Production of Low-cost Lactic Acid from Dairy Wastes and Dates Wastewater and Bioactive Silver-Poly (Lactic Acid) Nanocomposite for Biological Applications

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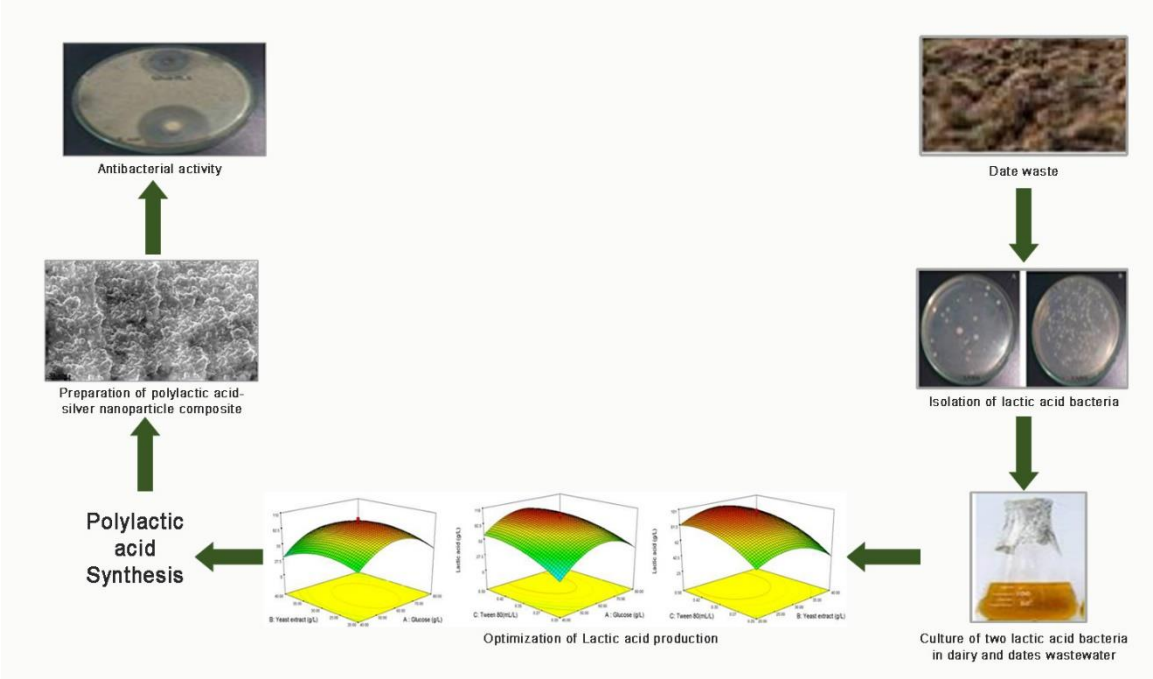
GRAPHICAL ABSTRACT



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L-Lactic acid-producing *Lactobacillus lactis* and *L. plantarum* were isolated from date wastes. The fermentation process was optimized using a one-variable-at-a-time approach. Dairy wastewater and wastewater from the date industry were utilized as low-cost culture media to produce lactic acid. The selected two bacterial strains were co-cultured in wastewater medium to produce L-lactic acid and D-lactic acid. Lactic acid production was significantly improved by glucose (carbon source), yeast extract (nitrogen source), initial inoculum level, and polysorbate 80. A central composite design and response surface methodology were used to optimize the variables and their levels to improve lactic acid yield. The supplemented yeast extract, glucose, and polysorbate 80 improved lactic acid. The predicted variables and their levels for maximum lactic acid production were glucose (67.5 g/L), yeast extract (10.28 g/L), and polysorbate 80 (0.48 mL/L). The prepared nanocomposites exhibited antibacterial activity against foodborne bacterial pathogens. The structural properties of the silver-polylactic acid nano compost materials were determined. The characterized compost materials exhibited a peak absorption wavelength of 430 nm. The silver and poly(lactic acid) were characterized using X-ray diffraction analysis and were 30 to 50 nm in size.

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Keywords: Lactic acid bacteria; Lactic acid; Poly lactic acid; Silver-polylactic acid composite; Antibacterial

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INTRODUCTION

Plastic production started in the late 1950s, and production has risen to about 8.3 to 9.1 million metric tons (Mt) globally (Pappa *et al.* 2021). These synthetic plastics are used in packing industries and are highly complicated to recycle. It has been estimated that more than 75% of prepared plastics accumulate in the soil environment or are discarded in landfills (Geyer *et al.* 2017). These plastics are slowly degraded, highly stable under environmental conditions, and form fragments of various sizes. The sizes of plastics range between 1 μm and 5 mm and are commonly termed microplastics. Microplastics are emerging pollutants that pose a serious threat to almost all living organisms in the environment. Microplastics are reported in sediments, oceans, sewage, and rivers (Huang *et al.* 2021). Emerging microplastic pollutants concern industrialists and scientists, leading

to a focus to developing eco-friendly polymers, which could be completely degraded in the soil environment. In recent years, great interest has been devoted to the development of biodegradable polymers instead of traditional petroleum-based polymers (Kian *et al.* 2019). Many waste management strategies, including composting, reusing, and recycling, decrease plastic pollution in the environment. However, the production of eco-friendly biopolymers is an alternate method because this method utilizes polymers from waste biomass.

Polylactic acid (PLA) is considered an important biopolymer, and the production of PLA from biowastes may reduce pollution in the natural environment (Balla *et al.* 2021). PLA is produced from lactic acid (LA), and LA is obtained by microbial processing using potato, cassava starch, and corn (Kervran *et al.* 2022). PLA, a linear aliphatic polyester, is considered an alternative material in the packing industry (John *et al.* 2023). PLA-based bioplastic would be an excellent substitute for the existing conventional plastics in various applications. It is widely used in the food industry, and due to its exceptional properties, it is recommended for use in the textile, medical, and agriculture industries (Sanusi *et al.* 2021). Polylactic acid can be degraded slowly, resulting in the generation of microplastics; moreover, microplastics generated from PLA do not pose any toxic effects on living organisms or soils in the environment (Ainali *et al.* 2022). In humans, it is considered a non-toxic biopolymer; hence, it is widely used in biomedical and medical applications (Vlachopoulos *et al.* 2022). In the human body, PLA degrades slowly and is converted into a non-toxic LA (Beslikas *et al.* 2011). However, the use of PLA shows certain disadvantages, such as high permeability to vapour and gas, fragility, low thermal stability, and low melt strength, while its biodegradation rate is very low compared to waste biomass such as yard waste and food waste (Mayekar *et al.* 2020).

Polylactic acid can be prepared by two different methods (chemical and fermentation methods) (Wee *et al.* 2006). In the chemical method of PLA synthesis, petrochemical resources are utilized, followed by the supplementation of hydrogen cyanide (HCN) and lactic acid catalysts (Wee *et al.* 2006). In the case of the fermentation method, renewable resources can be utilized from natural sources, including carbohydrates in the culture medium, in the production of lactic acid (Jamshidian *et al.* 2010). To prepare PLA, the pure forms of D (-) lactic acid and L (+) lactic acid are important. In the chemical method, both D (-) and L (+) lactic acids are prepared simultaneously. By contrast, in the fermentation process, only D (-) lactic acid or L (+) lactic acid is obtained (Wee *et al.* 2006). The advantages of PLA are its antimicrobial, biodegradability, and antioxidant activities (Jamshidian *et al.* 2010). The PLA is biodegradable and eco-friendly, and it can be degraded into carbon dioxide and water, which protects the environment from toxic pollutants. Anaerobic bacteria are utilized for the production of lactic acid (Ghaffar *et al.* 2014). Lactic acid bacteria utilize sugars such as lactose, glucose, and galactose without any heat generation. Batch fermentation and fed batch fermentation are more useful for the production of LA than continuous fermentation. In a continuous fermentation system, productivity is high under controlled pH and temperature (Ghaffar *et al.* 2014). Fungi and bacteria were utilized for the production of lactic acid; however, the productivity of LA is high in bacteria (Ghaffar *et al.* 2014). To decrease the production cost of lignocellulosic biomass, it was recommended due to sustainability and availability (Abdel-Rahman *et al.* 2011). The wastewater discharged from the dairy and date palm effluent pollutes the environment, and the disposal of waste poses a serious environmental threat. Considering the circular economy, the present study was performed to use dairy wastewater and date palm wastewater for the production of LA-L and LA-D lactic acids by lactic acid bacteria.

