauxi - Ha Giang Dau Xi - Vn), alkaline fermentation of soybeans (natto-like) (Thua Nao – Th). Fermentation of these beans can have an important nutrient and health impact (Cao et al. 2019; Jayachandran & Xu, 2019). However, production of traditional fermentation products in South-East Asia is now managed by experience, produced mainly by manual households, with rudimentary production equipment, mostly fermented by endogenous microorganisms. Raw materials as well as technologies have not been standardized. These products are characterized by the local cultural identity. Despite their important sensorial role in Asian food, bringing for instance the umami taste to the meals (Hajeb & Jinap, 2015), production management and quality measurement are needed to improve quality and minimize food safety hazards. Food safety issues in traditional fermented food from South-East Asia have been reviewed recently (Anal et al., 2020) and soybeans, as a protein-rich matrice, are exposed to a biogenic amine risk (Park et al., 2019). Lactic acid bacteria (LAB) are usually not considered as the most important bacteria in traditional fermentation of legumes although they have an important role and are numerous in

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3. Antinutritional factors: can metabolic properties of LAB respond the challenges?

the vietnamese *tuong* and in the cambodian *sieng*.

Anti-nutritional factors (ANFs) are one of the main limitations for the development of legume-based foods. Since the early 80's, spontaneous fermentation, generally household, has

been reported to degrade some leguminous ANFs such as phytates, trypsin inhibitors and lectins (Chompreeda & Field, 1984a; Reddy & Pierson, 1994). Most of the analyzed fermented soy derivatives contain very small amount of trypsin inhibitors and isoflavones, compared to the raw soybean, but still contain phytates (Anderson & Wolf, 1995). However, it was not clear if microorganisms, including lactic acid bacteria (LAB) and yeasts, played an important role and even less what was their way of action. Hence, controlled lactofermentations were carried out. For instance, Sindhu and Khetarpaul (2001) demonstrated that LAB (Lactobacillus casei and Lactobacillus plantarum) can reduce phytates, polyphenols and trypsin inhibitors in a food mixture containing green grams, with a higher effect when inoculated after a first fermentation with yeasts (Saccharomyces boulardii). However, LAB did not seem to have any effect on agglutinins (also called lectins) (Ayyagari et al., 1989; Barkholt et al., 1998; Motarjemi & Nout, 1996). Accurate characterizations of LAB activities are underway with the objective to develop appropriate starters and processes, particularly for tannins and phytates removing. Metabolism of tannins or other polyphenols by LAB has been characterized only in a few plant fermentations including tempeh (Starzyńska-Janiszewska et al., 2014) and sorghum (Svensson et al., 2010). Among LAB, L. plantarum, Lactobacillus paraplantarum, Lactobacillus pentosus seem to be the only species capable of degrading hydrolysable tannins through a tannase activity (tannin acyl hydrolase, EC 3.1.1.20) which hydrolyzes ester bounds of tannic acid, thus releasing glucose and gallic acid (Osawa et al., 2000; Vaquero et al., 2004; Rodriguez et al., 2009). Most of tannase producers were found in fermented vegetables but also in human feces. In L. plantarum, tannase is very well characterized. Its activity was demonstrated and characterized by Rodríguez et al. (2008) and genetic analysis showed it constitutes a novel family of tannases (Iwamoto et al., 2008). LAB tannases are intracellular. A L. plantarum strain has been reported to produce a very efficient tannase during anaerobic

