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Application of locally isolated lactic acid bacteria metabolites as bio-preservatives to increase shelf-life, safety and quality of some fruits

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Abstract

The bio-preservation of fruits using cell-free supernatant (CFS) of lactic acid bacteria (LAB) isolated directly from locally fermented dairy products was an innovative approach. This study aimed to increase the shelf life and quality of some fruit samples during storage at room temperature by coating samples with CFS of Lb24 (L. plantarum strain MZ409592). Forty LABs were isolated from 30 locally fermented dairy products. Six LAB isolates that had the highest antimicrobial activity against pathogenic microorganisms were identified by 16SrRNA phylogenetic identification. The results showed that the six LAB strains belonged to Lactobacillus plantarum. CFS of L. plantarum Lb24 had the highest antimicrobial activity against pathogenic microorganisms using the in-vitro method. Then, fruits were coated with CFS of Lb24 at different concentrations (10%, 20%, and 30%) by sprayed method for further study. The microbiological results showed that apples, pears, and fig fruits coated with 20% and 30% of CFS of Lb24 had higher shelf life over 14, 12, and 6 days, respectively. While the control fruit samples had lower shelf life during storage at room temperature. The increase in shelf-life of the different fruits suggests the possible use of Lb24 (L. plantarum strain MZ409592) as bio-preservatives in fruits.

Keywords: Lb24 (L. plantarum strain MZ409592), Antimicrobial activity, Cell-free supernatant, Bio-preservative, shelf life, fruit.

استخدام النواتج الايضية لبكتريا حامض اللبنيك المعزولة محليا كمواد حافظة حيوية لزيادة مدة الصلاحية، امان ونوعية بعض الفواكه

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الخلاصة

يعتبر الحافظة الحيوية للفواكه باستعمال الراشع الخالي من الخلايا لبكتريا حامض اللبنيك المعزولة مباشرة من منتجات الالبان المتخمرة من الطرق المبتكرة. تهدف هذه الدراسة الى زيادة مدة صلاحية ونوعية نماذج بحض الفواكه خلال مدة الخزن في درجة حرارة الغرفة عن طريق تغليف هذه النماذج براشع الخالي من البكتريا لبكتريا Lb24 (*L. plantarum strain* MZ409592) حوالي اربعين عزله تم جمعها من ثلاثين منتوج من الالبان المتخمرة. ستة عزلات والتي اظهرت اعلى فعالية ضد ميكروبية ضد الميكروبات المرضية تم تشخيصها باستخدام 16S RNA اظهرت النتائج بان ستة من عزلات البكتريا حامض اللبنيك تعود الى مجموعة *Lactobacillus plantarum*. الراشع الخالي من البكتريا، لبكتريا Lb24 اظهرت اعلى فعالية ضد البكتريا المرضية باستخدام الطريقة المختبرية. تم تغليف الفواكه براشع الخالي من البكتريا Lb24 بتراكيز مختلفة (10%، 20%، 30%) باستخدام طريقة الرش للدراسة اللاحقة. اظهرت النتائج المايكروبايوجية بان تغليف التفاح، العرموط والتين بتركيز 20%، 30% بهذا الرشع اعطى اعلى مدة صلاحية بواقع 14, 12, 6 ايام على التوالي. بينما اظهر نماذج السيطرة اقل مدة صلاحية خلال الخزن عند درجة الحرارة الغرفة ان زيادة مدة الصلاحية للفواكه المختلفة يشير الى امكانية استعمال Lb24 (*L. plantarum strain* MZ409592) كمواد حافظة حيوية للفواكه.

كلمات مفتاحية: (*L. plantarum strain* MZ409592) Lb24، فعالية ضد ميكروبية، الراشع الخالي من الخلايا لبكتريا، الحافظة الحيوية، مدة صلاحية، للفواكه.

Introduction

Lactic acid bacteria (LAB) have a long history of safe use in fermented foods. LAB plays an important fermentative role in many foods. The use of LAB has been widely reported in food fermentation processes of different types of foods, such as yogurt, cheese, and other fermented foods like vegetables, seafood, or meat products (27 and 38). LABs are mainly used in food products due to their contributions to flavor and aroma, increase shelf life of fermented products, and improve the nutritional and sensory characteristics of the products (10, 32 and 39). During fermentation of foods, LABs are capable of producing metabolites that may be inhibitory or lethal to other microorganisms in the fermenting medium (55).

