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ORIGINAL ARTICLE

DETERMINATION OF THE EFFICACY OF *LACTOBACILLUS PLANTARUM* AND *PEDIOCOCCUS PENTOSACEOUS* BACTERIA WITH PROBIOTIC PROPERTIES AGAINST FOOD ORIGINATED GRAM-POSITIVE AND GRAM-NEGATIVE PATHOGENS

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ABSTRACT

Background: Probiotic bacteria, especially lactic acid bacteria (LAB), are famous for their health benefits. This study examined the inhibitory effects of two LAB strains, *Pediococcus pentosaceus* and *Lactobacillus plantarum*, on several food pathogens.

Materials & Methods: This in vitro laboratory study conducted over a 3-month period, examines the inhibitory effects of two LAB strains, *P. pentosaceus* and *L. plantarum*, on several food pathogens. Cell-free supernatants (CFS), planktonic cells, and biofilms of the LAB strains were tested against bacteria. The microplate technique and crystal violet staining measured the antibacterial activities of the CFS. The spread plate method was used to evaluate the antagonistic action of planktonic cells and biofilms.

Results: The results showed that a 48-hour incubation was optimal for biofilm production. While the planktonic cells reduced pathogen growth by 0.25-1.17 log CFU/mL for *L. plantarum* and 0.38-1.56 log CFU/mL for *P. pentosaceus*. *P. pentosaceus* biofilm is more effective against *B. subtilis* and *S. Typhimurium*, reducing their growth by 1.49 and 1.17 log CFU/mL, respectively. The CFS of *P. pentosaceus* shown more inhibition of food pathogens than *L. plantarum*.

Conclusion: *L. plantarum* exhibited greater biofilm-producing ability compared to *P. pentosaceus*, and both biofilm and planktonic forms of these two LAB strains effectively inhibited the growth of foodborne pathogenic bacteria.

KEY WORDS: Antagonistic; Antiadhesion; Biofilm; Cell free supernatants; Foodborne; Pathogenic bacteria; Probiotic.

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INTRODUCTION:

Food safety has become a serious concern for both producers and consumers. With the globalization food supply rise, heightened attention to food safety.[1] Lactic acid bacteria (LAB), characterized as Gram-positive, non-spore-forming microorganisms, catalase-negative, primarily make lactic acid the key product of carbohydrate fermentation.[2] Common LAB species like *Pediococcus, Lactobacillus, Lactococcus, Leuconostoc,* and *Streptococcus* play pivotal roles in food fermentation. Due to their robust anaerobic fermentation capabilities and favourable physiological, survival, and probiotic properties, certain *Lactobacillus* species are particularly valuable for enhancing the stability, nutritional value, and organoleptic qualities of foods. These bacteria deploy many mechanisms to colonise and proliferate in niches in the environment, including adhesion, nutrient competition, toxic metabolite production, and the secretion of antimicrobial substances like antibiotics and bacteriocins.[3,4] Probiotics exhibit key functional features like acid and bile tolerances, epithelial adhesion, and antimicrobial activities against intestinal pathogens, potentially conferring health

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benefits by bolstering resistance to colonisation, enhancing the intestinal barrier, which modulates the immune system, and influencing the gut microbiota's composition and activities.[5] The current work studied the antagonistic features of planktonic, biofilms, and *Lactobacillus plantarum* cell-free supernatant forms f and *Pediococcus pentosaceous* against many pathogens, such as *Salmonella Typhimurium*, *Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Enterococcus faecium,* and *Pseudomonas aeruginosa.* The study also aimed to explore the inhibitory influences of these LAB on pathogen adhesion to Caco-2 cells, suggesting a potential protective role against enteric pathogens by targeting host cell binding sites and enhancing interactions with the host.

MATERIALS AND METHODS

This in vitro laboratory study, conducted from Mar 2023 to Nov 2024, examined the inhibitory effects of two LAB strains, *P. pentosaceus* and *L. plantarum*, on several food pathogens. The study was approved by Faculty of Science, Ankara University on 24 Nov 2024. Lactic acid bacteria and pathogenic strains originated from the prokaryotic Genetics Laboratory (Faculty of Science, Ankara University, Turkey). *L. plantarum* and *P. pentosaceous* were cultured on De Man–Rogosa–Sharpe (MRS) agar and broth (Oxoid, Milan, Italy). On Luria-Bertani (LB) agar and broth (Merck, Rahway, Italy, we cultured pathogens such as *S. Typhimurium* and *E. coli*, while *S. aureus, L. monocytogenes, E. faecalis, B. subtilis,* and *P. aeruginosa* were grown on Tryptic Soy Broth (TSB) and agar (Oxoid, Milan, Italy). Bacterial stocks were prepared with 40% sterile glycerol and stored at -20°C.