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fermentation (Aguilar-Zárate at al., 2015). Genes involved in tannins degradation are regulated in a coordinated way and are inducible by tannin and other phenolic compounds (Reveron et al., 2017). Characterization of fermented cassava LAB allowed identifying uncommon tannase producers such as Weissella cibaria and Leuconostoc mesenteroides ssp. mesenteroides (Kostinek et al., 2007). Degradation of phytates during food process is very inconstant, depending on the process (soaking, heating, milling ...) and on the plant species (Gibson et al., 2006). Soaking and germination can activate plant endogenous phytases in most cereals and legumes (Holzapfel, 1997). The role of endogenous plant phytases during fermentation is still under debate. For some authors, phytate reduction is rather the consequence of plant phytases activation during fermentation than of microbial phytases (Reale et al., 2007; Shirai et al., 1994). Indeed, LAB decrease and thus optimize the pH for plant phytases that are more active between pH 5 and pH 4 (Gänzle et al., 2014). Moreover, production of organic acids by LAB could play a synergic effect by complexing with minerals from phytates, thus making them more accessible for phytases (Maenz et al., 1999). On the contrary, other authors claim that phytase activity is very variable depending on the plant species, thus plant phytase activities would not be sufficient in most fermentations (Cossa et al., 2000). Phytases have been found in yeasts, fungi and bacteria. Generally, they are intracellular. Bacterial phytases have been detected in various ubiquitous genera such as Bacillus, Pseudomonas, Klebsiella, Enterobacter, Escherichia and in anaerobic ruminal bacteria (Konietzny & Greiner, 2002). A few LAB strains have been shown to degrade, at least partially, phytates. The strains belonged to Pediococcus pentosaseus in millet and soya (Raghavendra et al., 2011), Leuconostoc mesenteroides, L. plantarum, Lactobacillus acidophilus and P. pentosaseus in wheat (Lopez et al., 2000; Cizeikiene et al., 2015), some Lactobacillus species in soy milk (Tang et al., 2010), L. acidophilus, Lactobacillus buchneri

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and L. plantarum in lupin (Camacho et al., 1991; Fritsch et al., 2015), Streptococcus thermophilus and Bifidobacterium infantis in co-fermentation in soymilk (Lai et al., 2013). A few studies aimed to screen phytase activities among LAB strains. Anastasio et al. (2010) have found 29 phytase-producers among 150 LAB, most of which isolated from wheat fermentation, whereas Songré-Ouattara et al. (2008) have found 30 among 155 isolated from millet fermented gruel. According to these two works, one can expect about 20% of phytases producers among LAB isolated from plant fermentation, with the highest activities for some L. plantarum, Enterococcus faecium and Lactobacillus fermentum strains. These studies constitute preliminary reports that have to be strengthened. Indeed, another study has highlighted the importance of the substrate used for the screening since on the 40 tested LAB strains able to degrade calcium phytates, only two strains (Pediococcus pentosaceus) can degrade sodium phytates (Raghavendra et al., 2009). In addition, Fredrikson et al. (2002) warned about indirect methods to detect phytates and media composition for the screening. They did not observe reliable results for LAB. It is likely that some LAB species are considered as non- or low- phytase producers because the screening conditions do not permit phytase production in bacteria (Konietzny & Greiner, 2002). Some studies have been carried out with tighter methods. Sreeramulu et al. (1996) have reported that Lactobacillus amylovorus was a good producer of extracellular phytases and that extracellular activity was also detected for L. acidophilus, L. plantarum, Lactobacillus delbrueckii and L. casei. They did not detect any production with Lactococcus lactis or Lactobacillus helveticus. Moreover, Zamudio et al. (2001) have reported the ability of six strains of LAB (P. pentosaceus, Leuconostoc mesenteroides, L. casei, L. fermentum, L. delbrueckii and L. plantarum) to partially degrade phytate (from 6 to 11%). Whereas no intracellular and low extracellular phytase activities were measured, they proved that phytate degradation was due to a non-specific acid phosphatase. Palacios et al. (2005) have come to