Eating fresh fruits and vegetables is a healthy habit that should be adopted by everyone because of their nutritional value and functional properties (1 and 5), and low energy content (9). Heart disease, colon cancer, obesity, and diabetes are some of the diseases that can be reduced with a high intake of fruits and vegetables (31). These beneficial health effects are attributed to the presence of antioxidants in fruits and vegetables such as ascorbic acid, B carotene, and polyphenols (30). Fruit products are highly susceptible to microbial contamination that may affect their quality attributes and reduce their nutritional value during storage. The possible presence of microbial toxins or pathogenic microorganisms such as *Salmonella*, *Escherichia coli*, *Bacillus cereus*, *Campylobacter* spp., *Yersinia enterocolitica*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Clostridium perfringens*, and *Aspergillus niger* may even endanger consumer safety and contribute to foodborne illness (6). Also, the fungus *Penicillium expansum*, can cause important postharvest diseases of fruit (53).

Between 25% and 40% of fruits and vegetables are lost before consumption because of inadequate post-harvest treatments (28). After harvesting fruits and vegetables and during storage, and transportation, its sensorial, nutritional, and sensorial quality decreases due to its high moisture content, microbial growth, environmental factors, maturity, and senescence. Considering their very short shelf life, fruits and vegetables need immediate post-harvest care to reduce the microbial load and increase their shelf life (28 and 41). LABs are generally recognized as safe (GRAS), which are used in the preparation and preservation of many food products such as meat, dairy products, and vegetables (17).

According to (45), biopreservation can be defined as the extension of shelf life and enhancement of safety of foods by using natural or added microflora and their antimicrobial products. In this sense, LABs present a promising approach for several reasons which they naturally occur in foods such as fresh vegetables and fruit, are considered harmless to human health, have a GRAS status (generally recognized as safe), are widely used in the food industry and can act as biocontrol agents due to their ability to produce antimicrobial compounds and to colonize plant tissues vulnerable to infection (29). The antimicrobial effect of LAB and its safety for use as preservatives is widely accepted (49).

LABs exert a strong antagonistic activity against many food-contaminating microorganisms as a result of the production of organic acids (lactic acid and acetic acid) (16), hydrogen peroxide, diacetyl, carbon dioxide, and bacteriocins or bactericidal proteins that have been used to preserve food through carbohydrate fermentation and for their effect on texture, color, taste, and smell of food products (14, 18 and 35). Food preservation aims to maintain the quality of raw materials and their physical and chemical properties, to improve the quality of the final product, and to provide safe and stable products (7).

The aim of this study was to investigate the effect of LAB metabolites as bio-preservatives on the shelf life of some fruits, by reducing the growth of pathogenic microorganisms during the storage period, and also, to investigate the potential changes to chemical properties after treatments.

Materials and Methods

Sample Collection: Locally fermented dairy products (n=30) were collected in 20 different markets in Erbil. Each sample was placed in sterilized containers. The samples then were brought to the laboratory with an ice box and stored in a refrigerator at +4°C for further study.

Bacterial strains: The following indicator strains were obtained from the College of Biological Sciences/ culture collection (microbiology lab) at the University of Salahaddin, which were used for the inhibitory activity test, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*Staph. aureus*) and *Salmonella typhi* (*Sal. typhi*). The following indicator strains of fungi were obtained from the College of Agricultural Engineering Sciences/ culture collection (microbiology lab) at the University of Salahaddin also used for the inhibitory activity test, *Altrenaria alternate* (*A. alternate*), *Penicillium expansum* (*P. expansum*).

Isolation of LABs from Locally Fermented Dairy products: For isolation of LABs, 11 g of each sample of locally fermented dairy products was mixed with 99 ml of 1g/L sterilized peptone water. Then, a decimally diluted five times in the same sterilized peptone water, and 0.1 ml from the last three dilutions were spread on MRS agar containing 5g/L calcium carbonate ($CaCo_3$). For each sample, three plates of each dilution were made (total: nine plates per sample). The inoculated plates were incubated under anaerobic conditions using an anaerobic jar with Gas Generation Kit (GmbH, Germany) at 37°C for 48 hours. Then, distinct colonies were randomly picked from countable MRS plates for further study. The isolated colonies of LABs were transferred into about 10 ml MRS broth and purified by repeated streaking on MRS agar. The isolated colonies were first characterized phenotypically by Gram-positive, catalase-negative growth at different temperatures and growth tolerance at different salt levels. Gram-positive and catalase-negative isolates were considered to be LAB and then used in further studies. LAB isolates were kept in MRS broth with 20% (vol/vol) glycerol and frozen at -20°C. Stock cultures were reactivated by sub-culturing in MRS broth and incubating at 37°C in the 5% CO_2 incubator for 24h before use.

