Madras Agric. J., 2022; https://doi.org/10.29321/MAJ.10.000706

RESEARCH ARTICLE

Potential Protective Role of Probiotic Strains of lactobacilli Against Pesticide Toxicity

Palanisamy M* and Vijila K

Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore-641 003

Received: 20 June 2022

Revised: 07 August 2022

Accepted: 06 September 2022

ABSTRACT

Lactic acid bacteria (LAB) are commonly associated with agricultural produces like cereals, fruits, and vegetables. Probiotic lactobacilli are the potential microbes to reduce unavoidable pesticide absorption besides their ability to degrade pesticides in humans and wildlife. The present study aimed to evaluate the strains *Lactobacillus plantarum* Pb3, *Lactobacillus acidophilus* Pc1 and *Lactobacillus lactis* Pt4 for their antioxidant ability and tolerance to simulated gastric and intestinal juice to understand their effects against oxidative damage induced by the pesticides chlorpyriphos, imidacloprid and chlorantraniliprole. Among the three strains, *L. plantarum* Pb3 exhibited the highest antioxidant ability and tolerance to simulated gastric and intestinal juices, followed by *L. acidophilus* Pc1 and *L. lactis* Pt4. Intact cells possessed higher activity than cell-free extracts and cell-free supernatant. The lipid peroxidation inhibition ability of intact cells of *L. plantarum* Pb3 in the presence of chlorpyriphos, imidacloprid and chlorantraniliprole was 58.12%, 50.75% and 48.88% respectively, and it was 46.19% in the absence of pesticides. Hydroxyl radical scavenging abilities of intact cells of *L. plantarum* Pb3 in the presence of chlorpyriphos, imidacloprid, and chlorantraniliprole were 52.09%, 49.88%, and 49.15% respectively, as against 45.56% in the absence of pesticides. Under simulated gastric and intestinal juices, a 70 to 75% survival rate was recorded in *L. plantarum* Pb3. The antioxidant ability of LAB and the tolerance of simulated gastric and intestinal juices indicated the potential protective effects of *L. plantarum* Pb3 against the adverse effect of pesticides on human health.

Keywords: *Lactic acid bacteria; Pesticide toxicity; Antioxidant; Gastrointestinal tolerance*

INTRODUCTION

Pesticide contamination is a primary concern in food safety and health globally. Extensive efforts have been made in food industries to produce chemical-free, food products. Despite the efforts to produce safe food, food products can be contaminated through pesticide-contaminated raw materials and/or during various food processes. These pesticides, which impede the function of acetylcholinesterase are hazardous to the liver, kidney, heart, lung, blood, reproductive, and immunological systems and eventually make their way into human bodies through the food chain (Sidhu *et al.,* 2019). The use of pesticides cannot be completely banned without placing food production at risk, it has become an urgent task in the design process to eliminate pesticide residues from food. Oxidative stress and DNA damage caused by pesticide exposure have been the focus of toxicology research (Yuan *et al.,* 2019),). Pesticide exposure induces oxidative stress by increasing the creation of free radicals, which can build up inside of cells and harm biological macromolecules like RNA, DNA, and proteins or accelerate lipid peroxidation (Uriostegui-Acosta *et al.,* 2020).

As of now, there are several conventional processing techniques for reducing pesticide residues in food (washing with various agents, peeling, cooking, and chemical oxidants) and emerging advanced oxidation technologies (ozone, ultrasound, ultraviolet light, and non-thermal plasma), have been documented by numerous researchers (Azam *et al.,* 2020; Pandiselvam *et al.,* 2020). Satisfactory levels of pesticide reduction can be achieved by employing some treatments, but in many cases, toxic by-products are found in residue. Some severe treatments may affect the sensory quality of food and the loss of nutritious components (Cengiz *et al.,* 2018).