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the same conclusion by characterizing a L. pentosus non-specific acid phosphatase (EC 3.1.3.2) which was able to hydrolyze phytates. De Angelis et al. (2003) have gone further by characterizing an intracellular phytase of L. sanfranciscensis at the protein level. They did not identify the corresponding gene. This strain decreased phytate content of almost 75% in sourdough after an 8 h-incubation. Several groups have identified and characterized efficient phytases in the *Bifidobacterium* genera (Haros et al., 2007; Tamayo-Ramos et al., 2012; García-Mantrana et al., 2015) but this genus does not fit with legumes fermentation mainly because it is strict anaerobic and fastidious. To overcome this limitation, García-Mantrana et al. (2016) succeeded in expressing active bifidobacterial phytases in L. casei. Nevertheless, in sourdough, the bacterial activity was low, compared to the activated plant phytases. Actually, identifying new LAB phytase activities based on phenotypical screening seems hazardous. Otherwise, in silico analysis of LAB genomes based on known bacterial phytases (Bacillus, Escherichia spp) does not predict any phytase (personal data). Even bifidobacterial phytases found by García-Mantrana et al. (2015) are very specific. Thus, molecular screening is not feasible for LAB. However, characterizing enzymatic activities and identifying the corresponding genes in LAB strains already highlighted would be very informative to know if specific or non-specific phytases exist in some LAB strains and if these phytases would be

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4. Decrease of protein allergenicity through LAB-mediated hydrolysis

efficient in reducing the phytate content in fermented legumes.

The main legume allergens are the proteins. These complex structures are difficult to degrade. The selection of legumes natural variants or the use of specific biotechnological processes can eliminate these particular proteins. However, they might also induce some side effects such as an increase in the protein synthesis pathways of the seed and the synthesis of other proteins

that might be allergenic as well (Nowak-Wegrzyn & Fiocchi, 2009; Mils et al., 2009; 200 Kroghsbo et al., 2014; Rahaman et al., 2016). 201 Plant proteins exhibits low digestibility compared to animal proteins. Poor protein 202 203 digestibility can cause gastrointestinal disorders that may result in fecal excretion of proteins. Therefore, increasing protein digestibility could reduce the levels of undigested proteins 204 which can potentially cause food allergies due to poor absorption in the gut (Untersmayr & 205 Jensen-Jarolim, 2008). Several studies have showed that LAB fermentation increases the 206 207 digestibility of plant proteins (El-Haget al., 2002; Pranoto et al., 2013). For example, during fermentation of lupin and soybean, protein digestibility is improved by 208 using LAB cultures such as Lactobacillus sakei KTU05-6, Pediococcus acidilactici KTU05-209 07 and P. pentosaceus KTU05-8. This improvement is due to the role of microbial proteolytic 210 enzymes. These enzymes partially hydrolyze these proteins into peptides and free amino 211 212 acids. These simple and soluble products are easier to digest than the whole protein (Bartkiene et al., 2013a; Bartkiene et al., 2013b; Aguirre et al., 2014). Fermentation of soybean meal 213 214 with L. plantarum or Bifidobacterium lactis showed a significant increase in the total amino 215 acids and a low immunoreactivity. Thus, the use of fermentation to reduce or eliminate allergenicity of soy products represents an interesting opportunity to produce hypoallergenic 216

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5. Lactic acid bacteria as biopreservation agent against pathogenic bacteria

food products from legumes (Song et al., 2008a; Song et al., 2008b).

Besides decreasing antinutritional factors (ANFs) and allergy, lactic acid bacteria can fulfill a task of biopreservation. This word can be defined as the extension of shelf-life and food safety by the use of natural or controlled microbiota and/or their antimicrobial compounds (Ananou, Maqueda et al. 2007).

