Microbial Detoxification of Residual Pesticides in Fermented Foods

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The treatment of agricultural areas with pesticides is an indispensable approach to improve crop yields and cannot be avoided in the future. At the same time, significant amounts of pesticides remain in food and their ingestion causes serious damage such as neurological, gastrointestinal, and allergic reactions; cancer; and even death. However, during the fermentation processing of foods, residual amounts of pesticides are significantly reduced thanks to enzymatic degradation by the starter and accompanying microflora.

pesticide food lactic acid bacteria

1. Microbial Detoxification of Fermented Foods Containing High Amounts of Pesticides

The scientific research related to microbial degradation of pesticide residues began in the 1940s when people began to pay more attention to environmental protection [1]. Biodegradation is the use of microorganisms or their enzymes to degrade and detoxify xenobiotics in food, water, and soil. The method is an efficient and inexpensive option to deal with pesticide pollution [2][3]. Ideally, the result should be complete mineralization/degradation of the pesticide to H_2O and CO_2 without the accumulation of more toxic intermediates [4]. Pesticides can be degraded metabolically by microorganisms. In catabolite degradation, microorganisms use pesticides as the main source of energy (as a carbon, nitrogen, or phosphorus source). Co-metabolic degradation occurs when they are not used as a primary energy source [5].

The reduction of pesticide residues during fermentation has been studied in various food products [5][6][7][8][9][10]. Microorganisms with the highest pesticide degradation activity belong to the genera *Bacillus, Micrococcus, Arthrobacter, Corynebacterium, Flavobacterium, Pseudomonas*, and *Rhodococcus*, as well as fungi from the genera *Penicillium, Aspergillus, Fusarium*, and *Trichoderma* [11][12][13]. Although fungal bioremediation of pesticides has significant potential, it has received less attention than bacterial bioremediation. However, most of the soil bacterial isolates are not applicable for food detoxification because of their pathogenic nature.

1.1. Milk and Yogurt

A number of comprehensive studies have shown that strains of different LAB species possess the natural ability to degrade pesticides in vitro and alleviate pesticide poisoning in vivo [14][15][16]. The first studies of the natural degradation of pesticides in dairy products due to the action of autochthonous microflora date back to the 1960s with the works of Kallman and Andrews [17], investigating organochlorine residues. They reported that the conversion of DDT by yeast occurs rather because of a pH decrease. Then, a true degradation of DDT (1 mg/L in milk and cheese) was shown by Abou-Arab [6], who observed the activity of starters containing *Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophilus*, and yeasts. The maximum reduction in total DDT of contaminated Ras cheese and milk was achieved after 8 days, 10 days, and 7 days for streptococci, lactobacilli, and yeasts, respectively [6].

Much later, *Latilactobacillus sakei* strain pro7 reached 95.1% biodegradation of DDT with a concentration of 20 mg/kg [18]. Duan et al. [19] proved that starter cultures (*L acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *Lp. plantarum*, *Lp. rhamnosus*, *Lc. casei*, *S. thermophilus,* and *Bif. bifidum*) decrease the concentration of five different OCPs during yogurt and cheese production. Witczak and Mituniewicz-Małek [20] demonstrated a significant reduction of the level of organochlorine pesticide residues after 14 days in cold storage by the addition of a probiotic mixture of *L. acidophilus* LA-5 and *Bif. animalis* subsp. *lactis* BB-12 to the yogurt starter cultures. The most significant was the reduction of heptachlor—by 36.6%.

Considering OPP reduction, Zhou and Zhao [21] showed the effectiveness of five different species of LAB for the elimination of organophosphorus pesticides in skimmed milk. Due to the activity of LAB, OPP concentrations decreased by 7.0–64.6%. All nine investigated compounds were most susceptible to *L. delbrueckii* subsp. *bulgaricus*, which increased their degradation rate constants by 18.3–133.3%. Zhang et al. [22] tested ten LAB strains and four combinations of strains for the degradation of five organophosuphate pesticides in skimmed milk. *Lev. brevis* 1.0209 was found to possess the highest pesticide degradation activity. Similar results were obtained by Zhao and Wang [23], reporting a strong acceleration of OPP degradation in skimmed milk by *L. delbrueckii* subsp. *bulgaricus*, *Lc. paracasei*, and *Lp. plantarum*. They added seven organophosphate pesticides to milk samples and 24 h later observed reduced pesticide concentrations by 20.9% (of methyl parathion/methyl parathion incubated with *Lc. paracasei*), and by 46.9% (of malathion/malathion incubated with *Lp. plantarum*). The greatest degradation activity was observed by the use of *L. delbrueckii* subsp. *bulgaricus* and *L. plantarum*.