Lactic acids and silver nanoparticles were used in the present work for preparation of PLA-silver nanocomposite materials. The formulated PLA-silver nanocomposite materials were characterized, and antimicrobial properties against food-borne bacterial pathogens were determined.

EXPERIMENTAL

Isolation of Bacteria from the Date Palm Waste

De Man, Rogosa, and Lactobacilli (MRS) agar was used for the isolation of lactobacilli from date palm waste. Briefly, 5 g of date palm waste was collected from the field food processing industry and blended mechanically (0.5 cm in length). It was ground with a pestle and mortar, and a 1 mL sample was inoculated into De Man, Rogosa, and Lactobacilli broth (MRS broth) and incubated for two days at 30 ± 1 °C. It was serially diluted and streaked onto MRS agar plates. The MRS agar plates were prepared using 8 mM sodium dihydrogen phosphate and 100 mM disodium hydrogen phosphate (pH 7.2). The plates were incubated for 48 h at 37 °C. A total of 12 lactic acid bacteria (LAB) were isolated from the date palm waste (Balasubramanian *et al.* 2021).

Lactic Acid Screening-modified MRS Agar Medium Method

The bacteria isolated from the MRS agar medium (12 bacteria) were streaked on a MRS agar medium containing 2.5% and 5% $CaCO_3$. The plates were incubated for 24 h at 37 °C. $CaCO_3$ is used with MRS agar medium and is considered a neutralizing agent in LA production. The bacterial strain that showed a halo zone on MRS-modified medium was selected for fermentation (Zhang *et al.* 2020).

Lactic Acid Production and Assay

The isolated bacterial strain was inoculated into MRS broth medium and incubated for 24 h at 37 °C. The culture medium is composed of (g/L): meat extract, 5; peptone from casein, 10; (+)-glucose, 20; yeast extract, 4; dipotassium hydrogen phosphate, 2.0; di-ammonium hydrogen citrate, 2.0; manganese sulphate, 0.04; magnesium sulphate, 0.2; and sodium acetate, 5.0, and polysorbate 80. The culture was incubated for 72 h, and LA production was determined every 24 h. After 24 h, it was centrifuged at 10,000 rpm for 10 min. The amount of LA was determined using high-performance liquid chromatography (HPLC) (Park and Chang 2017). It was equipped with a C18 column and connected to a photodiode detector. About 20 µL of sample was injected into the HPLC column, and the spectrum was monitored for 10 min. The amount of lactic acid in the sample was calculated using a lactic acid calibration curve. The column temperature was maintained at 65 °C, and 5 mM H_2SO_4 was used as the mobile phase at a flow rate of 0.5 mL/min.

Morphological, Biochemical and 16S rDNA Sequencing

One L-Lactic acid producing LAB strain (LAB04) and one potent D-Lactic acid producing strain (LAB09) were selected for optimized production of lactic acid in submerged fermentation. These two bacteria were subcultured continuously on MRS agar slants and stored at 2 to 8 °C, and for long-term storage, they were cultivated in MRS broth medium and stored in 20% glycerol at -20 °C. The isolates were grown on nutrient agar plates and incubated at 37 °C for 24 h.

The morphological and biochemical tests were performed and tentatively characterized as LAB. DNA was extracted from these LAB strains, and the purity was tested using a nanodrop spectrophotometer (Thermo Fisher Scientific Ltd., Waltham, MA, USA). The universal primers (27F and 1429R) were used for the amplification of 16S rDNA. The amplified gene was sequenced and compared using the basic local alignment search tool, and gene bank accession numbers were assessed (Atif *et al.* 2020; Al-Dhabi *et al.* 2020).

Inoculum Preparation

The strains LAB04 and LAB09 were inoculated individually in an Erlenmeyer flask containing 100 mL of MRS broth medium (Himedia, Mumbai, India). The culture was incubated for 12 h, and cell density (colony-forming unit) (CFU/mL) was maintained between 10×10^6 and 10×10^7 CFU/mL. The cell density was determined by growing the cells on MRS agar plates (Wu *et al.* 2020).

Screening of Variables for the Production of LA

Lactic acid production was optimized using the traditional method. The strains LAB04 and LAB09 were used for LA production. In the one-variable approach method, five different variables (inoculums, carbon source, nitrogen source, and polysorbate 80) were selected. Carbon sources (50 g/L), such as sucrose, maltose, glucose, xylose, fructose, and lactose, were used, and beef extract, yeast extract, ammonium sulphate, and casein were used as nitrogen sources (25 g/L). Inoculum was tested at a 2 to 5% level, and polysorbate 80 was supplemented between 0.2 and 1.0%. The culture was incubated for 24 h at 37 °C, and the amount of LA was determined by the high-performance liquid chromatography (HPLC) (ThermoFisher Scientific Ltd., Waltham, MA, USA) method. Three significant variables influencing LA production were selected for the central composite design.

Central Composite Design and Response Surface Methodology

Glucose (carbon source), yeast extract (nitrogen source), and polysorbate 80 (inducer) were selected to determine the optimum response to LA production based on the one-variable-at-a-time approach. The central composite design (CCD) was comprised of 20 runs for three variables (Marraiki *et al.* 2020). Two potent LAB strains (LAB04 and LAB09) were applied for the production of LA. The experiment was performed in triplicate, and an average value was computed for analysis of variance (ANOVA). Design Expert (Version 8.0, Minneapolis, MN, USA) software was used to design the experiments and analyze the data. The three selected factors were coded according to Eq .1,

$$X_n = \frac{(X - X_0)}{\frac{X_{+1} - X_{-1}}{2}} \quad (1)$$

The variables and their levels are provided in Table 1. The experiment was performed in a 250-mL Erlenmeyer flask containing 50 mL of culture medium, 50 g/L of calcium carbonate (to control pH), and 5% inoculum. The culture was incubated in an orbital shaker incubator at 130 rpm for 24 h at 37 °C. After 24 h, LA production was determined by the HPLC method.

Table 1. Analysis Of Variables Influence on Lactic Acid Production in Submerged Fermentation Using Lactic Acid Bacteria

Name	Units	Low Actual	High Actual	Mean
Glucose	g/L	40	80	60
Yeast extract	g/L	20	40	30
Polysorbate 80	mL/L	0.2	0.5	0.35

Production of Lactic Acid in Fermenter

Batch fermentation was performed in a 7-L fermenter (Applikon, Delft, The Netherlands). The pH of the fermenter was maintained at 6.5 using NaOH (5 N), and a fermentation experiment was performed using milk wastewater and date processing wastewater. The milk wastewater was derived from cheese processing waste, and date wastewater was obtained from date syrup processing waste. Production of lactic acid in fermentation was performed using manual inoculation and sampling. The pH of the medium was controlled using 50 g/L $CaCO_3$. The selected two bacterial strains were inoculated (0.400 to 0.500 OD at 600 nm). The total operating volume was 4 L, with 130 rpm, and a pH of 6.5. The temperature was automatically controlled. In the culture medium, the optimum levels of glucose, yeast extract, and polysorbate 80 were supplemented based on statistical optimization and experimental results. The experiment was performed for 48 h, and the sample was withdrawn from the fermenter. It was centrifuged at 5000 ×g for 10 min, and the culture filtrate was filtered using a 0.20 μm filter. The concentrated LA was stored at -20º for further studies. The amount of LA was determined, and the result was expressed as g LA/L.