Preparation of Cell-free supernatant: Cell-free supernatant (CFS) was prepared from each inoculated LAB in MRS broth at 37°C for 48 h. CFS was obtained by centrifuging the bacterial culture at 10000rpm for 10min. CFS was sterilized by membrane filtration through a 0.22µm pore size filter (Sartorius Stedim Biotech GmbH, Goettingen, Germany).

Preparation of inoculum bacteria: A single colony of inoculum was taken from colonies grown previously as a pure culture on a plate and inoculated into 10ml nutrient broth. The broth suspension was incubated for 18h at 37°C, except for *Pseudomonas aeruginosa* which was incubated at 30°C. Bacterial cultures were diluted by using saline solution and were standardized to 10^7 -10^8 CFU/ml. The fungi were grown in an incubator at 25°C on Sabouraud dextrose agar (SDA) media for 3-5 days. Spores/cells suspension method was employed for inoculation of the fungi on the medium. Using sterile distilled water, the concentrations of spore/cells suspensions were diluted to 10^5 spores/cells ml^{-1} of fungi.

Antimicrobial activity test by agar well diffusion method: The agar well diffusion method was used to determine the antimicrobial activity of LAB isolates against bacterial strains such as *P.aeruginosa*, *E. coli*, *Staph. aureus*, and *Sal. typhi* (12). Stock cultures of all tested bacteria were grown in nutrient broth for 18h. Final cell concentrations were diluted to 10^7-10^8 CFU/ml using the McFarland standards by visually comparing the opacity of the bacterial suspension to the 0.5 McFarland standard. Then, 200µl of this inoculum was added to each universal tube containing 20ml molten brain heart infusion (BHI) agar, mixed well, and poured into a disposable Petri dish. A sterile cork borer was used to make wells (5mm diameter) after the agar was solidified. Forty µl of CFS was added to each well and was left to diffuse for one h at room temperature, then incubated at 37°C for 24h. After incubation, the diameter (mm) of the inhibition zone around the wells was measured in three directions using Vernier callipers and the averages were calculated (21 and 22). Three replications were made for the experiment.

Molecular identification of LAB strains: LABs that possessed strong antibacterial activity were selected for identification by sequence analysis of 16S rRNA (50). 16s rRNA gene sequences analysis was applied to identify the isolated LABs. Each LAB isolate's DNA was extracted using the GenEluteTM Bacterial Genomic DNA Kit, manufactured by Iran- No. PF230-050. Bacterial cells were collected by centrifugation (13000 × g, 10 min) from culture kept overnight at 37 °C and the DNA was extracted using the previously described method (42). Amplification of the 16S rDNA gene was done by polymerase chain reaction (PCR) under conditions (94 °C for 5 min, 30 cycles of 45 s at 55 °C, 1.5 min at 72 °C, 10 min at 72 °C) using Forward primer 27(F) (5'-AGAG TTTG ATCC TGGC TCAG-3') (20 bases) and Reverse primer 1492(R) (5'- GGCT ACCT TGTT ACGA CTT -3') (19 bases) which obtained from (Eurofins MWG Operon, Germany). The partial 16S rDNA sequences of approximately 1200 bp were used to search public databases (Genbank using BLAST and the Ribosomal Database Project) at http://blast.ncbi.nlm.nih.gov/Blast.cgi for the identification of the LABs with the closest species match being reported. As the result of antimicrobial activity, Lb24 (*Lactobacillus plantarum strain* MZ409592) was selected for further studies.

Application of Lb24 (*Lactobacillus plantarum*) as bio-preservatives in some fruits: CFS of Lb24 was served as a source of the biofilm coating agent. Fruit samples (apple, pear, and fig) were washed separately with water to remove any residue that might affect LAB and pathogen growth, then drained, dried, and placed separately on sanitized plastic grids. The CFS of Lb24 were manully sprayed at different concentrations (10%, 20%, and 30% vol/vol) on the fruit samples. While unsprayed fruits were considered as a control. After spraying treatments, fruits were air-dried to remove any surface moisture for 1 h. Both sprayed and unsprayed fruits were stored in polyethylene bags then kept at room temperature for different days until microbial growth was observed on the surface of fruit samples (40). Three replicates were measured for each treatment.