Fermentation by lactic acid bacteria (LAB) is one of the most common methods of food biopreservation. In this process, organoleptic properties (unique flavor, aroma compounds) of products are also developed and improved. This is an important advantage of fermentation compared to non-fermented products that have to be biopreserved without any organoleptic impact. In South-East Asia, biopreservation strategies have been designed to avoid pathogens contamination in various products, although fermented meat as well as vegetables other than legumes, two classes being often considered as the riskiest products. For instance, (Phan, Tang et al. 2017) determined the diversity of LAB in 25 samples of meat and vegetable fermented products in Vietnam including dua gia (bean sprouts), dua cai (cabbage), mang chua (bamboo shoots), nem chua (uncooked pork) and tre (cooked pork). The results showed that the major species found in vegetable-based products (L. plantarum, L. fermentum, and L. helveticus) were different from those identified in meat-based products (P. pentosaceus, Weissella cibaria, and Lactococcus lactis) (Phan, Tang et al. 2017). The LAB ranged approximately from 10^6 to 10^9 CFU/g, depending on the food items (Phan, Tang et al. 2017). In the case of legumes, it is important to use bacteria that can develop easily on these plant matrices rich in proteins but also to take into account the specificities of contaminations. From the field, legumes are likely to bring with them sporulating bacteria. Later, fungi can develop and produce mycotoxins. Finally, depending on the handling of the products, pathogens can occur through cross-contamination from meat or vegetables. The range of possible contaminants is thus rather wide. The inhibitory mechanisms of biopreservation by LAB against spoilage organisms are destabilization of cell membrane and subsequent interference with the proton gradient, inhibition enzyme activity and creation of reactive oxygen species. LAB strains are able to produce various antimicrobial substances such as low-molecular weight metabolites (reuterin, reutericyclin, diacetyl, fatty acids), hydrogen peroxide,

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antifungal compounds (propionate, phenyl-lactate, hydroxyphenyl-lactate and 3-hydroxy fatty 250 251 acids), and bacteriocins that may be exploited in the biopreservation of foods (Siedler, Balti et al. 2019). 252 253 There is a wide number of bacteriocins produced by LAB and they can be classified into three classes. Class I (Lantibiotics), class II (Non-Lantibiotics) and class III (Big peptides) 254 depending on their chemical and genetic characteristics. The antibacterial activity of nisin, the 255 most studied lantibiotics, has been demonstrated against Listeria sp., Micrococcus sp. and 256 257 sporulating bacteria such as *Bacillus* sp. and *Clostridium* sp. (Parada, Caron et al. 2007). Nguyen et al. (Nguyen, Elegado et al. 2009) isolated the LAB from nem chua and determined 258 259 their antimicrobial activity against pathogenic and sporulating strains such as *Bacillus cereus*, L. plantarum JCM 1149, Listeria monocytogenes, E. coli, and Salmonella Typhimurium. Five 260 strains NH3.6, NT1.3, NT1.6, NT2.9, and NT3.20 showed a broaden antimicrobial activity 261 against both pathogenic Gram-positive B. cereus and L. monocytogenes, and gram-negative E. 262 coli with the zone of inhibition ranging from 1,5-6 mm (D-d), however no inhibitory effect 263 264 against S. Typhimurium was detected (Nguyen, Elegado et al. 2009). L. plantarum HA2, HA3, HA5, HA8 and HA9 and L. fermentum HA6, HA7 and HA10 isolated from Vietnamese 265 fermented vegetables showed a strong and broad antifungal activity against 7 out of 11 266 indicator mold and yeast strains (Aspergillus terreus, A. fumigatus, A. niger, Absidia 267 corymbifera, Paecilomyces lilacinus, Geotrichum candidum, Fusarium sp., Scopulariopsis 268 brevicaulis, Curvularia lunata, Penicillium sp., and Candida albicans) (Ho, Luo et al. 2009). 269 From these two collections, biopreservatives LAB could be investigated for the specific 270 problems of legume fermented products. 271