Synthesis of Polylactic Acid (PLA) using LA

The PLA was prepared by the direct polycondensation method, as previously described. A white crystalline powder was obtained, and the final product was dissolved in chloroform. It was further purified in methanol, filtered, and dried at 60 °C under vacuum. The material was poured into clean Petri dishes, dried at 80 °C for 12 h, and the solvent was removed. Then, it was removed from the Petri dishes and maintained in desiccators for further chromatinization (Wang *et al.* 2009).

Preparation of Silver-PLA Nanocomposite Materials

Chloroform and dimethylformamide (DM)F were prepared at a 1:8 ratio (v/v), where the final concentration was 10%, and $AgNO_3$ was added (1.6 g). The $AgNO_3$/PLA suspension was continuously stirred for two days under an ice bath, and 10 mL of $NaBH_4$ (aqueous solution) was added dropwise for 120 min. The Ag-NPs developed, and the stable Ag-NP colloid was obtained due to the interactions of the hydroxyl groups of the PLA (Roucoux *et al.* 2002). The colloid material was removed from the aqueous phase, and silver ions were removed by shaking. It was dried at room temperature, and the Ag/PLA-NC films were suspended in chloroform. It was dried using the solution-casting technique and used for further experiments (Lu *et al.* 2008).

Determination of Antibacterial Activity

The antibacterial activity of the prepared silver-PLA nanocomposite was *in vitro* tested against bacterial strains (*Staphylococcus aureus* (ATCC 12600), *Escherichia coli* (ATCC 13706), *Enterobacter aerogenes* (ATCC 13048), and *Pseudomonas aeruginosa* (ATCC 25619)). Mueller-Hinton (MH) agar medium was prepared. Pathogenic strains cultivated in MH broth medium were swabbed on it. After 30 min, Ag/PLA-NC film was cut and placed on MH agar medium, incubated for 24 h at 37 °C, and the zone of inhibition analyzed (Atif *et al.* 2020). Chloroamphenicol was used as a positive control.

Characterization of Silver-PLA Material

The prepared Ag/PLA nanocomposite material was characterized by UV-visible spectroscopy (M/s. Shimadzu, Tokyo, Japan), X-ray diffraction (XRD) analysis (Model D8, M/s. Bruker, Germany), and scanning electron microscopy (SEM) (JSM-7600F,M/s. Jeol Ltd., Tokyo, Japan). The morphology of the Ag/PLA nanocomposites was determined using SEM, and XRD analysis was performed using a Philips, X'pert diffractometer that featured Cu Ka radiation. The degree of crystallinity, was calculated using Eq. 2:

$$\chi_c = A_c / A_c + A_a \quad (2)$$

where A_c=crystallized areas and A_a amorphous areas on the x-ray diffractogram.

UV-VIS spectra were performed using a UV-visible spectrophotometer (Shimadzu, Tokyo, Japan). The scheme of PLA-silver nanocomposite materials preparation, analysis, and antimicrobial properties were depicted in Fig. 1.

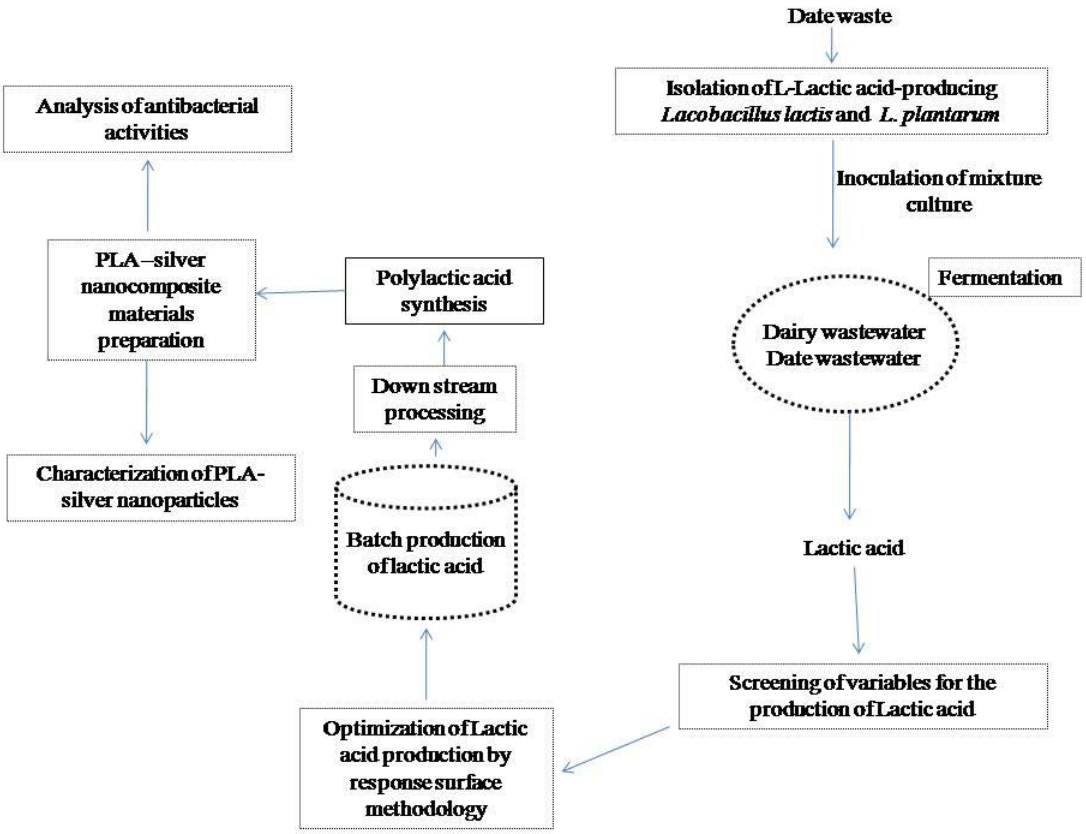


Fig. 1. PLA-silver nanocomposite materials preparation, analysis of composite materials and antimicrobial properties

Statistical Analysis

The amount of LA production was expressed as mean ± standard deviation, and the significance level was determined using one-way analysis of variance (ANOVA). Statistical analysis was performed using Statistical package for social sciences (SPSS) software (Chicago, IL, USA).

RESULTS

Isolation of Lactic Acid Bacteria from the Date Palm Waste

A total of 12 LABs were isolated from the date palm waste. Among 12 bacterial strains, 10 isolates were Gram-positive and fermented glucose. These glucose hydrolyzing isolates released acid in the culture medium. The rapidly growing glucose hydrolyzing bacterial strains were selected for batch production of LA.

Batch Production of LA

The culture medium was centrifuged, and the filtered cell-free supernatant was used for the determination of LA. The HPLC spectrum of LA is depicted in Fig. 2. The LA is the major end product produced using available nutrients in the wastewater. The isolated LAB strain, LAB04 produced L-lactic acid, and the strain LAB09 produced D-lactic acid. The amount of LA in the extract was determined using HPLC, and the result is described in Table 1. Bacterial biomass and LA production were found to be high in the LAB strains LAB04 and LAB09.