Microbial shelf life of fruit samples: The fruit samples were taken for microbiological analysis every two days during the storage period until microbial growth was observed on the surface of fruit samples. Ten g of each fruit sample was put in a sterile plastic bag. Then the sample was homogenized with 90 ml of 0.1% peptone water for 3min in a stomacher (GmbH, Germany). Aliquots were serially diluted in maximum recovery diluent and plated out following Microbiology Standard Method that reported the preparation of samples and dilutions, plating and sub-culture (19 and 37). Total bacterial counts (TBC) were determined on nutrient agar. *E. coli* was determined on MacConkey agar. *Staph. aureus* was determined Mannitol Salt agar. *Sal. typhi* was determined on *Salmonella* and *Shigella* agar. *pesduomonas areogenus* was determined on Cetrimid agar. The inoculated media were incubated at 37ºC for 48h. Mold and yeast counts were enumerated on the Sabouraud dextrose agar medium, and then the plates were incubated at 25°C (±2°C) for 5 days. This test was carried out in triplicate for each sample.

Statistical analyses: All data were analyzed by One-way Analysis of Variance (ANOVA) and by the Tukey test, the statistical significance ($P \leq 0.05$) program from Minitab 16 software was used.

Results

Biochemical Characterization and Identification of LAB: A total of 40 potential LAB strains were isolated from 30 different locally fermented dairy products. All isolated strains were gram-positive and catalase negative, cocci or rod in shape, as the results were described by (3). LABs were white or creamy colonies on the MRS agar plate. All strains grew at different concentrations of 2, 4, and 6.5% NaCl. Also, strains were grown at 15 and 45°C, which were similar to the results of (51). Morphological and biochemical properties of six LABs were shown as mentioned in (Table 1).

Table 1 Morphological and biochemical test of Isolated LABs.

Strains	Shape	Gram Stain	Catalase test	Temp °C		NaCl%			Color
				15	45	2	4	6.5	
Lb5	Rod	+	-	+	+	+	+	+	Creamy
Lb12	Rod	+	-	+	+	+	+	+	White
Lb21	Rod	+	-	+	+	+	+	+	White
Lb24	Rod	+	-	+	+	+	+	+	Creamy
Lb32	Rod	+	-	+	+	+	+	+	Creamy
Lb33	Rod	+	-	+	+	+	+	+	Creamy

Note: (+) = Positive reaction, (−) = Negative reaction

Determination of the antimicrobial activity of selected LABs by agar well diffusion method: Six LAB strains had higher antimicrobial activity from a total of 40 LAB isolates by using the agar well diffusion method, the results are shown in (Table 2). Lb24 had higher antimicrobial activity against pathogenic bacteria compared to other LAB stains. Lb24 had also higher antifungal activity against *A. alternate*, while Lb21 had higher antifungal activity against *P. expansum* compared to Lb24. According to the previous study, LABs have the ability to produce antimicrobial components such as organic acids, hydrogen peroxide, and bacteriocins, which have

an important role in inhibiting the growth of microorganisms (8 and 44). From the results of this experiment, Lb24 were selected for further studies.

Table 2 Antimicrobial activity* of CFS of LAB against bacterial and fungi food pathogens using an agar well diffusion method.

LAB Isolates	Diameter of inhibition zone (mm)					
	Staph. aureus	*E. coli*	*Sal. typhi*	*P. aeruginosa*	*A. alternata*	*P. expansum*
Lb5	++++	++	+++	++++	++	++
Lb12	++++	+++	+++	++++	+++	+
Lb21	++++	+++	+++	++++	++	+++
Lb24	++++	+++	++++	++++	+++	++
Lb32	++++	+++	+++	+++	+	+
Lb33	++++	+++	+++	+++	++	++

*** Diameter of inhibition zone: (–) no inhibition zone, (+) Inhibition zone of 6 – 9 mm, (++) Inhibition zone of 10 – 13mm, (+++) Inhibition zone of 14 –18 mm, (++++) Inhibition zone of>20 mm. Mean values from three replicates**

Identification of LAB isolates by 16S rRNA gene sequencing: The strains with the best antimicrobial activity were identified by sequencing the 16S rRNA. Six suspected isolates showed very clear bands in the gel electrophoresis of the DNA samples using 1500 base pairs ladder as indicated in (Fig. 1). The bands were more than 1000 base pairs., All six bands of the LAB strains that were found are *L. plantarum*. The identity of all bacterial species was 100% as the results shown in (Table 3). Our findings of the results of identification of LAB strains in agreement with the results of (2 and 15), where they found *L. plantarum* species in dairy products. According to results and FAO/ WHO guidelines, identification of microorganisms by 16SrRNA patterns can be considered as a more suitable technique than other costly and time-consuming molecular techniques (4). This technique has been effectively used for analyzing and isolating LABs from fermented dairy products (36 and 54).