Many researchers have discovered that lactic acid bacteria, including *Lactococcus lactis, Leuconostoc mesenteroides, Lactobacillus rhamnosus*, and *L.*

*Corresponding author's e-mail: mpalanisamy112131@gmail.com

brevis, can degrade organophosphorus in vitro (Pinto *et al.,* 2019; Wochner *et al.,* 2018). Moreover, there is considerable evidence that probiotic LAB has antioxidative properties that may be effective against pesticide-induced oxidative stress in vivo. Daily intake of probiotic LAB cells also could prove to be a protective dietary strategy for populations exposed to pesticides (Luti *et al.,* 2020). Therefore, the present study aimed to screen potential pesticide-degrading LAB strains for antioxidative activity against pesticide toxicity. Besides, the resistance of selected strains in simulated gastric juice and bile was investigated to ensure that the selected strains could survive in the human gut.

MATERIAL AND METHODS

LAB culture used in this study

Strains of Lactobacilli selected for their pesticide degrading ability viz., *L. plantarum* Pb3, *L. acidophilus* Pc1 and *L. lactis* were used in this study. The strains exhibited their growth in the presence of pesticides, namely chlorpyriphos, imidacloprid, and chlorantraniliprole, up to 100 mg L-1 in previous studies.

Preparation of intact cells, cell-free supernatant, and cell-free extracts

The sterile MRS broth was inoculated with 1% inoculum of 24 h old culture containing 106 CFU mL-1. The pesticides chlorpyriphos, imidacloprid and chlorantraniliprole were added at the concentration of 10 mg L^{-1} (maximum residue level in crops) after filtering through 0.22 µm pore size membrane filter into the MRS broth. The 24 h old LAB cultures were centrifuged at 6000g at 4 °C for 10 min to collect supernatant and bacterial precipitation. The supernatant was filtered with a 0.22 µm filter membrane to obtain cell-free supernatant (CFS). The cells were washed with Phosphate buffer solution (PBS) (0.01 mol L^{-1}, pH 7.2) and resuspended in phosphate buffer solution at a concentration of 106 CFUu mL^{-1}. One portion of the suspension was used as intact cells(IC), and the rest was broken by an ultrasonic ice bath and centrifuged at 4 °C for 10 min. The supernatant was filtered with a 0.22 µm filter membrane and kept as cell-free extracts (CFE). MRS broth without the addition of pesticides was considered a control.

Lipid peroxidation inhibition (LPI) assay

The LPI assay was conducted following the method of Tang et al. (2017) and as modified by Yuan et al. (2021). Linoleic acid emulsion (1 mL) was added to 0.5 mL of PBS (0.02 $molL^{-1}$, pH 7.4), then mixed with 1 mL of 0.01% FeSO4, 1 mL of H2O2 and 0.5 mL of sample (IC, CFE or CFS). After incubation at 37 °C for 12 h, 0.2 mL of 4% TCA and 2 mL of 0.8% thiobarbituric acid were added to the mixed solution, and the reaction solution was incubated at 100 °C for 30 min and cooled rapidly. The supernatant after centrifugation for 10 min at 6000×g was measured at 532 nm (Ax). In the control group, 0.5 mL sterile normal saline was used instead of the sample solution to determine the absorbance (A0). The inhibition rate of lipid peroxidation was calculated according to the following equation:

$$\text{Inhibition rate (\%)} = (1 - Ax / A0) \times 100\%$$

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging assay was conducted using a Fenton reaction method (Li *et al.,* 2012). The reaction mixture containing 1.0 mL of salicylic acid (0.435 mmolL-1, Sigma), 1.0 mL of sample, 2.0 mL of ferrous sulfate (0.5 mmolL-1, Sigma) and 1.5 mL of hydrogen peroxide (3.0%, w/v) was incubated at 37 °C for 15 min, and the absorbance was measured at 510 nm.

$$\text{Scavenging ability (\%)} = [(As - Ao)/ (A - Ao)] \times 100\%$$

Where, As is the absorbance in the presence of the sample, Ao is the absorbance of the control in the absence of the sample, and A is the absorbance without the sample and Fenton reaction system.