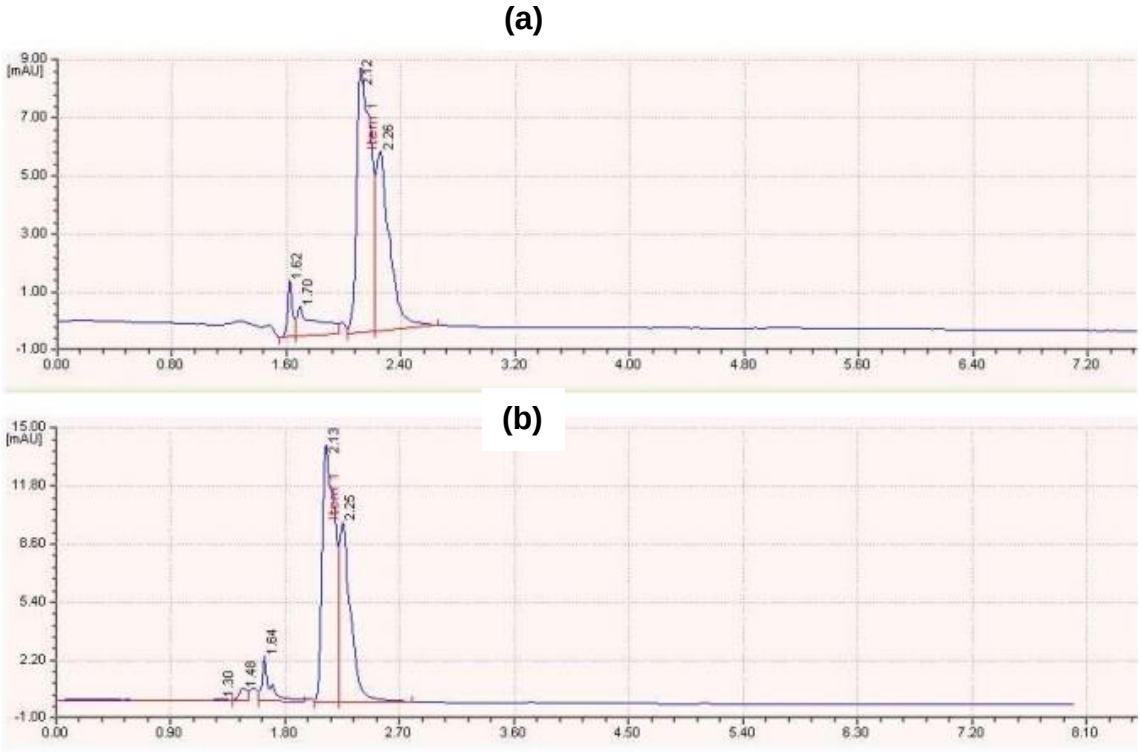


Fig. 2. High Performance Liquid Chromatography profile of lactic acid from the bacterial strains: (a) lactic acid standard, (b) sample

Table 1. Bacterial Biomass and LA Production by the LAB Strains Isolated from the Date Palm Waste

	Biomass (Dry Weight)	LA
LAB Strains	(g/mL)	(g/L)
LAB01	0.052 ± 0.005	14.02 ± 0.06
LAB02	0.0361 ± 0.11	18.5 ± 0.09
LAB03	0.0276 ± 0.05	4.01 ± 0.02
LAB04	0.076 ± 0.06	29.1 ± 1.1
LAB05	0.026 ± 0.004	16.4 ± 1.1
LAB07	0.0472 ± 0.02	19.5 ± 0.12
LAB08	0.028 ± 0.01	11.7 ± 0.06
LAB09	0.065 ± 0.06	33.5 ± 0.06
LAB10	0.022 ± 0.002	19.4 ± 1.1
LAB012	0.015 ± 0.01	2.7 ± 0.02

Table 2. Morphological and Biochemical Properties of LAB Strains Isolated from the Date Palm Waste

	Bacterial Strains	
Characters	LAB04	LAB09
Gram straining	Positive	Positive
Shape	Rod	Rod
Surface	Smooth	Shiny
Colony color	Creamy white	Creamy white
Opacity	Opaque	Opaque
Elevation	Convex	Convex
Margin	Entire	Entire
Indole	Negative	Negative
Oxidase	Negative	Negative
Catalase	Negative	Negative
Citrate	Positive	Positive
Gas production	Positive	Positive
Urease	Negative	Negative
Triple sugar iron	Negative	Negative
Methyl red	Positive	Positive
Glycerol	Positive	Positive
D-Glycose	Positive	Positive
Sorbitol	Positive	Negative
D-Fructose	Positive	Positive
Mannitol	Positive	Positive
D-xylose	Positive	Positive

Morphological and Molecular Characters of LAB Strains

Colony morphology, biochemical analysis, and molecular characterization were performed for the two selected LAB strains for optimization. The morphological and biochemical characters of LA-producing LAB strains are described in Table 2 and Fig. 3. These two strains were characterized by 16S rDNA sequencing, and strain LAB04 was characterized as *Lactobacillus lactis* and strain LAB09 was determined to be *L. plantarum*.

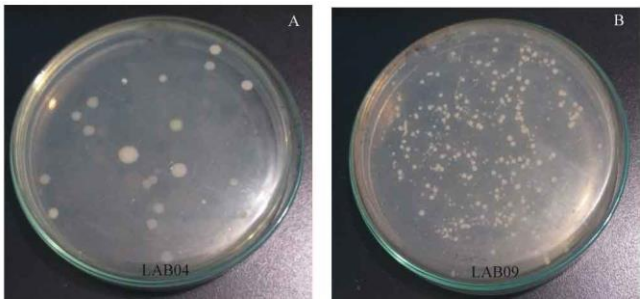
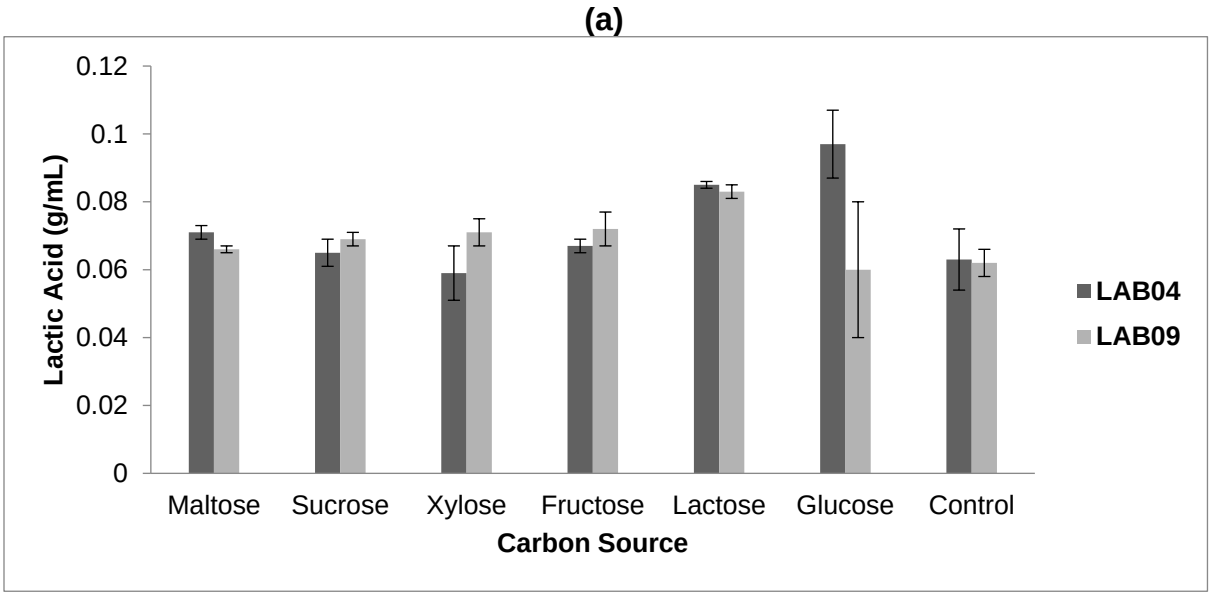


Fig. 3. Growth of *Lactobacillus lactis* LAB04 (A) and *Lactobacillus plantarum* LAB09 (B) on nutrient agar plates. The plates were incubated at 37 ºC for 24 h and visible growth was observed.