In the planktonic form assay, 1 mL of Brain Heart Infusion (BHI) broth method was used as described earlier and the viable bacteria were quantified in log CFU/mL. For biofilm antagonistic activity, LAB biofilms formed in microplates were challenged with 1 mL of BHI broth containing pathogenic bacteria. After 48 hours at 30°C, pathogen levels were determined by the spread plate method on selective media. Control biofilms were similarly assessed without LAB presence. CFS was prepared by culturing *L. plantarum* and *P. pentosaceus* in MRS broth at 30°C for one and two days. We centrifuged the cultures at 11,200 x g for 10 minutes at 4°C, and filtered the supernatants through 0.45 μm membranes. We also adjusted the pH of CFS to 6.5 with 4M NaOH to neutralize the antimicrobial activity of organic acids.

Antimicrobial activity was assessed by the agar well diffusion method. Test strains were standardized to 0.5 McFarland, spread on nutrient agar created with sterile cork borers. We added 100 μl of CFS every well, and incubated the plates at 37°C for 24 hours. Zones of inhibition were measured post-incubation. We cultured Caco-2 cells in Dulbecco's Modified Eagle's medium with 10% FBS and antibiotics under anaerobic conditions at 37°C, 5% CO2. We seeded the cells in 24-well plates and passaged bi-daily.

This experiment assessed the impact of two LAB strains on the adhesion of pathogenic bacteria to enterocyte-like Caco-2 cells. Active cultures of the bacterial isolates were incubated for 18 hours, centrifuging it at 8000 g for 5 minutes, and washed twice with PBS to prepare for the assay. We adjusted the concentrations of the cell cultures to 2x10 ^ 7 CFU/mL using DMEM and subsequently transferred into sterile tubes. A volume of 150 μL containing both LAB and pathogenic bacteria was mixed and added to the Caco-2 monolayer coatings for simultaneous incubation. The study incubated the cultures under anaerobic circumstances with 5% CO2 at 37°C for 90 minutes to facilitate interaction. Following this period, non-adherent bacterial cells were removed using a sterile pipette, washing the Caco-2 cell monolayers twice with PBS to clear any unbound bacteria. In addition, treatment of the adherent cells was with 300 μL of trypsin-EDTA at 37°C for 10 minutes to detach any bacteria bound to the Caco-2 cells. The detached bacteria were appropriately diluted, plating them on selective and differential agar media to quantify the viable, cell-associated bacterial colonies. *S. typhimurium* was cultured in 5 mL of sterilised Luria Bertani Broth (LB) without salt at a 1:100 volume/volume ratio, with constant agitation at 200 rpm at 37°C for 18 hours. Post-cultivation, 5 μL of each LAB cell-free supernatant (CFS)-both neutralized and non-neutralized-was added to distinct wells. This was followed by 190 μL of LB no-salt medium and 5 μL of overnight *S. typhimurium* inoculum. The setup was then incubated at 20°C for 48 hours. For controls, pathogenic bacteria were incubated with LB broth without LAB CFS; a negative control involved only LB broth.

The study conducted the statistical analyses using the R programming language, utilising both base R and the "lme4" library. The analysis focused on understanding the variability and significant differences across multiple testing conditions and time points. For parametric data, two sample t-test was used when comparing two samples while for more than two samples one way ANOVA was used followed by Tukey's posthoc test. For non-parametric data, the Chi-square test was conducted. A p-value less than 0.05 was considered significant.

RESULTS

This study examined the inhibitory effects of two LAB strains, *P. pentosaceus* and *L. plantarum*, on several food pathogens. The rates of biofilm formation initially increased, peaking after 48 hours, but subsequently decreased in both strains after 72 hours. Notably, *L. plantarum* demonstrated a higher biofilm formation rate than *P. pentosaceus.* The probiotic strain's antagonistic effects in both planktonic and

biofilm forms were assessed against various pathogens. The planktonic form of *L. plantarum* reduced pathogen growth by 0.25 to 1.17 log CFU/mL and *P. pentosaceus* by 0.38 to 1.56 log CFU/mL, as shown in Figure 1A. Specifically, the planktonic forms of *L. plantarum* notably decreased the development of *E. coli* and *P. aeruginosa* by 1.17 log CFU/mL, followed by *S. typhimurium* (1.10 log CFU/mL), *E. faecalis* (0.83 log CFU/mL), *S. aureus* (0.66 log CFU/mL), *L. monocytogenes* (0.62 log CFU/mL), and *E. faecium* (0.51 log CFU/mL).

