

PROBIOTIC PROSPECTS FROM PEELS: UNLOCKING GUT-FRIENDLY MICROBES IN FRUIT AND VEGETABLE WASTES

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ABSTRACT

The increasing interest in gut health has led to a surge in both Prebiotic and probiotic intake. While prebiotics are indigestible food elements that specifically promote the growth or activity of helpful microbes in the stomach, probiotics are the live bacteria that, when supplemented in adequate proportions, aids the host's wellness. The purpose of this investigation is to isolate and characterize probiotic bacteria from a fruits and vegetables peel, an underutilized food manufacturing byproduct. Native bacterial strains with probiotic potential, such as the capacity to withstand gastrointestinal disorders, suppress harmful bacteria, and satisfy fundamental safety standards for probiotic use, are found in fruit and vegetable peels. The peels from banana, orange, red apple, cucumber, and cabbage were collected under aseptic conditions. After homogenization, samples were serially diluted and cultured on de Man, Rogosa, and Sharpe (MRS) agar, incubated anaerobically at 37°C for 48 hours. Isolated colonies were preliminarily screened by Gram staining and catalase testing. The isolated LAB strains were characterized based on their ability to survive under simulated gastrointestinal conditions and bacteriocin production optimization. This study successfully identified a several strains of lactic acid bacterial strains, which exhibited strong probiotic potential. Out of the four bacterial isolates, two strains exhibited survival at pH 1.5-2.0 and 1.5% bile salts, indicating strong acid-bile resistance. Antibiotic susceptibility profiling confirmed sensitivity to most clinically relevant antibiotics, with no transferable resistance genes detected. Fruit and vegetable peels have the potential to be an affordable source for isolating probiotic bacteria.

Keywords: Probiotics, isolation, LAB, fruit and vegetable peels, Bacteriocin, Antibiotic sensitivity

INTRODUCTION

The increasing global demand for probiotics, coupled with the need for sustainable biotechnological solutions, has prompted the exploration of alternative microbial sources beyond traditional dairy environments. Fruit and vegetable peels, often considered agro-waste, are nutrient-dense substrates that naturally harbor diverse microbial communities. Despite their potential, these by-products remain underexplored as reservoirs of probiotic bacteria. Recognizing this gap, the present study focuses on isolating and characterizing beneficial lactic acid bacteria from selected fruit and vegetable peels, with the aim of identifying strains that exhibit essential probiotic traits—such as acid and bile tolerance, antimicrobial activity, and safety for human consumption.

Probiotics are live bacteria that support the organism's gut microbial balance while it passes through the body. In Greek probiotic is known as "protokos," which means "for life," (Lilly and Stillwell, 1965). Identification of the probiotic bacteria is determined as the concentration of viable cells average should contain CFU-10⁶ cells. The finding of novel probiotics emphasizes strain selection, culture's ability to survive during the isolation and storage of biomass. Probiotic bacteria have been used continuously for the past 10 years by both people and animals for their health benefits. Based on the health benefits, probiotics are used instead of antibiotics as an alternative medicine to fight against infectious diseases in humans and animals (AFRC, 1989). Consuming living microorganisms, known as probiotics, can enhance the microbial balance in the gut. These microbes are mostly made up of *Eubacteria*, *Lactobacilli*, and *Bifidobacteria*. By shielding the digestive system from harmful infections, they aid in the maintenance of gastrointestinal health and the management of inflammatory bowel illnesses.

Lactobacillus are gram-positive, catalase-negative, non-spore-forming rods that belong to the lactic acid bacteria group (Bernardeau et al., 2008). Six isolates from the *Lactobacillus* genera *Lactobacillus brevis*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus helveticus*, *Lactobacillus lactis*, and *Lactobacillus plantarum*—with populations of 10⁷–10⁹ cells/g of wet potato pulp were characterized by Mayer and Hillebrandt (1997). In 2006, Zlatica Kohajdova and colleagues investigated the feasibility of lactic acid fermentation for vegetable juices made from cabbage, tomatoes, pumpkin, and courgette. The authors found that all studied vegetable juices were appropriate for lactic acid fermentation.

Probiotics have gained prominence for their role in supporting gut health, immune function, and overall well-being. Traditionally derived from dairy products, the search for novel, sustainable, and non-dairy sources of probiotic bacteria has intensified—particularly in response to dietary restrictions, environmental concerns, and growing interest in plant-based alternatives. In this context, fruit and vegetable peels offer a compelling opportunity. Often discarded as waste, these peels are rich in fibers, polyphenols, and nutrients that can support the growth of beneficial microbes (Morelli, 2012). Yet, their potential as a natural reservoir for probiotic strains remains largely untapped. This study therefore focuses on isolating and evaluating lactic acid bacteria from common fruit and vegetable peels to assess their viability and safety as next-generation probiotic candidates.

