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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-02394820v1

Submitted on 20 Jul2022

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Version of Record: https://www.sciencedirect.com/science/article/pii/S0956713519305468 Manuscript_8f307cd0fe2ba90308589b706e28d141

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

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- 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 inihibit 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.

41	Key words: legumes,	lactic acid bacteria,	phytates,	biogenic	amines,	mycotoxins,	food	safety
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50 **1. Introduction**

51 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 52 micronutrients, they have a positive impact on the environment but on the other hand, they 53 might contain non-neglectable amounts of antinutritional factors (ANFs), toxic chemicals and 54 toxins. For their culture, agroindustry promotes the facilitated culture of GMO while scientific 55 studies reveal that the use of pesticide-resisting varieties results in many risks, including the 56 increase in the level of pesticide in culture, in the soil and, ultimately, in the food (Bonny 57 2016; Tsatsakis, Nawaz et al. 2017)). Some of these issues might find solutions in the future 58 59 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 60 time, especially in South-East Asia where farmers are often not enough trained and have 61 62 economic limitations to implement the required changes in culture practices. Microorganisms functionalities in fermented foods can bring several advantages to consumers (Tamang et al., 63 64 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 65 present work with a special focus on legumes. 66

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68 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 75 76 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 -77 Kh), fermented soybean curd (Chao - Vn), Tauxi - Ha Giang Dau Xi - Vn), alkaline 78 fermentation of soybeans (natto-like) (Thua Nao – Th). Fermentation of these beans can have 79 an important nutrient and health impact (Cao et al. 2019; Jayachandran & Xu, 2019). 80 However, production of traditional fermentation products in South-East Asia is now managed 81 by experience, produced mainly by manual households, with rudimentary production 82 equipment, mostly fermented by endogenous microorganisms. Raw materials as well as 83 84 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 85 umami taste to the meals (Hajeb & Jinap, 2015), production management and quality 86 87 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 88 89 et al., 2020) and soybeans, as a protein-rich matrice, are exposed to a biogenic amine risk 90 (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
the vietnamese *tuong* and in the cambodian *sieng*.

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

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 100 lectins (Chompreeda & Field, 1984a; Reddy & Pierson, 1994). Most of the analyzed 101 fermented soy derivatives contain very small amount of trypsin inhibitors and isoflavones, 102 compared to the raw soybean, but still contain phytates (Anderson & Wolf, 1995). However, 103 it was not clear if microorganisms, including lactic acid bacteria (LAB) and yeasts, played an 104 important role and even less what was their way of action. Hence, controlled 105 lactofermentations were carried out. For instance, Sindhu and Khetarpaul (2001) 106 107 demonstrated that LAB (Lactobacillus casei and Lactobacillus plantarum) can reduce phytates, polyphenols and trypsin inhibitors in a food mixture containing green grams, with a 108 higher effect when inoculated after a first fermentation with yeasts (Saccharomyces 109 *boulardii*). However, LAB did not seem to have any effect on agglutinins (also called lectins) 110 (Ayyagari et al., 1989; Barkholt et al., 1998; Motarjemi & Nout, 1996). Accurate 111 112 characterizations of LAB activities are underway with the objective to develop appropriate starters and processes, particularly for tannins and phytates removing. 113

114 Metabolism of tannins or other polyphenols by LAB has been characterized only in a few 115 plant fermentations including tempeh (Starzyńska-Janiszewska et al., 2014) and sorghum (Svensson et al., 2010). Among LAB, L. plantarum, Lactobacillus paraplantarum, 116 Lactobacillus pentosus seem to be the only species capable of degrading hydrolysable tannins 117 through a tannase activity (tannin acyl hydrolase, EC 3.1.1.20) which hydrolyzes ester bounds 118 of tannic acid, thus releasing glucose and gallic acid (Osawa et al., 2000; Vaquero et al., 2004; 119 Rodriguez et al., 2009). Most of tannase producers were found in fermented vegetables but 120 also in human feces. In L. plantarum, tannase is very well characterized. Its activity was 121 demonstrated and characterized by Rodríguez et al. (2008) and genetic analysis showed it 122 constitutes a novel family of tannases (Iwamoto et al., 2008). LAB tannases are intracellular. 123 A L. plantarum strain has been reported to produce a very efficient tannase during anaerobic 124

