doi.org/10.36721/PJPS.2023.36.3.SP.969-972.1

Antibiotic susceptibility and bioremediation potential of probiotic bacteria against lead and cadmium isolated from yogurt

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Abstract: Probiotic bacteria have capacity to bind with heavy metals. The present study was planned to assess the bioremediation potential of probiotic *Lactobacillus* species isolated from yogurt samples. *L. acidophilus* and *L. plantarum* were tested for acidic pH tolerance, bile salt resistance and gastric juice tolerance. The antibiotic susceptibility and antimicrobial activity was also checked. These *Lactobacillus s*pecies were also evaluated for degradation of lead (Pb) and cadmium (Cd) metals. The results indicated that *L. acidophilus* and *L. plantarum* were able to tolerate high acidic pH: 3. both showed significant growth after exposure to stimulated gastric juice from 0 to 24 hours. The significant plate count was observed at different bile salt concentrations (0.1%, 0.3%). The isolates showed resistance for all the tested antibiotics except *L. acidophilus* showed susceptibility for gentamicin and co-amoxiclave. The isolates depicted no antimicrobial activity against the indicator bacteria. *L. acidophilus* and *L. plantarum* were capable of tolerating Cd and Pb. Maximum tolerance and removal were observed for Pb by both *Lactobacillus* spp. The Cd removal was 11.50 and 3.50% while Pb removal was 42.70 and 35.50% for *L. plantarum* and *L. acidophilus*, respectively. In conclusion, *L. acidophilus* and *L. Plantarum* have potential for bioremediation of heavy metals.

Keywords: Bioremediation, probiotic, *Lactobacillus*, lead, cadmium.

INTRODUCTION

Clean water is essential for human and environmental survival. However, population growth, industrialization, urbanisation, and wasteful use of natural resources have degraded water quality in recent decades. Copper, cadmium, zinc, and lead are discharged most often (Zamora-Ledezma *et al*., 2021). Heavy metal ions are one of the most toxic water contaminants. High-density metals can be hazardous at low dosages. Due to urban, industrial, and agricultural growth, heavy metal pollution has increased by several folds. Sewage sludge, insecticides, metallic ferrous ores, fertilisers, municipal wastes and fossil fuels are the main sources of heavy metal toxicity (Tahoon *et al*., 2020).

Bioremediation is a promising method for removing heavy metals from water and damaged soil. Metal bioremediation requires microorganisms. Heavy metal intoxication can impair lungs, brain, liver, kidney, blood composition, and other organs and lower energy levels (Verma and Kuila, 2019). Physical and chemical remediation is expensive and wasteful. Hazardous metal balance has grown in popularity. Eco-friendly biosensor microbes are cost-effective. Thus, microorganisms have multiple metal sequestration methods that increase metal biosorption (Medfu Tarekegn *et al*., 2020).

Heavy metal-contaminated food and water kills. Heavy metals like lead and cadmium pollute the environment. This study will remove heavy metals with Pediococcus pentosaceu probiotic bacteria. Probiotics fight pathogens and live with organic acids, heavy metals, hydrogen peroxide and antifungal chemicals to boost the host's immune system (Jaafar, 2020; Tayang and Songachan, 2021). This study examined yoghurt probiotic bacteria's bioremediation ability.

MATERIALS AND METHODS

Procurement of bacterial species

The study was performed in of Institute of Microbiology, University of Agriculture Faisalabad, Pakistan. Species of *Lactobacilli; Lactobacillus acidophilus, Lactobacillus plantarum*, were isolated from yogurt samples and identified through Grams staining and biochemical tests. For antimicrobial testing, indicator bacteria including *E. coli*, *Pseudomonas aeruginosa (P. aeruginosa)*, *Staphylococcus aureus (S. aureus)* and *Salmonella typhi* (*S. typhi*) were procured from the same institute.

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Determination of probiotic properties
Lactobacilli were screened for their probiotic potential by following test; Bile salt resistance, Acidic pH tolerance, Gastric juice tolerance. The protocol was followed suggested by previous studies (Jafari *et al.,* 2011; Claus *et al.,* 2016).

Determination of antimicrobial activity
Well diffusion method was used to determine the antimicrobial activity of the *Lactobacilli* species against indicator bacteria (*E. coli*, *S. aureus*, *P. aeruginosa* and *S. typhi*) (Ahmed *et al.,* 2017).

Antibiotic susceptibility
Disk diffusion method was used to determine the antibiotic susceptibility of the *Lactobacillus* species against commonly used antibiotics including; kanamycin, gentamicin, clindamycin, co-amoxiclave, florfenicol, levofloxacin, chloramphenicol and ofloxacin. Results were recorded by measuring the zone of inhibition (Shoaib *et al.,* 2020).

Screening of Lactobacillus spp. for metal tolerance
Media enriched with heavy metals (Cd, Pb) of various concentrations was inoculated with *Lactobacilli* species and incubated for 48 hours at 37^{o}C (Jaafar, 2020).