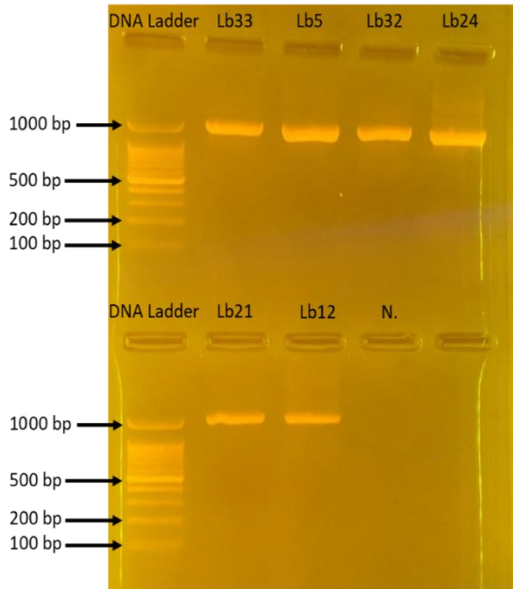

Figure 1 PCR amplified product of DNA templates of the samples

Table 3 Identification of LAB isolates based on 16S rDNA gene sequences.

Strains	Source	Identity%	Species	Accession
Lb5	Dairy product	100	*L. plantarum*	MZ409589
Lb12	Dairy product	100	*L. plantarum*	MZ409590
Lb21	Dairy product	100	*L. plantarum*	MZ409591
Lb24	Dairy product	100	*L. plantarum*	MZ409592
Lb32	Dairy product	100	*L. plantarum*	MZ409593
Lb33	Dairy product	100	*L. plantarum*	MZ409594

Microbial shelf life of fruit samples: The results didn't show more changes of microbial growth every day. So, the shelf life of the fruit samples was determined by microbial growth every two days during the storage period until microbial growth was observed on the surface of fruit samples. Figure 2 shows the growth of Total bacterial count (TBC), *E. coli*, and mold and yeast of apple samples over 14 days of storage at room temperature. The TBC was not observed in coated apple treatment with 30% over 14 days of storage. In coated apple treatments with 20% and 30%, *E. coli* and mold and yeast were not observed over 6 days and 4 days of storage, respectively. The highest growth of TBC and *E. coli* were observed in control samples over 8 days of storage. The control apple sample on day 8 and coated apple treatment with 10% on day 10 of storage were discarded and removed from the test due to the microbial growth on the surface of the samples. *Staph. aureus*, *Sal. typhi*, and *Pesduomonas areogenus* were not found in all treatments over 14 days of storage at room temperature.