Determination of tolerance of LAB strains to simulated gastric and intestinal conditions

The tolerance test of LAB strains to simulated gastric and intestinal conditions was conducted following the method of Roberts et al. (2018) and as modified by Yuan et al. (2021). Pepsin (1:10000, Sigma, USA) was suspended in sterile normal saline (0.8% w/v, adjusted to pH 3.0 with concentrated HCl) to a final concentration of 3 g L^{-1}, as the simulated gastric juice. Simulated intestinal juice was prepared by adding trypsin to sterile normal saline (0.8% w/v, adjusted to pH 8.0 with 0.1 $molL^{-1}$ NaOH) at a final concentration of 1 gL^{-1} with 0.3% bile salts (Sigma).

The cells of the LAB strains cultured for 24 h were harvested by centrifugation at 6000×g for 10 min and resuspended in simulated gastric juices to 106 CFU mL^{-1}. After incubation at 37 °C for 2 h, one mL of the culture was transferred to 10 mL of simulated intestinal juice and incubated at 37°C for 4 h. The viable counts of LAB after treatment with simulated gastric and intestinal juices were determined. The survival rate was calculated as follows:

$$\text{Survival rate (\%)} = (\log \text{CFUu Nt} / \log \text{cfu Nc}) \times 100\%$$

Where, Nt is the viable count of each strain after treatment with simulated gastric and intestinal juices, and Nc is the viable count of each strain before treatment.

Statistical analysis

The dataset was subjected to a two-way analysis of variance, and means were separated by Duncan's multiple range test (DMRT) at 0.05 level of probability using statistical software SPSS version 20.0. The PCA was performed at the 5% significance level to determine the differences and similarities between the variables using XLSTAT 2021.3.1 (Bressani *et al.,* 2021).

RESULTS AND DISCUSSION

Antioxidative activity of LAB strains

Exposure to pesticides can induce oxidative stress leading to a variety of adverse health effects. The pesticides, according to the category, can produce lipid peroxidation, stimulate free radical production and cause disturbance of the total antioxidant balance of the body. The creation of oxidative strains is conclusively related to pesticide toxicity in humans (Long *et al.,* 2020). Probiotic lactic acid bacteria can express antioxidant activity in the human host (Yuan *et al.,* 2021), and decreases the risk of accumulation of ROS during the ingestion of food. The antioxidant effect of probiotic strains is associated with inhibiting lipid peroxidation activity, scavenging free radicals, and decreasing DNA lesions in host tissues (Feng *et al.,* 2020). In our study, we examined the effects of three selected LAB strains to respond to oxidative stress induced by the pesticides viz., chlorpyriphos, imidaclopri,d and chlorantraniliprole. We investigated the lipid peroxidation inhibition, hydroxyl radical scavenging ability and survival in simulated gastric and intestinal juices of three LAB strains isolated initially from fresh vegetables and selected for their pesticide tolerance.

The lipid peroxidation inhibition ability of *L. plantarum* Pb3, *L. acidophilus* Pc1and *L. lactis* Pt4 was tested in the presence and absence of selected pesticides by using intact cells, cell free extracts, and cell free supernatants, and the results are shown in figure 1. All three cultures exhibited lipid peroxidation inhibition in the absence of all pesticides. In the presence of pesticides, the activity was enhanced, however, high response was noted with chlorpyriphos. The intact cells possessed more activity than cell free extracts and cell free supernatant. Among the three strains, *L. plantarum* Pb3 exhibited the highest activity. The lipid peroxidation inhibition ability of intact cells of *L. plantarum* Pb3in the presence of chlorpyriphos, imidacloprid and chlorantraniliprole was 58.12%, 50.75% and 48.88%, respectively. In the absence of pesticides, the strain exhibited 46.19% activity and was followed by *L. acidophilus* Pc1. The isolate *L. lactis* Pt4 showed the lowest percentage of lipid peroxidation inhibition ability of all three strains studied.