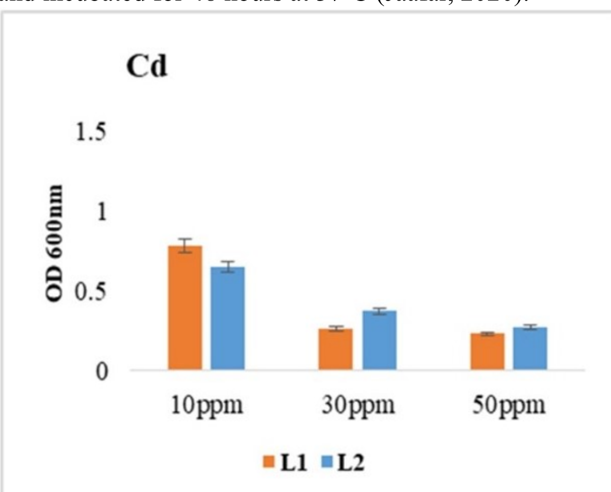

Fig. 1: Metal tolerance potential of L1 (*L. acidophilus*) and L2 (*L. plantarum*) against various concentration of Cd.

Bioremediation potential of lactobacillus spp
Bioremediation potential of bacteria was checked following methods described with slight modifications. Fresh culture was harvested by centrifugation and cells were washed with deionized water. Cell pellet was re-suspended in sterilized Cd and Pb solution. After incubation, samples were centrifuged and supernatants were filtered. Both Cd and Pb contents in supernatants were measured using atomic absorption spectrophotometer. The efficiency and percentage of metal removal was calculated using the equations described (Jaafar, 2020).

STATISTICAL ANALYSIS

The data obtained from each study was analyzed using GraphPad Prism 8 software by ANOVA (analysis of variance) with level of significance ($p<0.05$) (Al Azad *et al.,* 2020).

RESULTS

Probiotic properties
The selected *Lactobacillus* species were further subjected for evaluating their probiotic properties. The acid tolerance was checked at pH: 2, 3 and 7. It was observed that both *L. acidophilus* and *L. plantarum* species exhibited good growth at pH values of 2 and 3 and the level of acid tolerance for both species was found significantly variable ($P<0.05$) table 1.

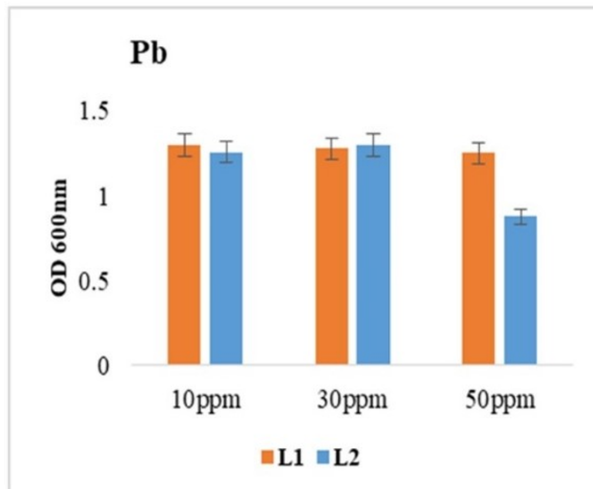

Fig. 2: Metal tolerance potential of L1 (*L. acidophilus*) and L2 (*L. plantarum*) against different concentration of Pb.

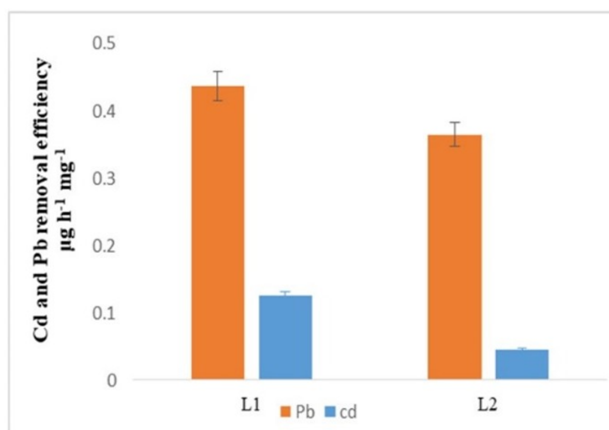

Fig. 3: Bioremediation potential (removal efficiency) of L1 (*L. acidophilus*) and L2 (*L. plantarum*) at 1ppm.