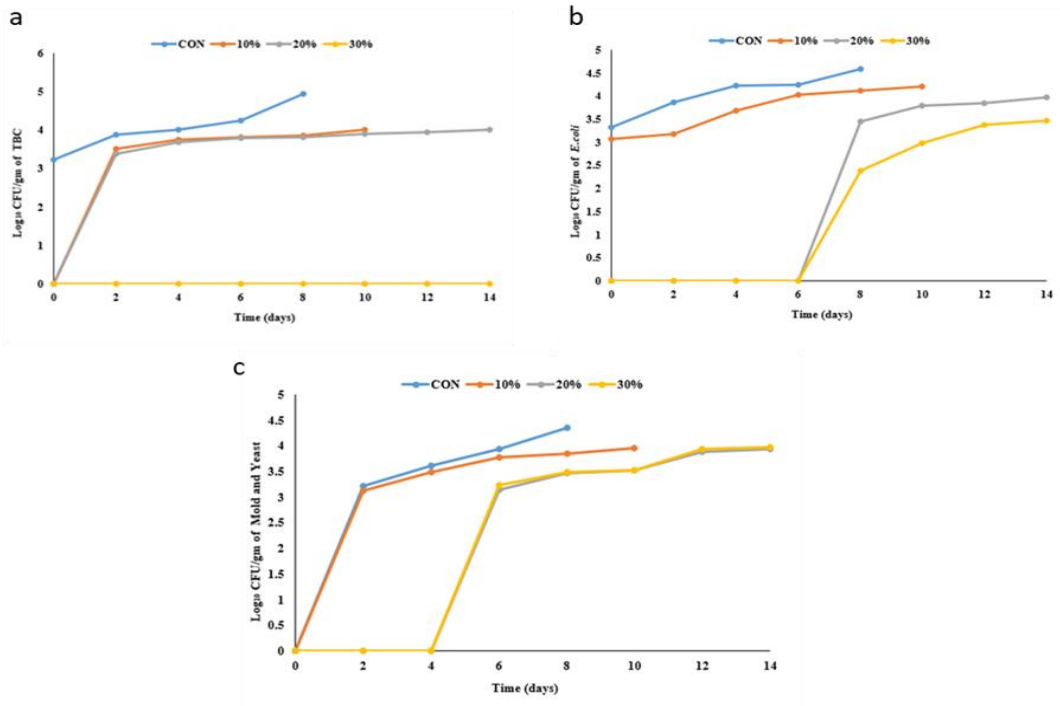

Figure 2 Microbial count of apple fruits treated with CFS of *L. plantarum* over 14 days of storage at room temperature (Log10 CFU/g).

The results are from present study are in agreement with the findings of (11) who reported that the shelf life of fruits was extended due to using bacteriocinogenic of LABs as bio-preservative of fruits, which inhibiting the growth of pathogenic microorganisms such as *E. coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeroginosa*, *Proteus vulgaris*, *Salmonella typhi*, *Xanthomonas campestris*. A total of 25 isolated bacterial fruit flora from apples, figs, bananas, sapodillas, kiwis, strawberrys, and pomegranates were analyzed using LABs isolated from curd and cow dung samples. The bacteriocinogenic of LAB coating forms a film on the surface of the fruit. Therefore, inhibiting the microorganisms that destroy the fruit.

Our results showed that the shelf life of apple coating with 20% and 30% separately had a shelf life over 14 days. While, the other treatments had a shelf life of 8 to 10 days when tested for the level of TBC, *E. coli* and mold and yeast. This extend might be due to the antimicrobial activity of CFS of *L. plantarum* on the apple samples. According to (25), who suggested that the antimicrobial activity of *Lactobacillus plantarum* could be the result of many organic acids; (lactic acid and acetic acid), and other compounds such as hydrogen peroxide, lactoperoxidase system with H_2O_2, lysozyme, reuterin, diacetyl, fatty acids, bacteriocins, and phenyllactic acids. The results were in agreement with the results of (56), who reported that the apple samples inoculated with *L. plantarum* TK9 had longer shelf life comparing to control samples.

Comparatively, the delayed growth of bacteria and fungi over 6 and 4 days for the treated apple samples compared to untreated apple controls proves the preservative potentials of 20% and 30%. This finding agrees with those obtained by (13) who reported that the treated tomato paste with *L. plantarum* Cs and *L. acidophilus* ATCC 314 CFS delayed growth of bacteria and fungi compared to untreated paste (Control). Several works supporting the biopreservation of foods by LAB metabolites have been reported (48). LAB metabolites application has shown promisings source of fruit preservative.

Figure 3 shows the growth of TBC, *E. coli, Staph. aureus* and mold, and yeast of pear samples over 12 days of storage at room temperature. The *Staph. aureus*, was not observed in coated pears treatment with 20% and 30% over 12 days of storage. In coated pear treatments with 20% and 30%, *E. coli* and mold and yeast were not observed over 6 days and 2 days of storage, respectively. The highest growth of TBC, *E. coli, Staph. aureus* was observed in control samples over 6 days of storage. The control pear sample on day 6 and coated pear treatment with 10% on day 8 of storage were discarded and removed from the test due to the microbial growth on the surface of the samples. *Salmonella typhi* and *Pseudomonas aeruginosa* were not found in all treatments over 12 days of storage at room temperature.