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 130 (soaking, heating, milling ...) and on the plant species (Gibson et al., 2006). Soaking and 131 germination can activate plant endogenous phytases in most cereals and legumes (Holzapfel, 132 1997). The role of endogenous plant phytases during fermentation is still under debate. For 133 134 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 135 decrease and thus optimize the pH for plant phytases that are more active between pH 5 and 136 137 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 138 139 accessible for phytases (Maenz et al., 1999). On the contrary, other authors claim that phytase 140 activity is very variable depending on the plant species, thus plant phytase activities would not be sufficient in most fermentations (Cossa et al., 2000). 141

Phytases have been found in yeasts, fungi and bacteria. Generally, they are intracellular. 142 Bacterial phytases have been detected in various ubiquitous genera such as Bacillus, 143 Pseudomonas, Klebsiella, Enterobacter, Escherichia and in anaerobic ruminal bacteria 144 (Konietzny & Greiner, 2002). A few LAB strains have been shown to degrade, at least 145 partially, phytates. The strains belonged to Pediococcus pentosaseus in millet and soya 146 (Raghavendra et al., 2011), Leuconostoc mesenteroides, L. plantarum, Lactobacillus 147 acidophilus and P. pentosaseus in wheat (Lopez et al., 2000; Cizeikiene et al., 2015), some 148 Lactobacillus species in soy milk (Tang et al., 2010), L. acidophilus, Lactobacillus buchneri 149

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) 152 153 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 154 millet fermented gruel. According to these two works, one can expect about 20% of phytases 155 producers among LAB isolated from plant fermentation, with the highest activities for some 156 L. plantarum, Enterococcus faecium and Lactobacillus fermentum strains. These studies 157 constitute preliminary reports that have to be strengthened. Indeed, another study has 158 159 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 160 degrade sodium phytates (Raghavendra et al., 2009). In addition, Fredrikson et al. (2002) 161 162 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 163 164 considered as non- or low- phytase producers because the screening conditions do not permit phytase production in bacteria (Konietzny & Greiner, 2002). 165

Some studies have been carried out with tighter methods. Sreeramulu et al. (1996) have 166 reported that Lactobacillus amylovorus was a good producer of extracellular phytases and that 167 extracellular activity was also detected for L. acidophilus, L. plantarum, Lactobacillus 168 delbrueckii and L. casei. They did not detect any production with Lactococcus lactis or 169 Lactobacillus helveticus. Moreover, Zamudio et al. (2001) have reported the ability of six 170 strains of LAB (P. pentosaceus, Leuconostoc mesenteroides, L. casei, L. fermentum, L. 171 delbrueckii and L. plantarum) to partially degrade phytate (from 6 to 11%). Whereas no 172 intracellular and low extracellular phytase activities were measured, they proved that phytate 173 degradation was due to a non-specific acid phosphatase. Palacios et al. (2005) have come to 174

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.

180 Several groups have identified and characterized efficient phytases in the *Bifidobacterium* 181 genera (Haros et al., 2007; Tamayo-Ramos et al., 2012; García-Mantrana et al., 2015) but this 182 genus does not fit with legumes fermentation mainly because it is strict anaerobic and 183 fastidious. To overcome this limitation, García-Mantrana et al. (2016) succeeded in 184 expressing active bifidobacterial phytases in *L. casei*. Nevertheless, in sourdough, the 185 bacterial activity was low, compared to the activated plant phytases.

Actually, identifying new LAB phytase activities based on phenotypical screening seems 186 187 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 188 189 phytases found by García-Mantrana et al. (2015) are very specific. Thus, molecular screening 190 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 191 specific or non-specific phytases exist in some LAB strains and if these phytases would be 192 efficient in reducing the phytate content in fermented legumes. 193

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195 **4. Decrease of protein allergenicity through LAB-mediated hydrolysis**

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;
Kroghsbo et al., 2014; Rahaman et al., 2016).