1.2. Pickled Vegetables

OPP residual concentrations are, as a rule, high in vegetables. Therefore, pesticide-contaminated fermented vegetable products such as kimchi, sauerkraut, olives, and pickles are correspondingly numerous.

The role of lactic acid bacteria in the degradation of chlorpyrifos during *kimchi* fermentation was profoundly investigated [24][25]. Chlorpyrifos was rapidly decreased by day 3 of the fermentation (83.3%), and completely degraded by day 9 [24]. Four species of lactic acid bacteria were isolated and identified as the cause: *Leuconostoc mesenteroides* WCP907, *Lev. brevis* WCP902, *Lp. plantarum* WCP931, and *La. sakei* WCP904. It was found that chlorpyrifos could be used by these four strains as the sole source of carbon and phosphorus [25].

Zhou et al. [26] reported the ability of *Lp. plantarum* to degrade four organophosphate pesticides, including chlorpyrifos, dichlorvos, phorate, and trichlorphon, in sauerkraut and *Mao-tofu*. The results showed that about

16.6–31.8%, 96.2–99.7%, and 79.7–99.5% of the OPPs were degraded after 5 h, 42 h, and 6 days, respectively.

Kumral et al. [27] investigated the degradation potential of two strains of *Lp. plantarum* (LB-1 and LB-2) isolated from fermented black olive brine to eliminate chlorpyrifos (OPP) and deltamethrin (a pyrethroid). LB-1 and LB-2 degraded 96% and 90% of chlorpyrifos and 24% and 53% of deltamethrin in three days, respectively. Maden and Kumral [28] successfully used *Lp. plantarum* 112 (previously isolated from the olive brine) for sauerkraut detoxication from malathion (2 mg/kg) and chlorpyrifos-methyl (4 mg/kg). *Lp. plantarum* strain 123 was efficient in pesticide removal during black olive fermentation, although the process of degradation was relatively slow. At the end of fermentation (after 60 days), 61% deltamethrin, 68% dimethoate, and 50% imidacloprid were removed by the strain [29].

1.3. Grains, Flours, and Sourdough

The amylolytic LAB species *Lp. plantarum* is indispensable for reducing pesticide levels in flours, sourdoughs, and silages. Zhang et al. [30] applied *Lp. plantarum* 1.0315, *Lp. plantarum* 1.0624, *Lp. plantarum* 1.0622, and their combination at room temperature for 10 weeks to detoxify corn silage from chlorpyrifos and phorate (0.36 mg/kg). The level of phorate reduction in the treated samples was between 24.9% and 33.4%, depending on the strain. The use of a combination of the three *Lp. plantarum* strains was found to be a more effective strategy in the degradation of OPPs than the use of single strains. Đorđević et al. [31] monitored the degradation of pirimiphos/pirimiphos-methyl by *Lp. plantarum* during wheat fermentation and observed 81% total OPP degradation without any influence on bacterial growth or fermentation activity. Low et al. [32] demonstrated that *Saccharomyces cerevisiae* can degrade glyphosate during bread fermentation, with 21% of the pesticide being degraded within 1 h. Engaging the same yeast species, Sharma et al. [33] reported dissipation of endosulfan (70%), deltamethrin (63%), malathion (60%), propiconazole (52%), chlorpyriphos (51%), and hexaconazole (46%) during dough fermentation. However, the role of the starter *Saccharomyces cerevisiae* in the process of detoxication was not elucidated.

An important success was achieved by Đorđević et al. [34] who revealed the possibility to decrease pesticide levels during the fermentation of wheat by yeasts and lactobacilli. When the amounts of pesticides were 15 times above MRL, the degradation rate constants increased by 594% for pirimiphos methyl in the presence of *Saccharomyces cerevisiae*, and 469% for chlorpyrifos due to lactic acid fermentation by *Lp. plantarum*.