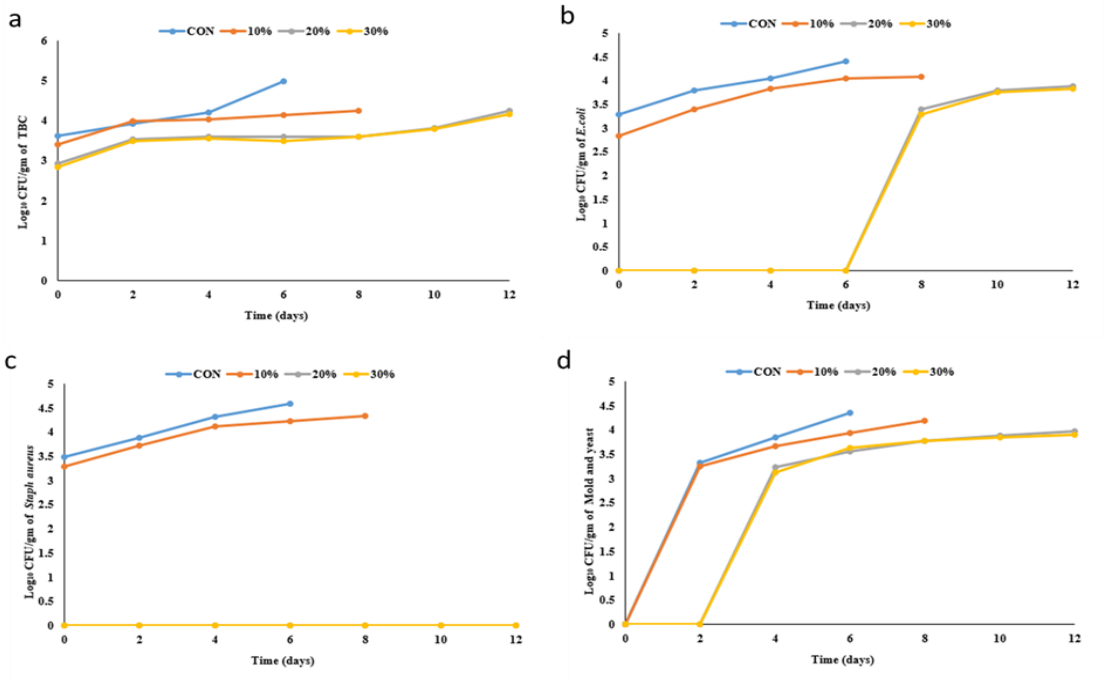

Figure 3 Microbial count of pear fruits treated with CFS of *L. plantarum* over 12 days of storage at room temperature (Log10 CFU/g).

The results in the present study are in agreement with the findings of (52), who investigated the efficacy of peptide-based coatings from *Lactobacillus plantarum* UTNCys5-4 and *Lactococcus lactis* subsp. lactis Gt28 strains against a pathogenic cocktail containing *E. coli*, *Salmonella*, and *Shigella* in fresh-cut slices of pineapple. The results, after 5 days preserved in refrigeration, showed a decrease in cell counts of the *E. coli*, *Salmonella*, and *Shigella*, respectively, indicating that these coatings are a good alternative to chemical compounds, increasing the shelf life and safety of fresh-cut pineapple.

Our results showed that the pears coating with 20% and 30% distinctly had a shelf life over 12 days. While the other treatments had a shelf life of 6 to 8 days when tested for the level of TBC, *E. coli*, *Staph. aureus*, and mold and yeast. This extend might be due to the antimicrobial activity of CFS of *L. plantarum* on the pear samples. According to (23) and (26), who suggested that the antimicrobial activity of *L. plantarum* could be the result of many organic acids such as lactic acetic and phenyllactic acids. Another study suggested that some soluble compounds in culture supernatant may be responsible for the inhibition (20).

The present results showed that *Staph. aureus* did not appear in coated pear treatment with 20% and 30% over 12 days of storage. Although *E. coli* and mold and yeast were not observed over 6 days and 2 days of storage, respectively. But the results were in agreement with the results of (33) and (34), who reported that LAB grows optimally at 30-37°C and produces metabolites like bacteriocin, which probably inhibits the growth of spoilage and pathogenic microorganisms such as *Staph. aureus* and other microflora in the pineapple juice. According to (43), who reported that suspension of

L. plantarum delayed the growth of *Aspergillus flavus*, *Fusarium graminearum*, *Rhizopus stolonifera*, and *Botrytis cinerea* on cucumber. CFS of *L. plantarum* was shown to have preservative properties against spoilage and pathogenic microorganism when applied in pears, thus representing an excellent applicant for food-related bio-preservative.

Figure 4 shows the growth of TBC, *E. coli*, *Staph. aureus* and mold, and yeast of fig samples over 6 days of storage at room temperature. Additionally, *Staph. aureus* was not observed in coated fig treated with 20% and 30% over 6 days of storage. In coated fig treatments with 20% and 30%, the *E. coli* and mold and yeast were not observed until 4 days and 2 days of storage, respectively. The highest growth of TBC, *E. coli, and Staph. aureus* were observed in control samples over 2 days of storage. The control fig samples and coated fig treated samples with 10% on day 4 of storage were discarded and removed from the test due to the microbial growth on the surface of the samples. *Salmonella typhi* and *Pseudomonas aeruginosa* were not found in all treatments over 6 days of storage at room temperature.