In bile salt resistance test, Both *L. acidophilus* and *L. plantarum*, were found to show significantly variable

Table 1: Mean values (±SE) of plate count of *Lactobacillus* species at different pH

pH	*Lactobacillus acidophilus* (CFU/mL)	*Lactobacillus plantarum* (CFU/mL)
2	1.8±0.005*	2.3±0.005*
3	1.5±0.005*	3.7±0.003*
7	3.7±0.004*	3.8±0.004*

Table 2: Mean values (±SE) of plate count of *Lactobacillus* species at different bile salt concentrations

Bile Salt Conc.	*Lactobacillus acidophilus* (CFU/mL)	*Lactobacillus plantarum* (CFU/mL)
0.1%	2.8±0.003*	2.6±0.003*
0.3%	2.1±0.003*	2.4±0.004*

Table 3: Mean values (±SE) of plate count of *Lactobacillus* spp. for gastric juice tolerance

Time (hours)	*Lactobacillus acidophilus* (cfu/ml)	*Lactobacillus plantarum* (cfu/ml)
0	2.1±0.002	2.2±0.001
2	1.7±0.0001	1.2±0.001
24	1.4±0.0003	1.4±0.001

* Level of significance ($p<0.05$)

Table 4: Mean Values of Antibiotic Susceptibility of *Lactobacillus* spp. (mm)

Isolates	Zone of Inhibition (ZOI)							
	K30	CN10	CD2	AUG30	FFC30	LEV5	C30	OFX5
Lactobacillus acidophilus	0(R)	19.5(S)	0(R)	28.5(S)	0(R)	0(R)	0(R)	0(R)
Lactobacillus plantarum	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)

R= resistant, S= susceptible, I= intermediate

($P<0.05$) resistance at different concentrations of bile salts (table 2). The gastric juice tolerance was assessed at 0, 2 and 24 hours of incubation and results were recorded in terms of viable count (table 3).

Antimicrobial activity

Antimicrobial activity of *Lactobacilli* species was evaluated against the indicator bacteria. The results depicted that both *L. acidophilus* and *L. plantarum* showed no antimicrobial activity for the indicator bacteria.

Antibiotic susceptibility

Antibiotic susceptibility of both species of *Lactobacillus* were determined against the above-mentioned antibiotics. It was found that *L. acidophilus* was resistant to all the tested antibiotics except for gentamicin and co-amoxiclave while *L. plantarum* showed resistance against all the tested antibiotics.

Screening for metal tolerance

The metal tolerance of *Lactobacillus* spp. for Cd and Pb was also tested at three different concentrations (10, 30 and 50ppm). The optical density (OD) values revealed that both *L. acidophilus* and *L. plantarum* were able to tolerate different concentrations of Cd and Pb metals. The gradual decrease in tolerance was observed with the increasing heavy metal concentration. The tolerance for Pb was observed to be higher as compared to the Cd metal (fig.1-2).

Bioremediation potential

Bioremediation ability of both *Lactobacillus* spp. was evaluated for Cd and Pb metals. The results indicated that bioremediation efficiency of *L. acidophilus* was higher as compared to *L. plantarum*. The maximum bioremediation was observed for Pb by both *L. acidophilus* and *L. plantarum.* The Cd removal was 11.50% and 3.50% while Pb removal was 42.70% and 35.50% for *L. acidophilus* and *L. plantarum*, respectively (fig. 3).

DISCUSSION

In the current research, two species of *Lactobacillus*; *L. acidophilus* and *L. planatarum* were evaluated for their bioremediation potential. Both of these species were procured from the institute and probiotic properties like acid tolerance, bile salt tolerance and gastric juice pH tolerance were determined. The results depicted that both *L. acidophilus* and *L. plantarum* showed highest tolerance at pH: 7. Both *L. acidophilus* and *L. plantarum*, were found to show significantly variable resistance at different concentrations of bile salts. In gastric juice tolerance, *L. acidophilus* showed more tolerant potential for gastric juice than *L. planatrum* even after 24h of incubation.

L. acidophilus and *L. plantarum* were resistant to all antibiotics except gentamicin and co-amoxiclave, respectively. Huet/Puchooa (2017). Streptomycin-resistant Lactobacillus species (Li *et al.,* 2020). Bacteria's metal tolerance explains antibiotic resistance. Another possibility is that constant exposure to heavy metal-contaminated environments causes heavy metal resistance and unanticipated antibiotic resistance in microorganisms.

In metal tolerance results, both species of *Lactobacillus* were found to be tolerant. However, the gradual decrease in tolerance was observed with the increasing heavy metal concentration. Both species depicted higher tolerance for Pb, compared to Cd metal. Bioremediation efficiency of *L. acidophilus* was higher as compared to *L. plantarum*.

Both *Lactobacillus* species depicted the maximum bioremediation potential for Pb. In another study, *Lactobacillus* species were used for bioremediation of copper and zinc and profound results were reported (Hasr Moradi Kargar *et al.,* 2020). *Bacillus* species have also potential to degrade heavy metals. In a study, *Bacillus* spp. were used for degradation of lead. The results were same like our present study (Sharma and Shukla, 2021)

CONCLUSION

The study concluded that both *L. acidophilus* and *L. plantraum* species bioremediation potential for Cd and Pb and can be applied for bioremediation of heavy metals in water.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Researchers supporting project number (RSP2023R190), King Saud University, Riyadh, Saudi Arabia.

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