Optimization of LA Production by Traditional Method

LAB04 and LAB09 were selected for optimization studies. The supplemented carbon sources in dairy wastewater and palm wastewater medium improved LA production (Fig. 4a) compared to the control ($p<0.05$). The supplemented nitrogen sources significantly influenced LA production ($p<0.05$) (Fig. 4b). Inoculum is one of the significant factors that directly affected LA production, and 4% was optimal for LA production ($p<0.05$) (Fig. 4c). The supplemented polysorbate 80 improved LA production (Fig. 4d).



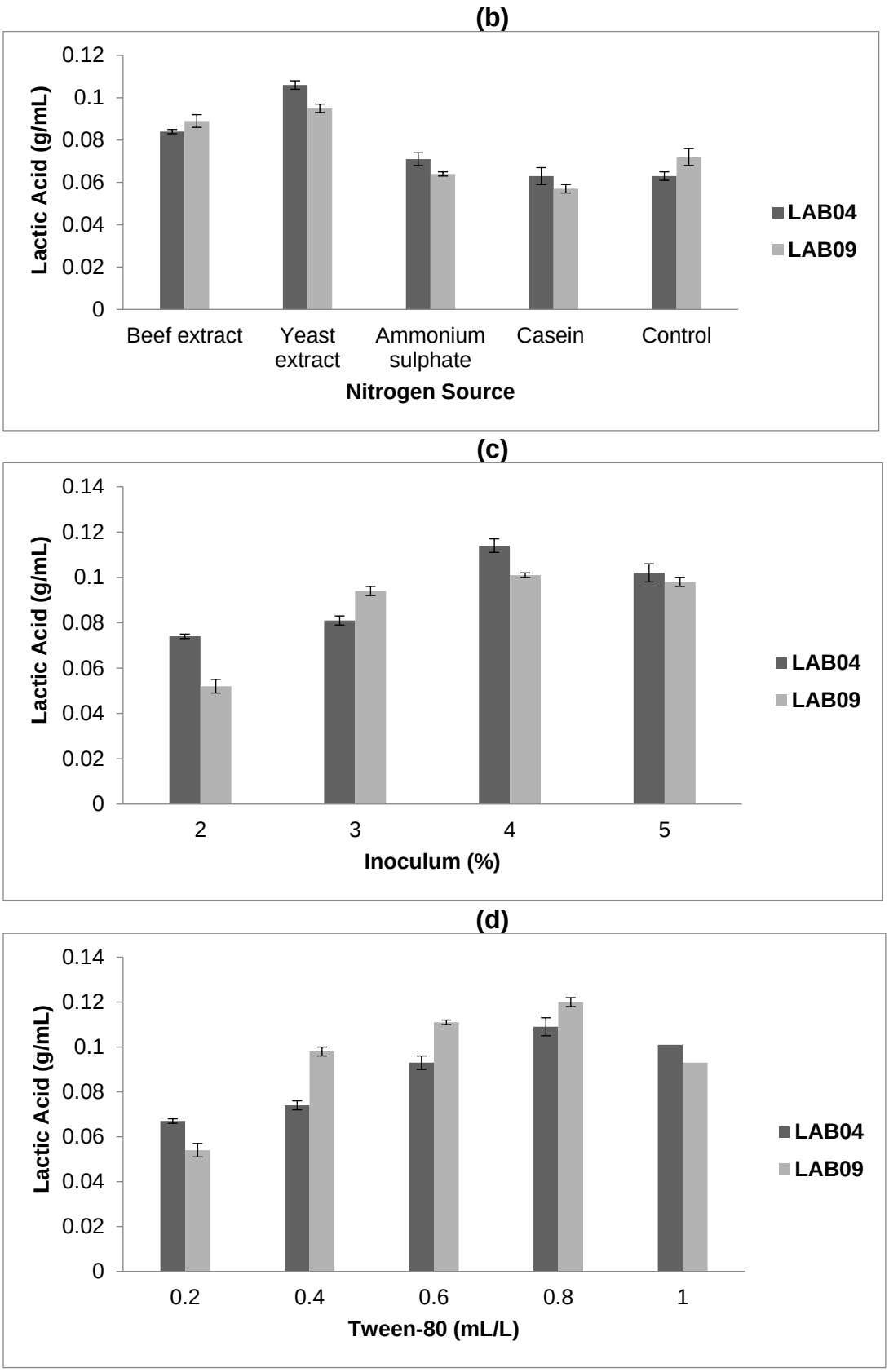


Fig. 4. Effect of carbon (a), nitrogen (b), inoculums (c), and polysorbate 80 (d) on lactic acid production

Optimization of Lactic Acid Production Using Central Composite Design

A central composite design and response surface methodology were used to optimize the significant variables affecting LA fermentation. The designed coded values and response (LA production) for co-culture (LAB04 and LAB09) are shown in Table 3. The amount of LA in the culture medium varied significantly. The coded variables and variation in the response revealed the significance of optimization for LA production. Fisher's test was performed to determine the significance level, and the model value was $p < 0.05$, which showed statistical significance (Table 4). The independent variable polysorbate 80 exhibited a significant effect ($p<0.05$), and the model p value was 0.0004. The designed model was fitted to a quadratic model, and the model F-value was 11.18. The interactive effects (A^2 and B^2) are significant. The lack of fit F-value of this model was 1.86, and the coefficient estimate was positive for glucose and Tween-80. The decreased level of yeast extract could improve the LA yield. The R^2 value of this model was 0.9, and the adjusted R^2 model was 0.82. The final equation in terms of coded factors is as follows:

$$\text{LA production} = +92.98+3.72 \times A-4.45 \times B+14.96 \times C+2.29 \times A \times B-5.04 \times A \times C+1.71 \times B \times C-33.44 \times A^2-16.79 \times B^2-12.34 \times C^2$$

Table 3. Central Composite Design and Response Surface Methodology on Lactic Acid Production

Runs	Glucose (A) (g/L)	Yeast Extract (B) (g/L)	Polysorbate 80 (C) (mL/L)	LA (g/L)
1	-1	-1	1	50.5
2	-1	-1	-1	4.7
3	0	0	0	98
4	-1	1	1	23.7
5	0	-1.68179283	0	59.2
6	0	0	-1.68179	23.5
7	1	1	-1	17.5
8	0	0	0	95.2
9	0	0	1.681793	98.4
10	1.681792831	0	0	0.7
11	0	1.681792831	0	37.5
12	1	-1	1	28.5
13	0	0	0	98
14	0	0	0	100.5
15	0	0	0	98.2
16	1	1	1	50
17	1	-1	-1	42
18	0	0	0	67
19	-1	1	-1	10.2
20	1.681792831	0	0	1.82

Table 4. ANOVA for Response Surface Quadratic Model for the Production of Lactic Acid

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	23432.4187	9	2603.602078	11.18446798	0.0004
A-Glucose	188.8410604	1	188.8410604	0.811217203	0.3889
B-Yeast extract	270.6348282	1	270.6348282	1.162584174	0.3063
C-Tween-80	3055.221474	1	3055.221474	13.1245197	0.0047
AB	41.86125	1	41.86125	0.179826178	0.6805
AC	203.01125	1	203.01125	0.872089036	0.3724
BC	23.46125	1	23.46125	0.100784064	0.7574
A^2	16115.47033	1	16115.47033	69.22830624	< 0.0001
B^2	4063.309608	1	4063.309608	17.45503148	0.0019
C^2	2193.310674	1	2193.310674	9.421951695	0.0119
Residual	2327.872977	10	232.7872977		
Lack of Fit	1513.944643	5	302.7889287	1.860046618	0.2561
Pure Error	813.9283333	5	162.7856667		
Cor Total	25760.29168	19			

Figure 5 shows that a concentration of <20 g/L of yeast extract improved lactic acid. The increasing concentration of glucose improved LA production, and 75.4 g/L was optimum for LA production. Among the three variables, supplemented polysorbate 80 significantly improved LA production.

