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

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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.

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

1. Introduction

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.

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

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 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

125 fermentation (Aguilar-Zárate at al., 2015). Genes involved in tannins degradation are
126 regulated in a coordinated way and are inducible by tannin and other phenolic compounds
127 (Reveron et al., 2017). Characterization of fermented cassava LAB allowed identifying
128 uncommon tannase producers such as *Weissella cibaria* and *Leuconostoc mesenteroides* ssp.
129 *mesenteroides* (Kostinek et al., 2007).
130 Degradation of phytates during food process is very inconstant, depending on the process
131 (soaking, heating, milling …) and on the plant species (Gibson et al., 2006). Soaking and
132 germination can activate plant endogenous phytases in most cereals and legumes (Holzapfel,
133 1997). The role of endogenous plant phytases during fermentation is still under debate. For
134 some authors, phytate reduction is rather the consequence of plant phytases activation during
135 fermentation than of microbial phytases (Reale et al., 2007; Shirai et al., 1994). Indeed, LAB
136 decrease and thus optimize the pH for plant phytases that are more active between pH 5 and
137 pH 4 (Gänzle et al., 2014). Moreover, production of organic acids by LAB could play a
138 synergic effect by complexing with minerals from phytates, thus making them more
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
141 be sufficient in most fermentations (Cossa et al., 2000).
142 Phytases have been found in yeasts, fungi and bacteria. Generally, they are intracellular.
143 Bacterial phytases have been detected in various ubiquitous genera such as *Bacillus*,
144 *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Escherichia* and in anaerobic ruminal bacteria
145 (Konietzny & Greiner, 2002). A few LAB strains have been shown to degrade, at least
146 partially, phytates. The strains belonged to *Pediococcus pentosaseus* in millet and soya
147 (Raghavendra et al., 2011), *Leuconostoc mesenteroides*, *L. plantarum, Lactobacillus*
148 *acidophilus* and *P. pentosaseus* in wheat (Lopez et al., 2000; Cizeikiene et al., 2015), some
149 *Lactobacillus* species in soy milk (Tang et al., 2010), *L. acidophilus*, *Lactobacillus buchneri*

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

175 the same conclusion by characterizing a *L. pentosus* non-specific acid phosphatase (EC
176 3.1.3.2) which was able to hydrolyze phytates. De Angelis et al. (2003) have gone further by
177 characterizing an intracellular phytase of *L. sanfranciscensis* at the protein level. They did not
178 identify the corresponding gene. This strain decreased phytate content of almost 75% in
179 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.
186 Actually, identifying new LAB phytase activities based on phenotypical screening seems
187 hazardous. Otherwise, *in silico* analysis of LAB genomes based on known bacterial phytases
188 (*Bacillus*, *Escherichia* spp) does not predict any phytase (personal data). Even bifidobacterial
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
191 corresponding genes in LAB strains already highlighted would be very informative to know if
192 specific or non-specific phytases exist in some LAB strains and if these phytases would be
193 efficient in reducing the phytate content in fermented legumes.
194
195 **4. Decrease of protein allergenicity through LAB-mediated hydrolysis**
196 The main legume allergens are the proteins. These complex structures are difficult to degrade.
197 The selection of legumes natural variants or the use of specific biotechnological processes can
198 eliminate these particular proteins. However, they might also induce some side effects such as
199 an increase in the protein synthesis pathways of the seed and the synthesis of other proteins

200 that might be allergenic as well (Nowak-Wegrzyn & Fiocchi, 2009 ; Mils et al., 2009 ;
201 Kroghsbo et al., 2014; Rahaman et al., 2016).
202 Plant proteins exhibits low digestibility compared to animal proteins. Poor protein
203 digestibility can cause gastrointestinal disorders that may result in fecal excretion of proteins.
204 Therefore, increasing protein digestibility could reduce the levels of undigested proteins
205 which can potentially cause food allergies due to poor absorption in the gut (Untersmayr &
206 Jensen-Jarolim, 2008). Several studies have showed that LAB fermentation increases the
207 digestibility of plant proteins (El-Haget al., 2002; Pranoto et al., 2013).
208 For example, during fermentation of lupin and soybean, protein digestibility is improved by
209 using LAB cultures such as *Lactobacillus sakei* KTU05-6, *Pediococcus acidilactici* KTU05-
210 07 and *P. pentosaceus* KTU05-8. This improvement is due to the role of microbial proteolytic
211 enzymes. These enzymes partially hydrolyze these proteins into peptides and free amino
212 acids. These simple and soluble products are easier to digest than the whole protein (Bartkiene
213 et al., 2013a; Bartkiene et al., 2013b; Aguirre et al., 2014). Fermentation of soybean meal
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
216 allergenicity of soy products represents an interesting opportunity to produce hypoallergenic
217 food products from legumes (Song et al., 2008a; Song et al., 2008b).
218
219
220 **5. Lactic acid bacteria as biopreservation agent against pathogenic bacteria**
221 Besides decreasing antinutritional factors (ANFs) and allergy, lactic acid bacteria can fulfill a
222 task of biopreservation. This word can be defined as the extension of shelf-life and food
223 safety by the use of natural or controlled microbiota and/or their antimicrobial compounds
224 (Ananou, Maqueda et al. 2007).