In comparison, the planktonic form of *P. pentosaceus* exhibited greater reductions in the development of *E. coli* and *S. typhimurium* (1.56 log CFU/mL and 1.40 log CFU/mL, in respect), and decreased *P. aeruginosa* growth by 1.29 log CFU/mL. It also reduced *E. faecalis* by 1.00 log CFU/mL, *L. monocytogenes* by 0.84 log CFU/mL, and *S. aureus* and *E. faecium* by 0.68 log CFU/mL and 0.59 log CFU/mL, respectively. Biofilm forms of these probiotics also demonstrated inhibitory effects on pathogens, albeit with variable efficacy. The biofilm of *L. plantarum* inhibited *S. aureus* growth by 0.80 log CFU/mL, *B. subtilis* by 0.58 log CFU/mL, *L. monocytogenes* by 0.46 log CFU/mL, and other pathogens to lesser extents. In contrast, the biofilm form of *P. pentosaceus* was more effective, significantly reducing *B. subtilis* by 1.49 log CFU/mL, *S. typhimurium* by 1.17 log CFU/mL, and other pathogens as noted in Figure 1B.

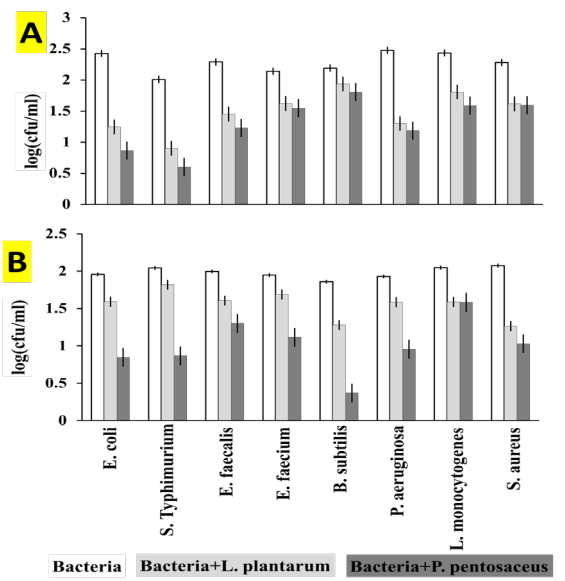

Figure 1. (A) Planktonic activities against pathogenic bacteria, **(B)** Biofilm activities against pathogenic bacteria. The histogram bar represents the mean and standard error of log10 colony-forming units per ml for each set of experiments.

L. plantarum exhibited significant pathogen inhibition, reflected in the inhibition zones measured in millimetres in bacterial broth cultures over 24 and 48 hours-specifically, 12mm and 18mm for *E. coli*. The inhibition zones for *E. faecalis* and *B. subtilis* were consistent at 13mm across both time points. There was also a variation in the growth inhibition of *E. faecium* and *S. aureus* at 13mm and 15mm, respectively. The inhibition zones for *P. aeruginosa* were 13mm and 14mm, while those for *L. monocytogenes* and *S. Typhimurium* were 12mm and 15mm, and 14mm and 15mm, respectively. Comparatively, *P. pentosaceus* exhibited greater inhibition of food pathogens than *L. plantarum*, with notable effects on Gram-positive and negative bacteria. For *E. coli*, the inhibition zones were 12mm and 18mm at 24 and 48 hours, respectively. For *E. faecalis* and *faecium*, the zones were 13mm, 15mm, and 21mm, 22mm; *S. aureus* showed 13mm and 17mm; *P. aeruginosa* had 15mm and 20mm; *B. subtilis* showed 16mm and 19mm; *L. monocytogenes* had 13mm and 16mm; and *S. Typhimurium* consistently showed 14mm across both time periods. This study confirms that the zone of inhibition increases with longer incubation times, corroborating the findings of, which indicated that maximum bacteriocin production occurs finally the late logarithmic phase and remains constant in the stationary phases, further supported by recent research from. The following statistical analysis approach was devised Out of 8 pairings, 3 display higher growth rate in the *Pediococcus* group (37.5%) than the Lactococcus group (Table 1).