6. Biogenic amines, fermentation may be the risk, but also the solution

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Microbial fermentation has been employed for millennia by mankind to prolong shelf life and improve texture and flavour of food stuffs. However, protein-rich raw materials can be a

source of aminoacids which can be converted into biogenic amines (BAs) by microorganisms. Biogenic amines are nitrogenous compounds of low molecular weight occurring in several fermented foods. The main BAs present in foods are histamine, tyramine, tryptamine, putrescine, cadaverine, and phenylethylamine (Gardini et al., 2016). Their accumulation is mainly due to microbial decarboxylation of aminoacids through the action of decarboxylases which remove the carboxyl group with the formation of the corresponding amine and CO₂, or through the amination and transamination of aldehydes and ketones (Alvarez et al., 2014). These compounds can cause toxic effects in consumers with several symptoms such as nausea, flushes, heart palpitation, headache, red rash, oral burning, and hypo- or hypertension which are more severe in consumers with an inefficient detoxification system and depend on the amount and type of BA ingested (Ladero et al., 2010). Biogenic amines are produced not only by Gram-positive and Gram-negative bacteria, but also by yeasts and molds (Gardini et al., 2016). However, lactic acid bacteria (LAB) are considered as the main BA producers in fermented foods and Enterococcus, Lactobacillus, Streptococcus, Lactococcus Oenococcus, Pediococcus, Weissella, Carnobacterium, Tetragenococcus, Leuconostoc, Sporolactobacillus are the main genera showing this trait (Barbieri et al., 2019). BAs production is a strain specific feature and some studies revealed that the involved enzyme is encoded by unstable plasmids (Lucas et al., 2005; Satomi et al., 2008). Therefore, horizontal gene transfer is essential to disseminate this ability in LAB (Lucas et al., 2005; Satomi et al., 2008). Although the factors influencing BAs production (e.g. temperature, salt concentration, and pH) are well known, it is difficult to prevent their accumulation in fermented foods, since the fermentation conditions cannot be easily modified and the aminobiogenic ability is straindependent. Several strategies have been proposed to prevent BAs accumulation in foods; however, they can decrease the nutritional value and the organoleptic features of foods (for a

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review see Naila et al., 2010). In the case of legumes, the boiling allows the elimination of BAs, unfortunately, for sprouted legumes, this approach permits only a reduction of their content (Shalaby, 2000). One of the most promising strategies is the use of amine oxidizing starter cultures. Three different classes of oxidases have been described: flavin-containing monoamine oxidases (FlavAO), copper containing amine oxidases (CuAO), and multi-copper oxidases (MCO). They catalyze the oxidative deamination of BAs forming the corresponding aldehydes, hydrogen peroxide and ammonia (Guarcello et al., 2016). Kim et al. (2012) isolated strains of Bacillus subtilis and Bacillus amyloliquefaciens from fermented soybean foods. They observed the ability of B. subtilis to degrade putrescine and cadaverine and of B. amyloliquefaciens to oxidize histamine and tyramine. Similarly, Kang et al. (2017) showed the ability of B. subtilis and B. amyloliquefaciens strains to reduce tyramine in Cheonggukjang. Eom et al. (2015) isolated from buckwheat sokseongjang, a Korean traditional fermented soybean food, 3 strains (B. subtilis HJ0-6, B. subtilis D'J53-4, and B. idriensis RD13-10) which were not only able to degrade histamine and tyramine but also unable to produce them. Finally, Lee et al. (2016) proposed the use of L. plantarum strains to reduce BAs content during Miso fermentation. The possibility to select new amine oxidizing starter cultures could represent a promising strategy to reduce BAs content in legumes based fermented foods where traditional methods are inadequate or it is necessary to eliminate BAs already formed during the fermentation process.

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7. Mycotoxins in leguminous: can fermentation degrade what fungi have made?