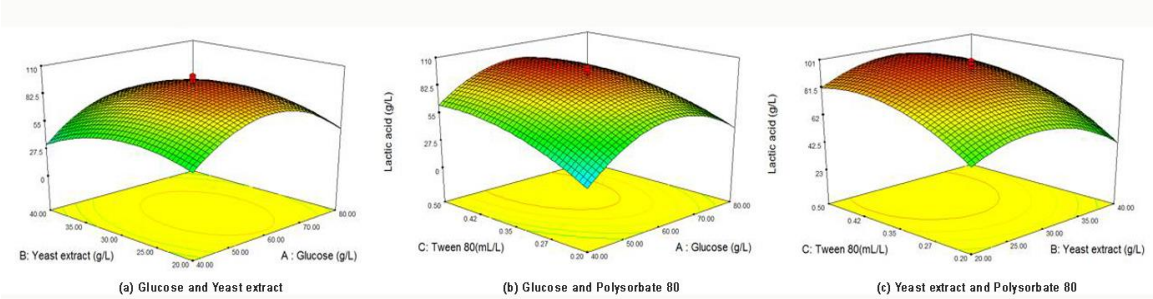


Fig. 5. Response surface graph shows the interactive effect between glucose and yeast extract (a), glucose and polysorbate 80 (b), and yeast extract and polysorbate 80 (c).

Production of Lactic Acid in a Fermenter

Batch fermentation was used to produce LA using the LAB strains LAB04 and LAB09. The selected bacterial strains utilized sugars from the milk wastewater and palm wastewater and converted them to LA. The supplemented optimized medium improved the yield twofold compared to the unoptimized medium. The productivity was improved in the optimized medium, but LA productivity declined after two days of fermentation, revealing a decrease in nutrients in the wastewater. The productivity was maximum after 20 h of culture (3.98 g/L/h).

Antibacterial Activity of Silver-PLA Nanocomposite Materials Against Food-Borne Bacterial Pathogens

The prepared silver-PLA nanocomposite exhibited antibacterial activity against food-borne bacterial pathogens such as *S. aureus*, *E. coli*, *E. aerogenes*, and *P. aeruginosa*. The zone of inhibition varied between 15 ± 2 and 21 ± 1 mm. The silver-PLA nanocomposite material showed maximum activity against *E. coli* (21 ± 1 mm zone of inhibition), and the least activity was observed against *E. aerogenes* (15 ± 2 mm zone of inhibition). It also showed activity against *S. aureus* (18 ± 2 mm zone of inhibition) and *P. aeruginosa* (17 ± 1 mm zone of inhibition) (Fig. 6a and b).

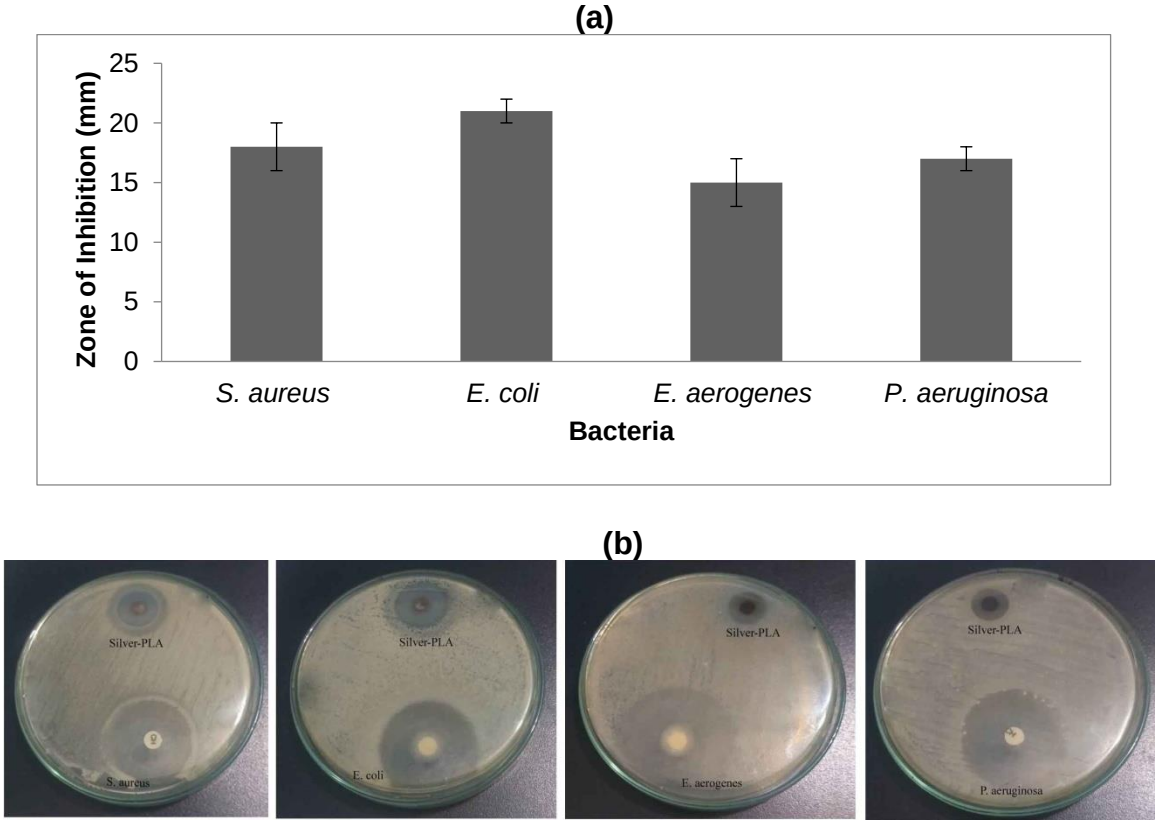


Fig. 6. Antibacterial activity of Silver-PLA nanocomposite materials against food-borne bacterial pathogens. (a) Antibacterial activity was expressed as zone of inhibition (mm); (b) Antibacterial activity of silver-PLA nanocomposite materials on Mueller Hinton agar plates against bacteria.

Characterization of Silver-PLA Nanocomposites

The prepared Ag/PLA nanocomposite material was characterized by analytical methods. Figure 7 shows the relationship between the absorbance of the sample and the wavelength of the compost material. UV-vis absorption was performed within the wavelength range 300 to 800 nm for the prepared Ag/PLA nanocomposite material. The electronic transition of the Ag/PLA nanocomposite occurred in the visible region with λ_{max} values ranging from 360 to 580 nm. The fabricated Ag/PLA nanocomposite film was broader in the visible range revealing that the PLA in the film contributed less to the overall UV absorption in the visible range. The XRD analysis was used to determine the crystal structure of the prepared silver-PLA nanocomposite material. The atomic arrangement revealed the presence of silver ions and PLA (Fig. 8) with various intensities.