Hydroxyl radical is a type of active oxygen with strong oxidation properties, which can destroy the permeability of the cell membrane and lead to oxidative damage to DNA (Luti *et al.,* 2020). In the present study Hydroxyl radical scavenging ability, of the three LAB strains was tested in the presence and absence of pesticides by using intact cells, cell free extracts and cell-free supernatants and the results are shown in figure 2. The strain *L. plantarum* Pb3 recorded stronger hydroxyl scavenging ability compared *L. acidophilus* Pc1 and *L. lactis* Pt4. The intact cells of *L. plantarum* Pb3 showed 45.56% activity and enhanced the presence of all three selected pesticides. The hydroxyl radical scavenging ability of the strains were in the order of *L. plantarum* Pb3> *L. acidophilus* Pc1> *L. lactis* Pt4. Also, in the presence of pesticides, a concomitant increase in hydroxyl radical scavenging ability was noted in CFE and CFS of all three strains. The present results are in agreement with earlier reports wherein, the strains of *Lactobacillus acidophilus* CICC2024, *Levilactobacillus brevis* CICC20014, *Limosilactobacillus reuteri* CICC23151, *Lacticaseibacillus casei* CICC23184, *Lactiplantibacillus plantarum subsp. plantarum* CICC20261, *Lacticaseibacillus rhamnosus* CICC20257, *Bifidobacterium animalis* CICC21717, *Streptococcus thermophilus* CICC6038, *Lactobacillus helveticus* CICC6032, and *Lactobacillus delbrueckii subsp. bulgaricus* CICC6047 were tested for lipid peroxidation inhibition and hydroxyl radical scavenging ability. Among the strains, *L. plantarum* CICC20261 showed the highest lipid peroxidation inhibition ability in IC, CFE and CFS groups. However, the strain S. thermophiles CICC6038 and *L. plantarum* CICC20261 showed a stronger hydroxyl radical scavenging ability in both the IC and CFE groups as reported by Yuan et al. (2021). The antioxidant activity of cell free supernatant was tested in the strains of *Leuconostoc mesenteroides* MG860, *L. citreum* MG210, *Pediococcus acidilactici* MG5001, *P. pentosaceus* MG5078, *Weissella cibaria* MG5223, MG5090, MG5215, MG5285, *Levilactobacillus brevis* MG5250, MG5280, MG5306, MG5311, *Latilactobacillus curvatus* MG5020, and *Latilactobacillus sakei* MG5048, MG5031 food-derived bacterial strains was measured through the DPPH and ABTS radical scavenging method. All LAB has DPPH and ABTS radical scavenging activity. Among them, MG5020 had the highest radical scavenging activity, as reported by Kim et al. (2022).

Because of the complex data on the antioxidative activity of LAB, a principal component analysis was used to find a correlation among the data. The principal components, Eigen values, variability (%), and cumulative variance (%) were computed. As shown in Figurer 3, two principal components whose eigenvalues are greater than 1 are extracted and these two principal components contain 98.72% of the total variance. F1 contributed 93.42% in the variance, F2 explained 5.30% of the variance. F1 variance which was

characterized by the antioxidative activity of the LAB strains, *L. plantarum* Pb3, *L. acidophilus* Pc1and *L. lactis* Pt4. F2 variance was characterized by the bacterial cell-free supernatant, intact cells and cell-free extracts of the three strains. As can be seen from the principal component scores shown in Figurer 3, *L. plantarum* Pb3 had the highest contribution to F1. These strains showed high lipid peroxidation inhibition and Hydroxyl radical scavenging ability on intact cells.

Table 1. Tolerance of pesticide degrading strain of lactobacilli to simulated gastric and intestinal conditions

Isolates	Survival rate (%)	
	Simulated gastric juice at pH 3	simulated intestinal juice at pH 8
***L. plantarum* Pb3**	75.05±0.90[a]	70.18±0.12[a]
***L. acidophilus* Pc1**	60.21±1.53[b]	57.09±1.06[b]
***L. lactis* Pt4**	54.57±0.48[c]	49.29±1.05[c]

Data are Mean ± Standard error; Mean values differ significantly at $p < 0.05$

a/b/c referred to significant different data within column determined by Duncan's test