Fruits are essential to human nutrients and wellness, and there is a constant demand for them. However, during fresh food consumption and food processing massive amounts of different fruit unused materials (skin, kernel, and spoiled fruits) are formed (Ilce Gabriela and Girish 2017). The majority of fruits have between 10% and 20% peel, which is wasted during post-harvest mechanisms and bio processing technology. Because of their advantageous nutritional and functional qualities. These fruit scraps have the potential to be converted into beneficial compounds. Fruit peels (FP) contain a number of important and functional components, such as dietary fibers (DF), polysaccharides, polyphenols, and flavoring agents. Which are beneficial to human health as well as in the food and pharmaceutical industries (Bharat Helkar and Sahoo 2016) as potential antioxidants and prebiotics. Numerous reasons have contributed to the rise in demand for functional foods, including growing awareness of the decline in personal health caused by a busy lifestyle with bad food choices and little exercise, a rise in the prevalence of self-medication, and an increase in knowledge (El-Sohaimy and Hussain, 2023).

According to FAO/WHO (Tomé et al., 2002), probiotics are live bacteria that consumption of prebiotics leads to improving a health advantage beyond those of basic nutrition. The majority of probiotic foods that are now on the market are made from milk. Nowadays, consumers prefer plant origin dietary supplements and other alternative sources of probiotics with nondairy origins due to the detrimental effects of cholesterol on health.

From a broader perspective, these findings contribute to the growing narrative around circular bioeconomy and waste valorization. Utilizing fruit and vegetable peels not only addresses sustainability by reducing agro-waste but also opens up

low-cost avenues for local probiotic production, particularly in resource-limited regions where access to commercial probiotics is constrained.

METHODOLOGY

Collection and processing of the samples

Peels were collected from five commonly consumed fruits and vegetables: banana (*Musa spp.*), orange (*Citrus sinensis*), red apple (*Malus domestica*), cucumber (*Cucumis sativus*), and cabbage (*Brassica oleracea*). The samples were then thoroughly cleaned with tap water and surface-sterilized using 70% ethanol and aseptically cut and homogenized in sterile phosphate-buffered saline (PBS) for microbial isolation (El-Sohaimy and Hussain, 2023).

Phytochemicals and Antioxidant Properties of Fruit and vegetable peel extract

With a few minor modifications, the extract was prepared by the method of Wichienchot *et al.*, 2010. The outer layer of the fruits and vegetables were washed with tap water before cutting into pieces and grinded into slurry with a mixer grinder and named as orange peel powdered (OPP), Apple peel powder (APP) and vegetable peel powder (VPP). 100 ml of 95% ethanol was added into the peel slurry and covered with foil to being extracted at standard conditions for 72 hours. The organic solvent was separated from the solid followed by centrifugation and subjected to evaporation in Rota evaporator at 30h Pa and 60 RPM before being used for further biological assays.

PHYTOCHEMICAL ANALYSIS

Total Polyphenolic Content (TPC)

In a reaction mixer with FC reagent (25µl), water (200µl), and sodium carbonate (10% w/v), an extract of fruit and vegetable peel (20–200µg/ml) was reacted. The phenolic content was absorbed at 760nm using a Spectrophotometer after an incubation for 60 minutes at room temperature. By comparing with standard Gallic acid at concentrations ranging from 20 to 200 µg/ml, the absorbance was computed. On a dry weight basis, the samples' total polyphenolic content (TPC) was reported as mg GAE/g (Subbaiah *et al.*, 2020).

Determination of Flavonoid Content (TFC)

The flavonoid content of the peel extracts were assessed by the method of (El-Sohaimy *et al.*, 2023). A known concentrations of extracts ranging from 20-200 µg/ml was added to 100 µl of 5% sodium nitrite solution and 100 µl of 2% aluminium chloride. The solution was kept in a room temperature for 150 min. The flavonoids content in a extracts were interpreted at 440nm, and the results were stated as mg quercetin equivalents per gm dry samp.