Table 1. Cell-free supernatant antimicrobial activities (inhibition zone diameters in mm)

Incubation time (hr)	24		48	
Lactic acid bacteria	L. plantarum	P. pentosaceus	L. plantarum	P. pentosaceus
E. Coli	13	12	17	18
E. Faecalis	13	13	13	15
E. Faecium	13	21	15	22
S. aureus	13	13	15	17
P. aeruginosa	13	15	14	20
B. subtilis	13	16	13	19
L. monocytogenes	12	13	15	16
S. typhimurium	14	14	15	14

The anti-adhesive effects of *L. plantarum* and *P. pentosaceus* on *E. coli* resulted in reducing 0.21 log CFU/ml and 0.64 log CFU/ml, respectively. Similarly, adhesion of *S. typhimurium* was diminished by 0.30 log CFU/ml and 0.17 log CFU/ml for each strain, respectively. The impact on *E. faecalis* and *E. faecium* was modest, with reductions of 0.12 log CFU/ml and 0.17 log CFU/ml, and 0.32 log CFU/ml and

0.18 log CFU/ml, respectively. *B. subtilis* adhesion decreased by 0.20 log CFU/ml and 0.16 log CFU/ml for each strain.

Notably, the highest reduction in adhesion was observed with *P. aeruginosa*, with decreases of 0.65 log CFU/ml and 0.35 log CFU/ml, while the least effect was on *L. monocytogenes* at 0.07 log CFU/ml and 0.23 log CFU/ml. The impact on *S. aureus* was 0.26 log CFU/ml and 0.14 log CFU/ml for each strain, respectively (Figure 2).

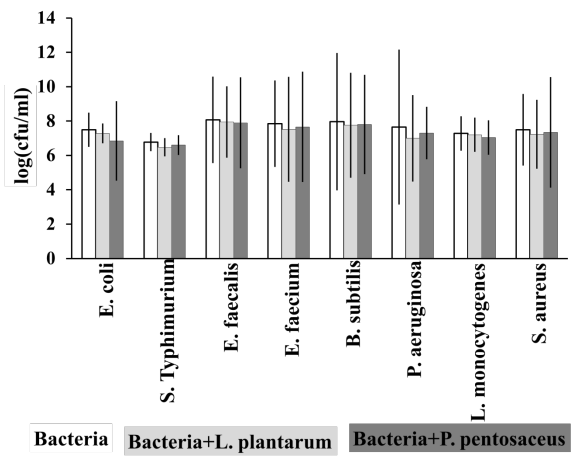

Figure 2. Inhibition of pathogenic bacteria's adhesion to Caco-2 cells

DISCUSSION

L. plantarum demonstrated the ability to inhibit both Gram-negative bacilli (*S.* Typhimurium and *E. coli, P. aeruginosa*) and Gram-positive strains, echoing findings from previous researches,[6,7] that highlight its role in interfering with pathogen infection capabilities and foodborne pathogens to prevent colonization.[8] Both planktonic and biofilm forms have proven to inhibit pathogens effectively, underscoring their potential in food preservation. A Poisson regression model confirmed that the differences in pathogen inhibition between groups were statistically significant ($p < 0.001$). This variance suggests that the wild-type bacterial isolates used in this study, possibly due to their origin and resistance to extreme conditions, behave differently from those mentioned in Rezaei et al. study, where similar experiments yielded different results.[5]

This study's results align with previous findings, which have confirmed that antimicrobial compounds like phenyl-lactic acid and lactic acid effectively target several pathogenic bacteria, including *E. coli*, *E. faecalis, S. aureus*, *P. aeruginosa*, and *B. subtilis*.[9,10] This study confirms that the zone of inhibition increases with longer incubation times, corroborating the findings of Chowdhury et al.[11] Moreover, the Caco-2 cells results confirmed in previous studies; however, variations due to different isolate sources and pathogen strains were observed.[12,13] The adhesion of lactobacilli to the intestinal epithelium facilitates pathogenic bacteria competitive exclusion, not solely through specific receptor blocking but also via competitive mechanisms including enhancing mucosal barrier function, modulating the immune response, competing for substrates, reducing luminal pH through lactic acid production, and secreting bacteriocins.[14,15]

The strength of the study represented by the direct application to food safety using well-characterized probiotic strains against comprehensive pathogen panel via investigation of cell-free supernatants (CFS). Limitations of the present study which need to acknowledged include *in vitro* conditions do not fully replicate food matrices. Lack of metabolite identification, absence of mechanistic insight, and no *in vivo* or food model validation.