1.4. Tea, Wine, and Fruit Juices

The only report concerning microbial detoxication of tea is that of Deng et al. [35]. The study proved the degrading ability of the *Aspergillus niger* strain YAT in Chinese brick tea. The strain could degrade 54.83% of β-cypermethrin in 7 days and 100% of 3-phenoxybenzoic acid in 22 h during tea fermentation. These results indicate that the *A. niger* YAT strain has great potential for bioremediation of pyrethroid insecticides in fermented foods. However, due to safety reasons, this is hardly possible.

Multiple pesticide residues were found in grapes and wines, such as fungicides boscalid, penconazole, pyrimethanil, fenhexamid, and iprovalicarb [36]. Actually, 79 different pesticides could be detected in grapes [37], most of them hindering the proper fermentation process of wine and irreversibly changing its aroma [38].

LAB species from the genera *Lactobacillus*, *Leuconostoc*, and *Pediococcus* were found to detoxicate red wine. *Oenococcus oeni*, the most promising species, was able to significantly reduce the concentrations of chlorpyrifos, dicofol, chlorothalonil, and procymidone by 70, 40, 35, and 25%, respectively [39]. Another study by González-Rodríguez et al. [40] reported about 86% decrease in tebuconazole during coupled fermentation of red wine by *Saccharomyces cerevisiae* and *O. oeni*.

Rezaei et al. [41] investigated the ability of probiotic *L. acidophilus* to detoxify apple juice from diazinon (1–5 mg/L). The strain efficiently reduced the pesticide concentration after 72 h and eliminated all traces of it after 28 days of cold storage.

1.5. Meat and Sausages

Abou-Arab et al. [42] investigated the effect of starter cultures on the degradation of DDT and lindane during the fermentation of meat products and sausages. When *Lp. plantarum* and *Micrococcus varians* were used as a starter culture and the meat fermentation was prolonged for 72 h, the DDT amount was reduced by 10%, and lindane by 18%.

2. Molecular Mechanisms of Pesticides Degradation

Many microbial species involved in food fermentation can metabolize a broad spectrum of pesticides and use them as carbon and energy sources [43]. The major enzymes involved in pesticide degradation belong to the group of phosphoric monoester hydrolases (EC 3.1.3), such as alkaline phosphatase (EC 3.1.3.1), acid phosphatase (EC 3.1.3.2), and phosphoric triester hydrolases (EC 3.1.8), for example, organophosphate hydrolase (OPH, EC 3.1.8.1) and organophosphorus acid anhydrolase (OPAA, EC 3.1.8.2) [44][45]. OPH is a Zn-containing enzyme most effective in hydrolyzing P–O bonds, to a lesser extent also P–F, P–CN, and (least of all) P–S, while OPAA shows 90% similarity with OPH but differs in substrate specificity, being unable to hydrolyze P–S bonds at all, for instance [46]. Carbamates and pyrethroids share with OPP an ester bond in their structure that can be hydrolyzed by esterases. At least 30 esterases with confirmed pesticide-degrading activity have been isolated from plants, animals, and bacteria, but very few of them belong to LAB species traditionally involved in the preparation of fermented foods [47]. Different OPH are encoded by *opd* genes (from organophosphate degrading), while for OPAA synthesis are responsible *opaA* genes [4].

The initial and most important step in the degradation of organophosphate pesticides (OPP) is the hydrolysis of the phosphoesteric (P–O–C) or phosphothiesteric (P–S–C) bond. Three of the most common OPPs, parathion, diazinon, and chlorpyrifos, all share a common P–O–C bond, which is hydrolyzed to diethylthiophosphoric acid

(DETP); *p*-nitrophenol, 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP) and 3,5,6-trichloro-2-pyridinol (TCP), respectively [46].

Dialkylphosphate (DAP) metabolites, a group of OPP metabolic products to which DETP belongs, have been associated with increased exposure in recent decades and various neurological pathologies, including impaired intellectual development and attention-deficit disorders in children [48][49][50]. TCP and DETP are toxic and persistent in nature. TCP has been linked with several harmful effects, including reduced testosterone levels in men [51], while DETP has been shown to have a negative influence on sex hormones in women [52]. TCP is known to have an anti-microbial activity that inhibits the growth of chlorpyrifos-degrading microorganisms. Soil bacteria such as *Pseudomonas* and *Enterobacter* can use TCP and DETP as sole sources of carbon, phosphorus, and energy [4][53]. The metabolic fate of TCP and DETP is poorly understood in probiotic strains. Among the relatively few bacteria able to mineralize TCP and DETP, as of yet, none have been confirmed in fermented foods.