Plant proteins exhibits low digestibility compared to animal proteins. Poor protein
digestibility can cause gastrointestinal disorders that may result in fecal excretion of proteins.
Therefore, increasing protein digestibility could reduce the levels of undigested proteins
which can potentially cause food allergies due to poor absorption in the gut (Untersmayr &
Jensen-Jarolim, 2008). Several studies have showed that LAB fermentation increases the
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 food products from legumes (Song et al., 2008a; Song et al., 2008b). 217

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220 5. Lactic acid bacteria as biopreservation agent against pathogenic bacteria

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 230 contamination in various products, although fermented meat as well as vegetables other than 231 legumes, two classes being often considered as the riskiest products. For instance, (Phan, 232 Tang et al. 2017) determined the diversity of LAB in 25 samples of meat and vegetable 233 234 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 235 that the major species found in vegetable-based products (L. plantarum, L. fermentum, and L. 236 237 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 238 approximately from 10^6 to 10^9 CFU/g, depending on the food items (Phan, Tang et al. 2017). 239 240 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 241 the field, legumes are likely to bring with them sporulating bacteria. Later, fungi can develop 242 and produce mycotoxins. Finally, depending on the handling of the products, pathogens can 243 244 occur through cross-contamination from meat or vegetables. The range of possible contaminants is thus rather wide. The inhibitory mechanisms of biopreservation by LAB 245 246 against spoilage organisms are destabilization of cell membrane and subsequent interference with the proton gradient, inhibition enzyme activity and creation of reactive oxygen species. 247 LAB strains are able to produce various antimicrobial substances such as low-molecular 248 weight metabolites (reuterin, reutericyclin, diacetyl, fatty acids), hydrogen peroxide, 249

antifungal compounds (propionate, phenyl-lactate, hydroxyphenyl-lactate and 3-hydroxy fatty
acids), and bacteriocins that may be exploited in the biopreservation of foods (Siedler, Balti et
al. 2019).

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) depending on their chemical and genetic characteristics. The antibacterial activity of nisin, the most studied lantibiotics, has been demonstrated against *Listeria* sp., *Micrococcus* sp. and 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

273 Microbial fermentation has been employed for millennia by mankind to prolong shelf life and274 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. 275 276 Biogenic amines are nitrogenous compounds of low molecular weight occurring in several fermented foods. The main BAs present in foods are histamine, tyramine, tryptamine, 277 putrescine, cadaverine, and phenylethylamine (Gardini et al., 2016). Their accumulation is 278 mainly due to microbial decarboxylation of aminoacids through the action of decarboxylases 279 which remove the carboxyl group with the formation of the corresponding amine and CO₂, or 280 through the amination and transamination of aldehydes and ketones (Alvarez et al., 2014). 281 These compounds can cause toxic effects in consumers with several symptoms such as 282 nausea, flushes, heart palpitation, headache, red rash, oral burning, and hypo- or hypertension 283 284 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). 285

Biogenic amines are produced not only by Gram-positive and Gram-negative bacteria, but 286 287 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, 288 289 Streptococcus, Lactococcus Oenococcus, Pediococcus, Weissella, *Carnobacterium*, 290 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 291 that the involved enzyme is encoded by unstable plasmids (Lucas et al., 2005; Satomi et al., 292 2008). Therefore, horizontal gene transfer is essential to disseminate this ability in LAB 293 (Lucas et al., 2005; Satomi et al., 2008). 294