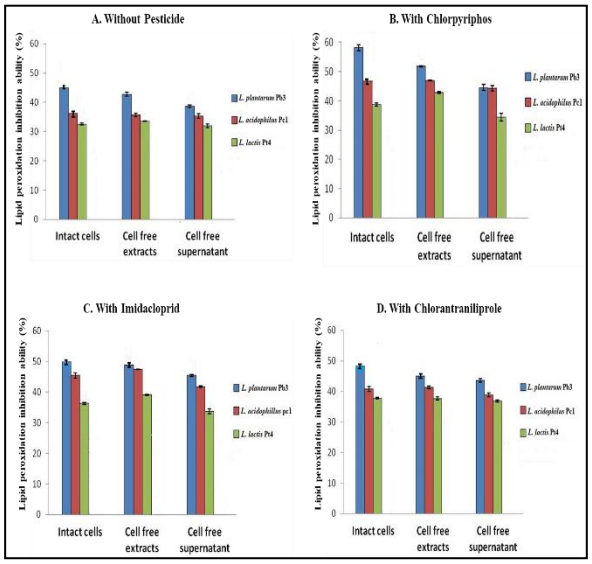

Figure 1. Lipid peroxidation inhibition (LPI) ability of pesticides degrading strains of lactobacilli in the presence of 10 mg L -1 pesticides at 24 h.

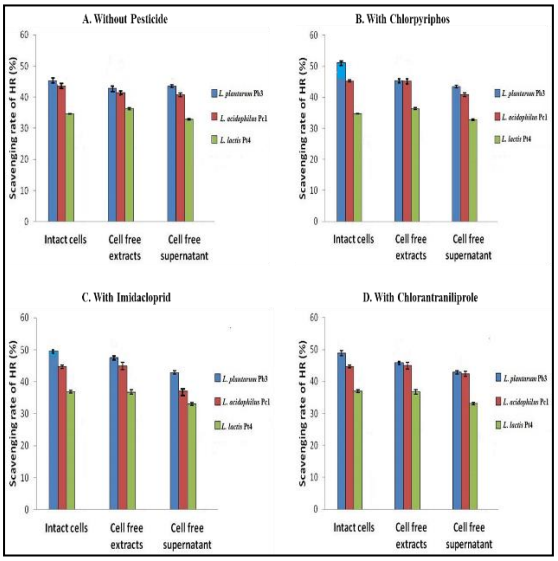

Figure 2. Scavenging rate of hydroxyl radical (HR) by pesticides degrading strains of lactobacilli in the presence of 10 mg L -1 pesticides at 24 h.

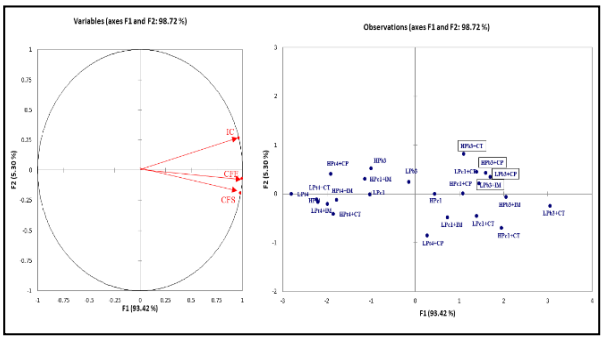

Figure 3. Principal component analysis on antioxidant ability (lipid peroxidation inhibition and hydroxyl radical scavenging) of pesticide degrading strains of lactobacilli.

CONCLUSION

In this investigation, the antioxidant ability of pesticides degrading LAB strains that would reduce the harm of pesticides to the human body is studied. As, no specific treatment for pesticide poisoning has been clinically confirmed, the use of probiotic LAB with antioxidant properties is to be considered a novel strategy against pesticide toxicity in the human body. In this circumstance, the strain *L. plantarum* Pb3 is a promising probiotic to deliver through food to minimize pesticide toxicity in humans.

Funding and Acknowledgment

The authors acknowledge the research facilities provided by the Department of Agricultural Microbiology, Coimbatore, TNAU.

Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Originality and plagiarism

This is original research work and any work and/or words of others, has been appropriately cited.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There was no conflict of interest in the publication of this content.

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through the corresponding official mail; mpalanisamy112131@gmail.com

Author contributions

Idea conceptualization-Vijila, Experiments- Palani, Guidance – Vijila, Writing original draft - Palani Writing- reviewing &editing – Vijila and Palani

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