CONCLUSION

P. pentosaceus showed strong antibacterial activity in both planktonic. *P. pentosaceus* reduced pathogen growth up to 1.56 log CFU/mL, while *L. plantarum* reached 1.17 log CFU/mL. Anti-adhesion tests showed the highest reduction against *P. aeruginosa*. The results suggest different survival and inhibition strategies between the two LAB strains.

REFERENCES

1. Dahdouh B, Basha O, Haggag Y, Khalil S, Tanekhy M. Antibacterial effects of nano-silver suspension against some fish pathogens (in vitro). Alexandria Journal of Veterinary Sciences. 2020;67(2):75.
2. Salih AY, Al-Taii HA, Ismael NS, Merkhan MM. Inhibition of biofilm formation and Pyocyanin production from multidrug resistance P. aeruginosa by Using Vitamin C, Salicylic Acid, and Multisera. Texila Int. J. Public Health. 2024;12(4):1-2.
3. Piatek J, Krauss H, Ciechelska-Rybarczyk A, Bernatek M, Wojtyla-Buciora P, Sommermeyer H. In-vitro growth inhibition of bacterial pathogens by probiotics and a synbiotic: product composition matters. International journal of environmental research and public health. 2020 May;17(9):3332.
4. Zhou J, Yuan Z, Yang R, Liu T, Lu X, Huang W, Guo L. Coaggregated E. faecalis with F. nucleatum regulated environmental stress responses and inflammatory effects. Applied Microbiology and Biotechnology. 2024 Dec;108(1):336.
5. Re Rezaei Z, Khanzadi S, Salari A. Biofilm formation and antagonistic activity of Lacticaseibacillus rhamnosus (PTCC1712) and Lactiplantibacillus plantarum (PTCC1745). Amb Express. 2021 Nov 25;11(1):156.
6. Zawistowska-Rojek A, Kośmider A, Stępień K, Tyski S. Adhesion and aggregation properties of Lactobacillaceae strains as protection ways against enteropathogenic bacteria. Archives of Microbiology. 2022 May;204(5):285.
7. Sadeghi M, Panahi B, Mazlumi A, Hejazi MA, Komi

DE, Nami Y. Screening of potential probiotic lactic acid bacteria with antimicrobial properties and selection of superior bacteria for application as biocontrol using machine learning models. Lwt. 2022 Jun 1;162:113471.

8. Mgomi FC, Yang YR, Cheng G, Yang ZQ. Lactic acid bacteria biofilms and their antimicrobial potential against pathogenic microorganisms. Biofilm. 2023 Dec 1;5:100118.
9. Yazgan H. Effects of Cell Free Supernatants of Lactobacillus reuteri ATCC55730 and Lactobacillus plantarum FI8595 Against Selected Food-Borne Pathogens and Fish Spoilage Microorganisms. Avrupa Bilim ve Teknoloji Dergisi. 2020(20):485-9.
10. Husejnagić D, Hodžić S, Avdić A, Širanović S, Vilušić M. Antimicrobial activity of the cell free supernatants of the lactic acid bacteria isolated from fresh cow cheese produced in Tuzla region. Technologica Acta. 2016 Dec 1;9(2):49-55.
11. Chowdhury A. Screening of Lactobacillus spp. from buffalo yoghurt for probiotic and antibacterial activity. Journal of Bacteriology & Parasitology. 2012;3(08):1-15.
12. Vasiee A, Falah F, Behbahani BA, Tabatabaee-Yazdi F. Probiotic characterization of Pediococcus strains isolated from Iranian cereal-dairy fermented product: Interaction with pathogenic bacteria and the enteric cell line Caco-2. Journal of Bioscience and Bioengineering. 2020 Nov 1;130(5):471-9.
13. Fonseca HC, de Sousa Melo D, Ramos CL, Dias DR, Schwan RF. Probiotic properties of lactobacilli and their ability to inhibit the adhesion of enteropathogenic bacteria to Caco-2 and HT-29 cells. Probiotics and antimicrobial proteins. 2021 Feb;13(1):102-12.
14. Monteagudo-Mera A, Rastall RA, Gibson GR, Charalampopoulos D, Chatzifragkou A. Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. Applied microbiology and biotechnology. 2019 Aug 16;103(16):6463-72.
15. Wan ML, Forsythe SJ, El-Nezami H. Probiotics interaction with foodborne pathogens: a potential alternative to antibiotics and future challenges. Critical reviews in food science and nutrition. 2019 Nov 13;59(20):3320-33.

CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	MBK, ANK
Acquisition, Analysis or Interpretation of Data:	MBK, ANK, ÖO
Manuscript Writing & Approval:	MBK, ANK, ÖO

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.