Fermentation is the chemical process which converts substrates into product(s) by using microorganisms for example fungi, yeast and bacteria. There are many undesirable substances contaminating foods and feeds that are harmful to human and animal health. These include

mycotoxins, which are widely present in food and feeds commodities. Microorganisms including fungi, yeast, and bacteria are able to eliminate mycotoxins. Additionally, various studies have been extensively reporting about their mycotoxin degradation mechanisms such as cell wall-binding, enzyme degrading or structure modification. However, these mechanisms of actions depend on strains of microorganisms. (El-Sharkawy & Abul-Hajj, 1988; Kakeya et al., 2002; Takahashi-Ando et al., 2002; Vekiru et al., 2010; El-Nezami et al., 2002; Zhao et al., 2015). Lactic acid bacteria (LAB) can be extensively found in various fermented foods (meat, vegetable, and milk). They are widely used to ferment and preserve foods and feeds in place of chemical preservatives due to their lower cost and better safety. They can produce antimicrobial and antagonistic compounds for the control of pathogenic bacteria/fungi and spoilage microflora. Additionally, the LAB are widely applied to reduce mycotoxins. The capability of LAB to remove mycotoxin has been reported more than two decades ago for several toxins such as aflatoxin B₁ (Haskard et al., 2000; El-Nezami et al., 2002; Lahtinen et al., 2004; Taheur et al., 2017), zearalenone (El-Nezami et al., 2004; Adunphatcharaphon et al., 2013; Long et al., 2012), ochratoxin A (Fuchs et al., 2008), fumonisins (Niderkorn et al., 2009), and patulin (Fuchs et al., 2008; Wang et al., 2015). Numerous LAB (Lactobacillus rhamnosus, L. plantarum, L. casei, L. curvatus, L. coryniformis, L. brevis, L. mucosae, and L. pentosus) exhibit mycotoxin reduction ability in which binding is their main mechanism. (El-Nezami et al., 2002; El-Nezami et al., 2004; Long et al., 2012). The cell wall of LAB has the typical Gram-positive structure made of a complex assemblage of glycopolymers and proteins. It consists in a thick peptidoglycan that surrounds the cytoplasmic membrane and is decorated with proteins, polysaccharides, and teichoic acids (Chapot-Chartier et al., 2014; Delcour et al., 1999). The sugars and amino acids in glycopolymer or protein structures onto bacterial cell wall is different in each species of bacterial cell (Chapot-Chartier et al., 2014). Moreover, polysaccharides have been suggested

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to be the important elements responsible for the binding of aflatoxin B₁ (Haskard et al., 2000) and fumonisins (Niderkorn et al., 2009) by LAB. Polysaccharides in Gram-positive bacteria can be found in 3 parts: peptidoglycan, teichoic acids, and natural polysaccharides which include exopolysaccharides, capsular polysaccharides, and cell wall polysaccharides. Bacterial polysaccharides exhibit great diversity, not only in sugar composition but also in linkage, branching, and substitution (Chapot-Chartier et al., 2014). This means that peptidoglycan and teichoic acids are also related to mycotoxin reduction and the difference in mycotoxin removal capacity in each bacterial strain arises from the diversity of polysaccharides. Hernandez-Mendoza et al. (2009) exhibited that the protoplast of Lactobacillus reuteri and L. casei Shirota also showed the capacity to reduce aflatoxin B₁ in vitro. Both data implied that lipids were one of the components which were able to attach to the mycotoxin. All of these studies supported that the mycotoxin removal by bacterial cell was strain specific because of the diversity of each element structures onto bacterial cell wall, which includes proteins, polysaccharides, and lipids. Therefore, this class of microorganism has demonstrated great potential in fungal inhibition and mycotoxin removal in fermented legumes food products.