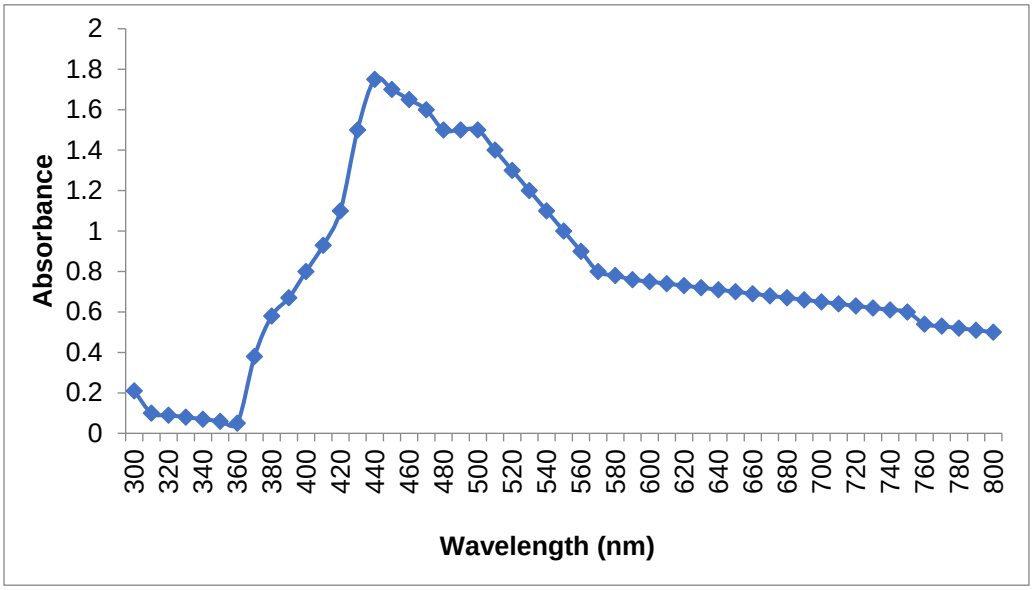


Fig. 7. UV–visible absorbance spectra of Ag/PLA nano composite material

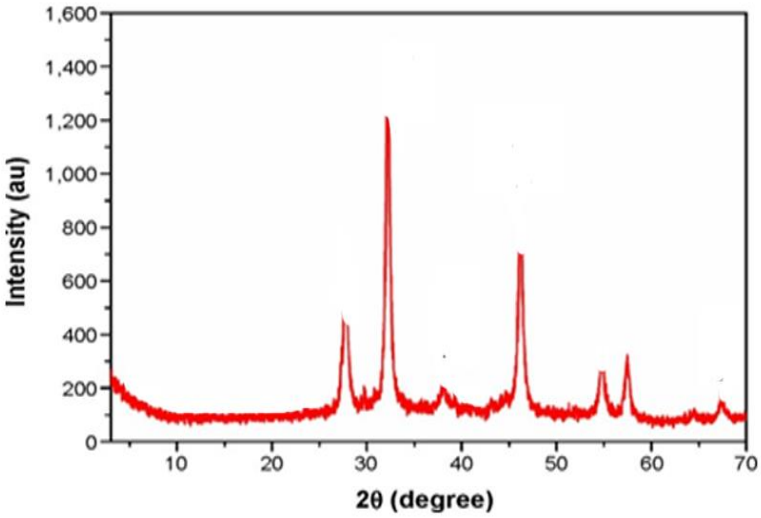


Fig. 8. X-ray diffraction analysis of prepared silver-PLA nanocomposite material

The XRD patterns of Ag/PLA nanocomposite material showed 32.4% crystallinity. As shown in Fig. 5, peaks reveal that the PLA-based composites were crystalline materials. The diffraction peak was observed at approximately 2θ=28°, and was ascribed to the crystal structure of PLA. Other intense peaks were detected at 32°, 47°, 56°, and 58°, indicating the characteristics of the composite materials. Scanning electron microscopy (SEM) images of microstructures of the Ag/PLA nanocomposite films are described in Fig. 9. The nanocomposite materials were aggregated, with particle sizes ranging from 30 to 50 nm. The presence of aggregates indicated the incomplete dispersion of the nanoparticles in the film. Scanning electron microscopy study revealed small voids on the fractured surface of Ag/PLA nanocomposites.

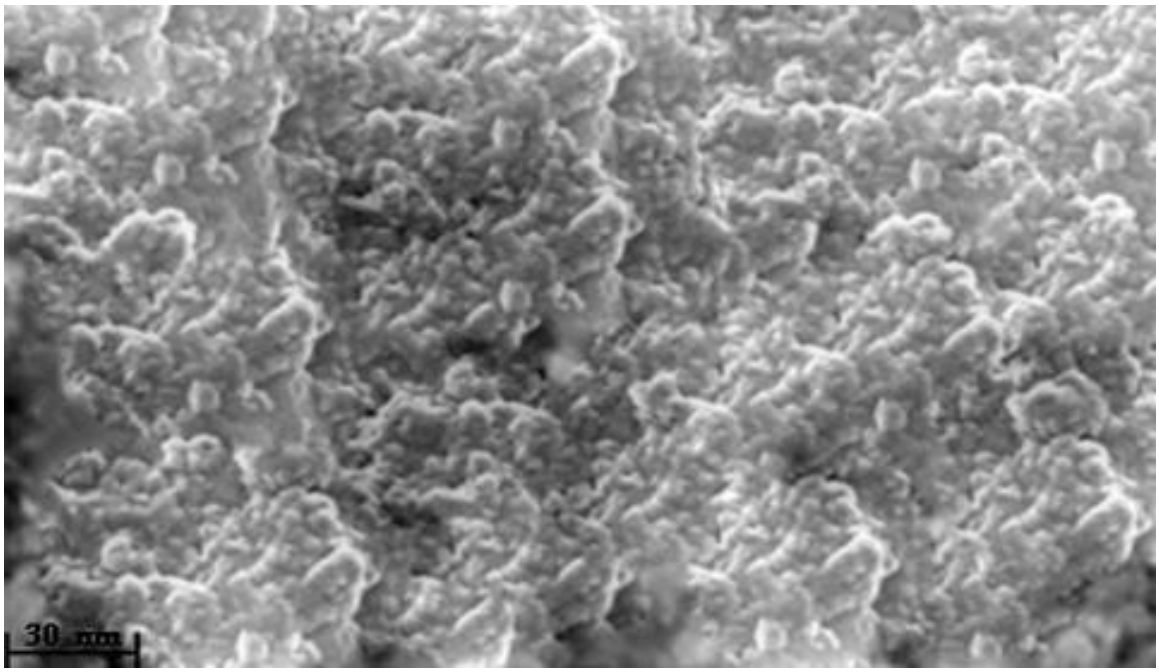


Fig. 9. SEM Images of Ag/PLA nano composite material

DISCUSSION

A total of 12 LABs were screened and isolated for LA production by the MRS agar plate method. A clear zone was observed around the bacterial colony, which was considered LA-positive. It was found that 10 bacteria fermented glucose. All these 10 isolates were subjected to LA production, and the strains LAB04 and LAB09 showed significantly higher LA production than other strains. All the bacterial strains showed a decline in LA production after 48 h, which may be due to the reutilization of LA produced by bacteria (feedback mechanisms) (Xu and Xu 2014). Milk wastewater and date processing wastewater were used as the culture medium to produce LA. The production of low-cost LA to minimize the production cost of the product is an important issue in the fermentation bioprocess. The formulation of low-cost culture media must meet the industrial requirements to improve the growth of bacteria selected to produce LA. The low-cost culture media may improve product yield and biomass production; however, the toxic heavy metals or other effluents may affect the growth of bacteria. Hence, the selection of low-cost and non-toxic food processing wastes is an important approach in bioprocessing. Wastewater discharge from the fruit or food processing industries consists of various residues that favor bacterial biomass production (Sathya *et al.* 2023). Corn cobs and dairy wastewater (David *et al.* 2022), dairy wastewater (Moradi *et al.* 2023), and cassava wastewater (Coelho *et al.* 2010) were used to produce LA.