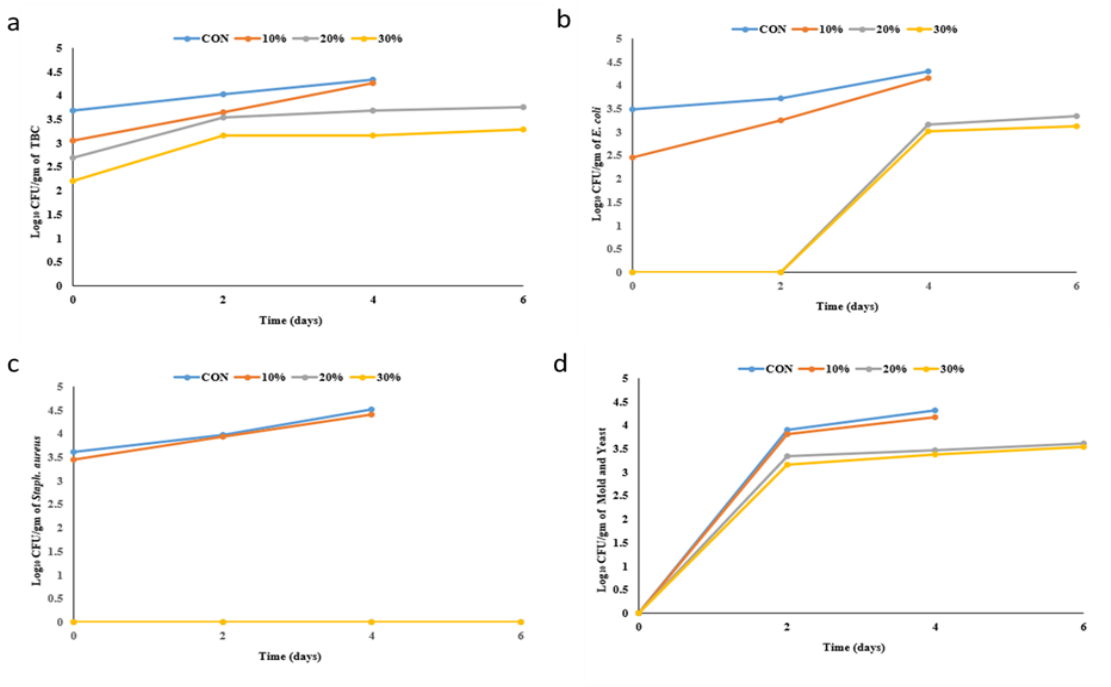

Figure 4 Microbial count of fig fruits treated with CFS of *L. plantarum* over 6 days of storage at room temperature (Log10 CFU/g).

These results in the present study are in agreement with the findings of (11), who reported that the shelf life of fruits was extended due to using biofilm coat of *B. smithii*, *which* was found suitable for extending the shelf life of strawberry up to seven days. While biofilm coats of *P. cineris* and *B. smithii* were found suitable for fig and preserved up to nine days, while untreated was deteriorateed within four days. Our results showed that the figs coating with 20% CFS and 30% distinctly had a shelf life over 6 days. While the other treatments had a shelf life of 4 days when tested for the level of TBC, *Staph. aureus*, *E. coli*, and mold and yeast. This extend might be

due to the antimicrobial activity of CFS of *L. plantarum* on the fig samples. The results were in agreement with the results of (24), who reported that LAB can inhibit the growth of fungi, and can enhance the shelf life of grapes for 6 days.

Moreover, *L. plantarum* is promising alternatives to chemical preservatives, as the antimicrobial compounds produced or excreted by *L. plantarum* and have the potential to overcome foodborne pathogens (46). *L. plantarum* is natural preservatives that are effective, safe, biodegradable, and has additional health benefits. Additionally, *L. plantarum* has been extensively used as biopreservatives for extending the shelf life of foods during storage (47).

Conclusions: In conclusion, the results from this study revealed that CFS of Lb24 had an influence on the quality and delayed shelf life of fruits samples. The highest antimicrobial activity of CFS of Lb24 was determined against pathogenic and spoilage microorganisms using the *in-vitro* method. The highest shelf life of fruits was found when the fruit samples were coated with 20% and 30% of CFS of Lb24. The microbiological results showed that apples, pears, and fig fruits coated with 20% and 30% of CFS of Lb24 had higher shelf life over 14, 12, and 6 days respectively, by reducing the growth of pathogenic and spoilage microorganisms. These impacts on the fruit samples are due to the activity of metabolites of Lb24, which have antimicrobial activity. Further study is needed, such as chemical properties and microbial counts to achieve the effect of the CFS of Lb24 on the safety and quality, and shelf life of other fruit products.

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