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8. Fermentation against chemicals from agriculture

The presence of chemicals and pesticides in plant products is a growing societal concern. Some studies point out that following recommendation to increase the dietary uptake of fruit and vegetables means often increasing the uptake of chemicals. For instance in Brasil, the cumulative intake of organophosphorus and carbamate pesticides by high consumers of fruits and vegetables has been evaluated to represent up to 169% of the Acute reference dose (ARfD) (Caldas and Jardim 2011). This type of data gives rise to a debate whether benefits of eating vegetarian products are higher than risks (Richter ED and N. 2002). In any case, there

is a consensus that the level of contamination by pesticide has to be significantly decreased. However, despite several Integrated Pest Management (IPM) programs, decreasing the use of pesticides in agriculture and therefore in food products is difficult. One transient possibility (before an agricultural control of persistent organic pollutants) is to degrade pesticides or chemical degradation products through fermentation. Considering the diversity of the earth microbiome, many chemicals can be transformed. However, when limiting the diversity to the safe bacteria that can be encountered in fermented products, the choice of biocatalytic activities is narrowed. Degradation of pesticides during fermentation has already been analyzed in several studies showing a good potential of degradation of organophosphorus insecticides like chlorpyrifos during the fermentation of Kimchi by strains belonging to L. mesenteroides, L. brevis, L. plantarum, and L. sakei (Cho et al., 2009). Lactobacillus brevis was also seen as an active catalyst against the same family of products during the fermentation of milk products (Zhang et al., 2014). The degradation of organochlorine pesticides has also been investigated in milk during yogurt and cheese production showing the effect of starters (Duan et al., 2018). Other examples show the capability of Micrococcus varians to degrade DDT to DDD and lindane to 2,4-, 2,5-, 2,6- and 2,3,4-2,3,5-trichlorophenol; 3,4-dichlorophenol; and hexachlorobenzene; and pentachlorophenol. However in sausage, the degradation was only of 10 to 18% during the 72 h of sausage fermentation (Abou-Arab, 2002). With the use of soy from the world market (mainly glyphosate resistant genetically-modified soy) to manufacture fermented soy in South-East Asia, it is likely that glyphosate is a main herbicide that has to be degraded although it can be combined with several other chemicals. The screening of strains able to degrade pesticides from fermented food is thus a potential way to decrease the contamination of foods by chemicals. Up to now, this subject of bioremediation of pesticides has been more focused on environmental microbiomes (Zhan et

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al., 2018) and the screening can also be extended to environmental lactic acid bacteria strains as in the following example for which *Lactococcus lactis* subsp. *lactis* able to degrade dinitrotoluene isomers has been isolated from earthworm intestine (Shin et al., 2005).

9. Conclusion

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Lactic acid bacteria are more and more used as a biopreservation agent against the development of pathogens and spoilage microorganisms in food products. They have also a potential to metabolize many compounds that are present in legumes and should be avoided in food products (Figure 1). They can thus decrease antinutritional factors and allergens as well as chemicals and pesticides used for culture. They can eventually remediate the presence of toxins from other microorganisms like mycotoxins and can oxidize biogenic amines. Although they already fulfill these roles in many fermented products, their role for bioremediation is still underutilized due to a lack of knowledge about precise genes and mechanisms involved. The screening of LAB functions helping to decrease the concentration of all these potentially toxic molecules is thus of great potential to improve food safety of products, not only fermented. Some questions remain, as for instance how to orient metabolism towards the targeted compounds? Can we find, in LAB diversity, strains able to degrade all types of compounds? Is the metabolization of toxic compounds sufficient to decrease significantly the risk? Although decreasing contamination is the first food safety strategy, lactic acid bacteria show a great potential through bioremediation functionalities that can help to decrease consumers' exposition to many toxic compounds. Some strains have already been identified in fermented foods but the development of starters with these functionalities has still to be developed.

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Acknowledgements: This work was funded by the Erasmus+ AsiFood «Universities as key partners for the new challenges regarding food safety and quality in ASEAN» project, the

- Bualuang ASEAN chair professorship of Thammasat University and the French Embassy in
- 426 Thailand. The author are thankful to Dr Giorgia Perpetuini for her active participation to the
- 427 redaction of this text from bibliography on biogenic amines to the editing of the final
- manuscript, and to Dr Guillaume Da for his precious help to establish the network.

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