Relatively few OPP-degrading enzymes in probiotic bacteria have been studied so far in some detail. Five different OP hydrolases (OpdB, OpdD, OpdA, OpdE, OpdC) from four different LAB species (*Lev. brevis*, *La. sakei*, *Leuc. mesenteroides*, *Lp. plantarum*) have been isolated from kimchi, heterologously expressed in *E. coli* and characterized [24][47][48][49]. They all share certain structural features, such as a serine residue critical for their activity and the ‘Gly-X-Ser-X-Gly’ motif typical for serine hydrolases, and they all belong to the GDSVG family of esterolytic enzymes. On the whole, both the original strains in vivo and the recombinant enzymes in vitro show similar abilities for OPP degradation. They are most effective in degrading chlorpyrifos, coumaphos, parathion, methylparathion, and diazinon: well over 50% for nine days at 100 mg/L initial concentration. They are least effective (well below 50% under the same conditions) in degrading dyfonate, cadusafos, ethoprophos, and fenamiphos. The first three of these contain another sulfur atom bound to the phosphorus, that is to say, a P–S–C bond, a somewhat unusual feature that explains at least partly their resistance to OP hydrolases. Fenamiphos has the remnants of an amino group (–NH–) attached to the phosphorus, quite a rare thing for OPP.

Alkaline phosphatase from *Lc. casei* 355 has been purified and characterized. It has the ability to degrade in vitro the organophosphate insecticide and acaricide dimethoate (1–2 mg/kg) almost in half after four hours that, in combination with its broad tolerance to physical conditions (at least 70% activity at 22–42 °C and pH 7.5–10), makes it a promising candidate for food detoxification [54]. Significant degradation of dimethoate, chlorpyrifos, methylparathion, and trichlorphon (50–87% at 24 h) has also been achieved with *Lp. plantarum* subsp. *plantarum* CICC20261. A positive correlation between this effect and the crude phosphatase activity of the medium was established. Interestingly, however, in vitro degradation by the crude phosphatase was lower and more uniform—around 50% for all four OPP—suggesting an additional mechanism besides the enzymatic degradation, possibly adsorption on the cell surface or perhaps even selective uptake [55]. Phosphatase activity on the level of crude extract has also been confirmed in the degradation of dimethoate by *Lp. plantarum* CICC20261 in milk, which yielded five products of estimated lower toxicity such as omethoate and trimethyl phosphodithioate, [44]. A positive correlation between phosphatase activity and pesticide degradation was also found for *Lev. brevis* 1.0209 [22] and *L. bulgaricus* [21], but in these cases, not even a crude enzyme was isolated and tested in vitro. Moreover, such statistical methods have been questioned by another study that did not find a significant correlation between OPP degradation and both intracellular and extracellular acid phosphatase activities in *Lp. plantarum* P9 isolated from sour porridge [56].

The experimental design in a number of studies concerned with OPP degradation by probiotic bacteria completely lacks any investigation into the molecular mechanisms. Sometimes the existence of an esterase [24] or an alkaline phosphatase [57] is no more than merely suggested, thus leaving impressive achievements in pesticide detoxification essentially incomplete. Sometimes an adverse effect (i.e., decelerated degradation) is observed, for instance, when *Lp. plantarum* 112 is added as a starter in sauerkraut fermentation [28], but again no mechanism has been suggested.

Pesticide adsorption has been reported for *Lc. casei* WYS3 [58] and *Lc. rhamnosus* GG [15]. Both LAB species showed some ability to sequester chlorpyrifos from the medium. In both studies, adsorption on the cell surface was in some way related to the better studied and more robust mechanism of enzymatic degradation. In the case of *Lc. casei* WYS3, hydrolysis products were detected by GC–MS, and an upregulation of the *opd* gene in the presence of chlorpyrifos was confirmed by RT-PCR. Significantly, *Lc. casei* WYS3 was rather more successful in chlorpyrifos removal: 80% after four days at 50 mg/L initial concentration. An OP hydrolase was predicted in the genome of *Lc. rhamnosus* GG but was not found to be functional. Compared to enzymatic degradation, pesticide adsorption is a less effective way of detoxification and seems less likely to engender any scientific breakthroughs in the future.