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

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 their antimicrobial activity against pathogenic and sporulating strains such as *Bacillus cereus*, *L. plantarum* JCM 1149, *Listeria monocytogenes, E. coli*, and *Salmonella* Typhimurium. Five strains NH3.6, NT1.3, NT1.6, NT2.9, and NT3.20 showed a broaden antimicrobial activity against both pathogenic Gram-positive *B. cereus* and *L. monocytogenes*, and gram-negative *E. coli* with the zone of inhibition ranging from 1,5-6 mm (D-d), however no inhibitory effect 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 fermented vegetables showed a strong and broad antifungal activity against 7 out of 11 indicator mold and yeast strains (*Aspergillus terreus, A. fumigatus, A. niger, Absidia corymbifera, Paecilomyces lilacinus, Geotrichum candidum, Fusarium* sp., *Scopulariopsis brevicaulis, Curvularia lunata, Penicillium sp.,* and *Candida albicans*) (Ho, Luo et al. 2009). From these two collections, biopreservatives LAB could be investigated for the specific problems of legume fermented products.

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

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 CO_2, 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 strain-dependent. 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

300 review see Naila et al., 2010). In the case of legumes, the boiling allows the elimination of
301 BAs, unfortunately, for sprouted legumes, this approach permits only a reduction of their
302 content (Shalaby, 2000). One of the most promising strategies is the use of amine oxidizing
303 starter cultures. Three different classes of oxidases have been described: flavin-containing
304 monoamine oxidases (FlavAO), copper containing amine oxidases (CuAO), and multi-copper
305 oxidases (MCO). They catalyze the oxidative deamination of BAs forming the corresponding
306 aldehydes, hydrogen peroxide and ammonia (Guarcello et al., 2016). Kim et al. (2012)
307 isolated strains of *Bacillus subtilis* and *Bacillus amyloliquefaciens* from fermented soybean
308 foods. They observed the ability of *B. subtilis* to degrade putrescine and cadaverine and of *B.*
309 *amyloliquefaciens* to oxidize histamine and tyramine. Similarly, Kang et al. (2017) showed
310 the ability of *B. subtilis* and *B. amyloliquefaciens* strains to reduce tyramine in
311 Cheonggukjang. Eom et al. (2015) isolated from buckwheat sokseongjang, a Korean
312 traditional fermented soybean food, 3 strains (*B. subtilis* HJ0-6, *B. subtilis* D'J53-4, and *B.*
313 *idriensis* RD13-10) which were not only able to degrade histamine and tyramine but also
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.
316 The possibility to select new amine oxidizing starter cultures could represent a promising
317 strategy to reduce BAs content in legumes based fermented foods where traditional methods
318 are inadequate or it is necessary to eliminate BAs already formed during the fermentation
319 process.
320

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

322 Fermentation is the chemical process which converts substrates into product(s) by using
323 microorganisms for example fungi, yeast and bacteria. There are many undesirable substances
324 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 B_1 (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

to be the important elements responsible for the binding of aflatoxin B_1 (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 B_1 *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.

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

375 is a consensus that the level of contamination by pesticide has to be significantly decreased.
376 However, despite several Integrated Pest Management (IPM) programs, decreasing the use of
377 pesticides in agriculture and therefore in food products is difficult. One transient possibility
378 (before an agricultural control of persistent organic pollutants) is to degrade pesticides or
379 chemical degradation products through fermentation.
380 Considering the diversity of the earth microbiome, many chemicals can be transformed.
381 However, when limiting the diversity to the safe bacteria that can be encountered in fermented
382 products, the choice of biocatalytic activities is narrowed. Degradation of pesticides during
383 fermentation has already been analyzed in several studies showing a good potential of
384 degradation of organophosphorus insecticides like chlorpyrifos during the fermentation of
385 Kimchi by strains belonging to *L. mesenteroides*, *L. brevis*, *L. plantarum*, and *L. sakei* (Cho et
386 al., 2009). *Lactobacillus brevis* was also seen as an active catalyst against the same family of
387 products during the fermentation of milk products (Zhang et al., 2014). The degradation of
388 organochlorine pesticides has also been investigated in milk during yogurt and cheese
389 production showing the effect of starters (Duan et al., 2018). Other examples show the
390 capability of *Micrococcus varians* to degrade DDT to DDD and lindane to 2,4-, 2,5-, 2,6- and
391 3,4-dichlorophenol; 2,3,4- and 2,3,5-trichlorophenol; hexachlorobenzene; and
392 pentachlorophenol. However in sausage, the degradation was only of 10 to 18% during the 72
393 h of sausage fermentation (Abou-Arab, 2002).
394 With the use of soy from the world market (mainly glyphosate resistant genetically-modified
395 soy) to manufacture fermented soy in South-East Asia, it is likely that glyphosate is a main
396 herbicide that has to be degraded although it can be combined with several other chemicals.
397 The screening of strains able to degrade pesticides from fermented food is thus a potential
398 way to decrease the contamination of foods by chemicals. Up to now, this subject of
399 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).