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

review see Naila et al., 2010). In the case of legumes, the boiling allows the elimination of 300 BAs, unfortunately, for sprouted legumes, this approach permits only a reduction of their 301 content (Shalaby, 2000). One of the most promising strategies is the use of amine oxidizing 302 303 starter cultures. Three different classes of oxidases have been described: flavin-containing monoamine oxidases (FlavAO), copper containing amine oxidases (CuAO), and multi-copper 304 oxidases (MCO). They catalyze the oxidative deamination of BAs forming the corresponding 305 aldehydes, hydrogen peroxide and ammonia (Guarcello et al., 2016). Kim et al. (2012) 306 307 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.* 308 309 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 310 Cheonggukjang. Eom et al. (2015) isolated from buckwheat sokseongjang, a Korean 311 312 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 313 314 unable to produce them. Finally, Lee et al. (2016) proposed the use of L. plantarum strains to 315 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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321 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 325 including fungi, yeast, and bacteria are able to eliminate mycotoxins. Additionally, various 326 studies have been extensively reporting about their mycotoxin degradation mechanisms such 327 as cell wall-binding, enzyme degrading or structure modification. However, these 328 mechanisms of actions depend on strains of microorganisms. (El-Sharkawy & Abul-Hajj, 329 1988; Kakeya et al., 2002; Takahashi-Ando et al., 2002; Vekiru et al., 2010; El-Nezami et al., 330 2002; Zhao et al., 2015). Lactic acid bacteria (LAB) can be extensively found in various 331 fermented foods (meat, vegetable, and milk). They are widely used to ferment and preserve 332 foods and feeds in place of chemical preservatives due to their lower cost and better safety. 333 334 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 335 mycotoxins. The capability of LAB to remove mycotoxin has been reported more than two 336 337 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; 338 339 Adunphatcharaphon et al., 2013; Long et al., 2012), ochratoxin A (Fuchs et al., 2008), 340 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. 341 coryniformis, L. brevis, L. mucosae, and L. pentosus) exhibit mycotoxin reduction ability in 342 which binding is their main mechanism. (El-Nezami et al., 2002; El-Nezami et al., 2004; 343 Long et al., 2012). The cell wall of LAB has the typical Gram-positive structure made of a 344 complex assemblage of glycopolymers and proteins. It consists in a thick peptidoglycan that 345 346 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 347 in glycopolymer or protein structures onto bacterial cell wall is different in each species of 348 bacterial cell (Chapot-Chartier et al., 2014). Moreover, polysaccharides have been suggested 349

to be the important elements responsible for the binding of aflatoxin B₁ (Haskard et al., 2000) 350 and fumonisins (Niderkorn et al., 2009) by LAB. Polysaccharides in Gram-positive bacteria 351 can be found in 3 parts: peptidoglycan, teichoic acids, and natural polysaccharides which 352 353 include exopolysaccharides, capsular polysaccharides, and cell wall polysaccharides. Bacterial polysaccharides exhibit great diversity, not only in sugar composition but also in 354 linkage, branching, and substitution (Chapot-Chartier et al., 2014). This means that 355 peptidoglycan and teichoic acids are also related to mycotoxin reduction and the difference in 356 357 mycotoxin removal capacity in each bacterial strain arises from the diversity of polysaccharides. Hernandez-Mendoza et al. (2009) exhibited that the protoplast of 358 359 Lactobacillus reuteri and L. casei Shirota also showed the capacity to reduce aflatoxin B_1 in vitro. Both data implied that lipids were one of the components which were able to attach to 360 the mycotoxin. All of these studies supported that the mycotoxin removal by bacterial cell 361 362 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 363 364 has demonstrated great potential in fungal inhibition and mycotoxin removal in fermented 365 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. 380 However, when limiting the diversity to the safe bacteria that can be encountered in fermented 381 products, the choice of biocatalytic activities is narrowed. Degradation of pesticides during 382 fermentation has already been analyzed in several studies showing a good potential of 383 384 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 385 al., 2009). Lactobacillus brevis was also seen as an active catalyst against the same family of 386 387 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 388 389 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 390 2,3,4-2,3,5-trichlorophenol; 391 3,4-dichlorophenol; and hexachlorobenzene; and pentachlorophenol. However in sausage, the degradation was only of 10 to 18% during the 72 392 h of sausage fermentation (Abou-Arab, 2002). 393

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

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).

403 9. Conclusion

Lactic acid bacteria are more and more used as a biopreservation agent against the 404 development of pathogens and spoilage microorganisms in food products. They have also a 405 potential to metabolize many compounds that are present in legumes and should be avoided in 406 407 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 408 409 toxins from other microorganisms like mycotoxins and can oxidize biogenic amines. Although they already fulfill these roles in many fermented products, their role for 410 bioremediation is still underutilized due to a lack of knowledge about precise genes and 411 412 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 413 414 products, not only fermented. Some questions remain, as for instance how to orient 415 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 416 decrease significantly the risk? 417

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.

422

423 Acknowledgements: This work was funded by the Erasmus+ AsiFood «Universities as key
424 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 Thailand. The author are thankful to Dr Giorgia Perpetuini for her active participation to the 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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