Microbial degradation of organochlorine pesticides [59], carbamates [60], and ne-onicotinoids [61] has been studied extensively for decades. A number of bacterial species isolated from soil and water have been implicated in the process. As of yet, however, there are no probiotics from fermented foods among them. Considering the remarkable results achieved with such bacteria in the degradation of organophosphates, their application for biodegradation of other pesticides may prove to be an exciting area of future research.

3. Prospects in Fermented Food Detoxification

Pesticides are a threat to human health of global magnitude. Chlorpyrifos, for example, is found in fruits and vegetables all over the world, from cucumbers in Thailand (275 µg/kg) to apples in Slovakia and Poland (21–93 µg/kg), even though in all three countries, the use of chlorpyrifos is banned [62]. Some fermented foods can be detoxified from pesticides thanks to the activity of the bacterial microflora present. Lactic acid bacteria are known for their antagonistic activity against diseases caused by fungi and for which plants are treated with tons of fungicides [63][64][65][66][67]. In these cases, the biological approach should be preferred to chemical methods of pesticide removal. On the other hand, the selection of strains with detoxification potential can occur in natural habitats. For example, LAB isolated from the gut of bees exposed to pesticides such as chlorpyrifos, coumaphos, and imidacloprid are capable of binding and neutralizing them in vitro as well [68].

Functional foods rich in probiotic LAB have the potential of combating accidentally ingested pesticides in the GIT directly, by degradation or absorption as already discussed, or indirectly by neutralizing the adverse effects of pesticides [69]. Many lactobacilli have potent antioxidant properties and may be able to alleviate the oxidative stress and damage caused by chronic exposure to OPP [70]. One recent study showed the antioxidant capacity of *Lp. plantarum* Pb3 is increased in the presence of chlorpyrifos, imidacloprid (a neonicotinoid), and chlorantraniliprole (an insecticide from the ryanoid class). A high survival rate (70–75%) in simulated gastric and intestinal juices also makes the strain a suitable candidate for combating the adverse effects of ingested pesticides. It should be noted, however, that both the ability to inhibit lipid peroxidation and to scavenge hydroxyl radicals were slightly increased (5–10%) in the presence of the pesticides [71]. In vivo studies with rats show that *Lp. plantarum* BJ0021 can alleviate most harmful effects of endosulfan (an organochlorine insecticide and acaricide) yet has no positive effect on major antioxidant enzymes such as SOD and CAT [14]. The antioxidant properties of LAB should be treated with caution.

Another indirect influence is the ability of many LAB species to enhance the gut barrier function and thus prevent the absorption of pesticides. This is only one of the numerous beneficial effects of LAB on intestinal health that are supported by a great and growing body of evidence [69][72][73]. *Lp. plantarum* MB452 has been shown to affect the expression of 19 genes related to tight junctions, thus improving the integrity and signaling in human colon cancer (Caco-2) cells, a common model for intestinal epithelium [74]. *Lc. rhamnosus* strains GG and GR-1 reduced the absorption of 100 µM parathion and especially chlorpyrifos within 60 min in Caco-2 Transwell model of the small intestine epithelium [15]. Far from being merely preventive medicine, probiotic bacteria can also help once the damage is done. A cocktail of four *Lactobacillus* species (JUP-Y4) isolated from traditional Chinese fermented foods was shown to improve the recovery of antibiotic-induced intestinal disruption in mice, including enhanced integrity of the gut barrier, reduced inflammation, lower levels of endotoxins in the blood, and restored numbers of beneficial gut bacteria [75].

Neurodegenerative diseases, especially Parkinson's disease, have also been linked with pesticide exposure and, consequently, probiotic bacteria may add to their health benefits a neuroprotective effect [76]. In addition to pesticides, probiotics are studied as a potential weapon against many other toxic substances [77]. Innovative microbial processes are developed with the certain potential to detoxify foods from mycotoxins, polycyclic aromatic hydrocarbons, perfluoroalkyl and polyfluoroalkyl compounds, phthalates, bisphenol A, and heavy metals.

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