9. Conclusion

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.

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

425 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
428 manuscript, and to Dr Guillaume Da for his precious help to establish the network.
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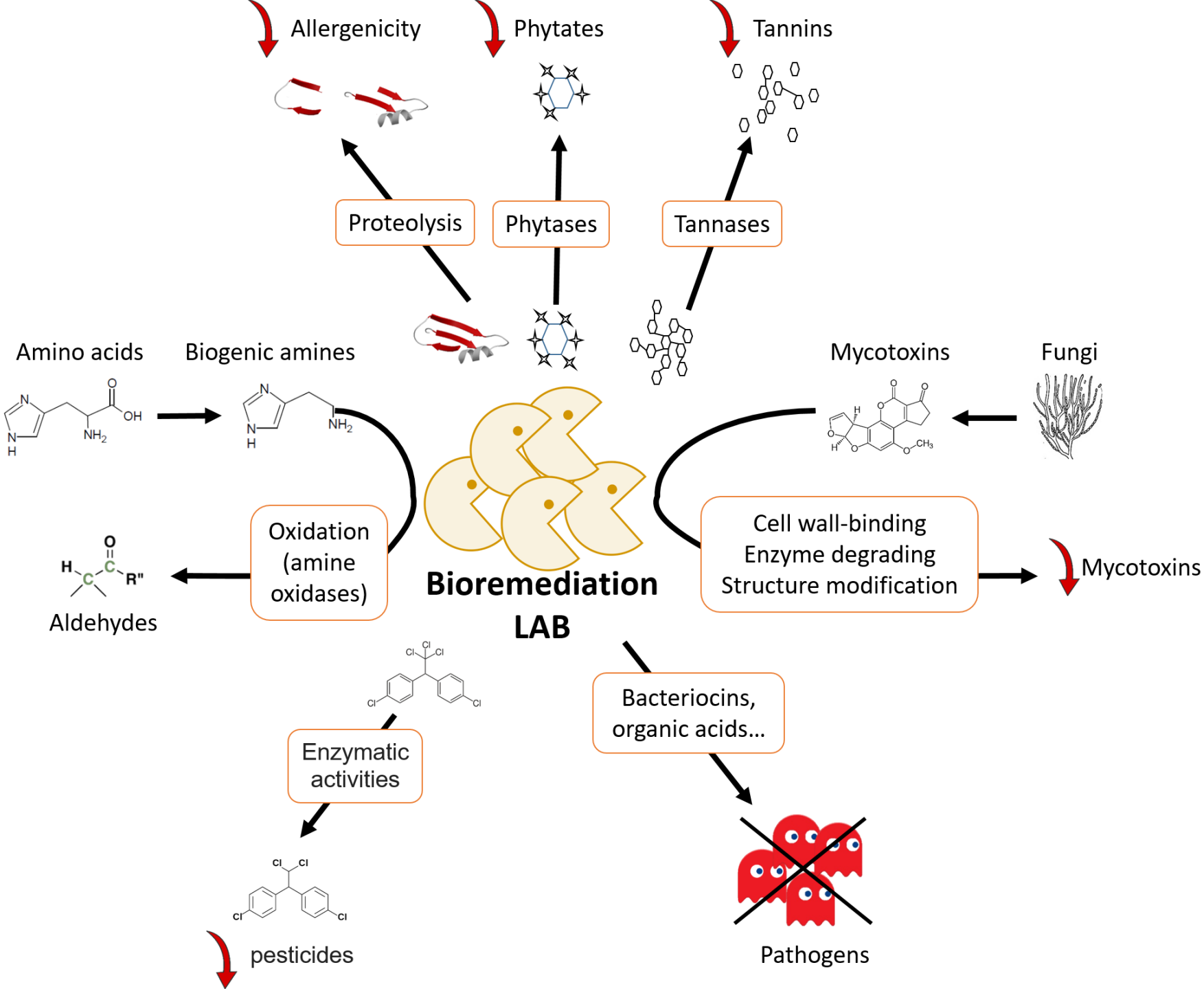
Allergenicity
Phytates
Tannins
Proteolysis
Phytases
Tannases
Amino acids
Biogenic amines
Mycotoxins
Fungi
Oxidation (amine oxidases)
Aldehydes
Bioremediation LAB
Cell wall-binding
Enzyme degrading
Structure modification
Mycotoxins
Enzymatic activities
pesticides
Bacteriocins, organic acids…
Pathogens