In this study, two LABs were selected to produce LA in submerged fermentation using dairy wastewater and palm wastewater. The LAB utilized available carbon and nitrogen sources from the wastewater for biomass conversion and metabolite production. Unlike other bacterial genera, LAB are fastidious bacteria with respect to available nutrients and growth. The MRS medium is considered an ideal culture medium to produce LA by LAB; however, its high cost and hazardous downstream processing make it unviable for commercial productions. In this study, the selected LAB strains (*L. lactis* and *L.*

plantarum) were used for optimized production of LA. To improve the production of LA, carbon, nitrogen, and polysorbate 80 were supplemented. Lactic acid bacteria required sugars for lactic acid production. In addition, vitamins, minerals, nitrogen and inorganic compounds were useful for bacterial growth, maintenance, and lactic acid production. The fermentation capacity of the sugars varied based on bacteria. Hence, lactic acid production varied among bacteria (Zhang *et al*. 2020). In LAB, the optimal growth range varied based on strains, however 30 to 45 °C was reported as the optimal temperature. Likewise, pH was reported as a major factor influencing lactic acid production and the LAB can grow in pH values between 4 and 7. In LAB, lactic acid production was growth-associated and the pH value of the liquid medium decreased due to lactic acid production (Al-Dhabi *et al*. 2020). The supplemented carbon and nitrogen sources enhanced 1.1-fold LA production. Polysorbate 80 significantly improved the production of LA and glucose (carbon source), and yeast extract (nitrogen source) improved LA production. AristimuñoFicoseco *et al*. (2018) reported that glucose, yeast extract, Mg and Mn salts, sodium acetate, ammonium citrate, and polysorbate 80 are required for the growth of probiotic LAB. The supplemented maltose, sucrose, xylose, fructose, and lactose also improved the growth of LAB and LA production. In LAB, osmotic stress is one of the important factors, and increased concentrations of sugars affect bacterial growth (Linko and Javanainen 1996). However, in the current study, the microbial growth was not affected at higher concentrations of glucose (>50 g/L) in the culture medium. In the current study, the supplemented yeast extract significantly improved LA production. It has been reported that the C/N ratio significantly affects LA production (Wang *et al.* 2015).

Central composite design and response surface methodology were used for optimization of LA production using dairy wastewater and date wastewater. In the current study, the model P-value was less than 0.05, which shows that the designed model was statistically significant. The increased F-value and the p-value<0.05 were considered significant models (Law *et al.* 2020). The R^2 value of the model shows the reliability of the designed model and is highly recommended, and a value >0.9 reflects good model quality (Jeong and Kim 2020). In response surface models, the differences in the adjusted R^2 value should be less than 0.2, and the adjusted value obtained in the current study showed the reliability of the model design (Ying *et al.* 2020). In statistical optimization methods, the coefficient of variation (CV) is used to analyze the variance of the result, and a variance within 10% is recommended for any model design and is considered reliable and accurate (Kim and Yeom 2020). The adequate precision of the designed model was >4, which measures the signal-to-noise ratio. The increased adequate precision value indicated that the designed model is useful to study the designed space (Behera *et al.* 2018). The response surface method graph was used to analyze the interaction between selected variables (Sari *et al.* 2020).

The PLA was prepared by the direct polycondensation method, and the $AgNO_3$/PLA nanocomposite material was characterized. The PLA production was performed by direct polycondensation (Kim and Woo 2002), the microwave-assisted single-step method (Nagahata *et al.* 2007), and the 2-step direct polycondensation process (Pivsa-Art *et al.* 2013). The prepared Ag/PLA nanocomposite material was characterized, and the λ_{max} value was observed between 360 and 580 nm. The XRD analysis revealed crystallinity of composite materials. The particle size of the composite material ranged from 30 to 50 nm. Chen *et al.* (2012) observed the strongest diffraction peak for PLA at $2\theta = 16°$, which corresponds to the reflection of α-form crystals, which was similar without study. In addition to this peak, other intense peaks observed at $2\theta = 22°$, 19° were strongly

consistent with the crystal structure of the PLA sample. The present findings revealed that the addition of silver nanoparticles with PLA did not affect the crystallinity of the composite materials. The peak in the XRD spectrum revealed the crystallinity of the composite materials. The decreasing trend of crystallinity of composite was reported previously with increased addition of inorganic or organic silica with PLA. Wang *et al*. (2015) stated decreasing crystallinity of polyvinylamine (PVAm)/silica composite by increased addition of inorganic silica. Yan *et al*. (2007) reported decreased peak intensity due to the poor crystallized macromolecules in XRD analysis. The metastable and small crystals might be strongly confined by the rigid silica material, thus showing in the decreased degree of the composite. PLA and nanoparticles were used previously for the preparation of biodegradable polymer composite materials. The supplemented nanoparticles improved the physical and mechanical properties of biodegradable film and biological properties. Dorigato *et al*. (2012) reported the improved mechanical properties of PLA-silica nanoparticles. Ketabchi *et al*. (2016) applied cellulose nanoparticles with polylactic acid and reported improved mechanical properties. Pietrzykowska *et al.* (2020) prepared nanocomposites using polylactic acid and hydroxyapatite nanoparticles. Luo *et al.* (2009) prepared nanocomposites using poly(lactic acid) and functionalized TiO_2 to improve their mechanical and biological properties.

The prepared silver-PLA nanocomposite exhibited antibacterial activity against food-borne Gram-positive and Gram-negative bacteria. The antimicrobial properties of polylactic acid-silver nanoparticle nanocomposite were described previously, and the screened *Escherichia coli* were susceptible to nanocomposite material (Rashedi *et al.* 2020). Vidakis *et al*. (2020) reported significant antimicrobial activity of polylactic acid (PLA)-silver nanoparticle against *Escherichia coli* and *Staphylococcus aureus*. Khan *et al*. (2016) prepared silver nanoparticle-loaded hemp hurd/poly (lactic acid) materials and antibacterial activity was reported. The antibacterial mechanism of silver nanoparticle is that the nanoparticle might accumulate in the cytoplasmic membrane of bacteria, thus inducing membrane permeability; such alteration of plasma membrane function may lead to cell death (Rhim *et al.* 2013; Tsou *et al.* 2017).

CONCLUSIONS

1. Dairy wastewater and date palm wastewater are rich sources of carbon, nitrogen, minerals, and other micronutrients.
2. The present results suggest that wastewater could help reduce the production cost of lactic acid and can be considered as an alternate substrate.
3. Response surface methodology improved lactic acid production (75.4 g/L).
4. The co-culture of lactic acid bacteria produced lactic acid in the fermenter with an increased productivity.
5. The prepared silver-PLA composite was effective against food borne bacterial pathogens.
6. The silver-PLA nanocomposite material showed maximum activity against *E. coli* (21 ± 1 mm zone of inhibition), and *S. aureus* (18 ± 2 mm zone of inhibition).

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