ANTIOXIDANT ACTIVITY OF PEELS EXTRACT

ABTS Scavenging Capacity

ABTS protocol was used to determine antioxidant activity (Wichienchot *et al.*, 2010). The working solution for the ABTS assay was prepared by combining an equal amount of 7.4 mM ABTS and 2.45 mM potassium persulfate. It was then incubated in the dark for 12 to 16 hours to react and form an active ABTS⁺ radical cation, which reacted with the extract solution to measure antioxidant activity. The methanol was used to dilute the ABTS solution. After mixing 50 µl of the sample with 1.9 ml of ABTS solution, the mixture was left to dark-incubate for six minutes. Absorbance at 734 nm was measured following incubation.

DPPH Scavenging Assay

DPPH assay of extracts was analyzed by Ali *et al.*, (2021). The reaction was performed in 96 well plate where 50 µl of sample and 1ml of DPPH solution in methanol was mixed and kept in the dark for 30min. The radical scavenging activity was subjected and measured at 517nm. Quantification of the sample was calculated as mg ascorbic acid equivalent per gram sample (mg AAE/g dw).

Lactic acid bacteria (LAB) isolation and characterization

Serial dilutions of the peel homogenate by transferring 1 mL into 9 mL of sterile saline or buffer. Typically, dilutions from 10⁻¹ to 10⁻⁶ are prepared. Each dilution was inoculated on MRS (De Man, Rogosa, and Sharpe) agar plates using the spread plate method and the plates were allowed to incubation in an anaerobic conditions Pundir *et al.*, (2013) for 48-72 hours. MRS broth is a selective for lactic acid bacteria because of its low pH and high nutrient content (lactose and peptones). After incubation, the colonies were observed with the characteristics of round, convex, white to cream-colored, and smooth appearance. After obtaining the desired characteristics, 3-5 distinct colonies from the highest dilution were

transferred to fresh MRS agar plates for purification and incubated for 24-48 hours at 37°C (Than *et al.*, 2022).

Identification of probiotic bacteria (LAB)

Probiotic bacteria isolated on MRS agar added with bromocresol purple which produces yellow color zone after an incubation of 24-48 h at 37°C. Based on morphological variations, single bacterial colonies which generated a yellow zone were chosen. For selected colonies Gram stain and catalase test was done (Amenu *et al.*, 2023 & Rajyalakshmi *et al.*, 2016). As per the reports of Collee *et al.*, (2006) other IMVIC test were also performed for identification of isolated probiotic bacteria including Indole, methyl red, citrate utilization, oxidase and polysaccharides degradation (Gupta *et al.*, 2021), urea amidohydrolase, Ornithin and basic amino acids hydrolysis tests (Keerthini *et al.*, 2021). Prior to experimental usage, only colonies that were catalase-negative and Gram-positive were subcultured in MRS broth and stored in glycerol.

Characteristics of probiotic LAB in *invitro* when subjected to stomach conditions (temperature, bile, acid, and salt).

When probiotics, specifically probiotic bacteria are subjected to in vitro stomachic environment, several key attributes are assessed to determine their potential efficacy and survival in the gastrointestinal (GI) tract.

Acid Tolerance test

The methodology Dhanvanti *et al.*, (2023) generally involves exposing the bacteria to low pH conditions and measuring their survival rate over time. The isolated LAB were cultured in MRS broth at room temperature for 18 hr. The cell suspension was made into pellet by centrifugation (10,000 g) in refrigerated centrifuge at 4°C for 15 min. Followed by washing at 10,000 g × at 4°C for 15min with sterile 0.89 w/v NaCl, a pure pellet was obtained for each strains. Adjust the pH of the solution to between 1.5 to 3.0 using hydrochloric acid (HCl), depending on the degree of acidity being tested. Re-suspend the bacterial pellet in the acidic solution to achieve a standardized bacterial concentration (e.g. 10⁸–10⁹ CFU/mL). Incubated the bacteria at 37°C (to mimic human body temperature) for a specific time period, typically ranging from 1 to 3 hours. During incubation, for every 30 minutes bacterial growth was measured at 620nm in spectrophotometer

Bile Salt, Salts and Temperature Tolerance test

After studying the surviving rate at stomach acid, probiotics must also tolerate bile salts, which are released in the small intestine and can have a detergent-like effect on bacterial membranes.

Hence to know the stability of LAB the various concentrations of bile salts were added to culture medium and measured the survival rate at 620 nm in Spectrophotometer (Garcia *et al.*, 2016). Salt concentration (4, 5, 8 and 12% w/v NaCl) and temperature at 10°C, 20°C, 30°C and 40°C and incubated for 24-48 hours. Calculated the percentage survival using the formula:
Survival rate (%) = X/100

Antibiotic sensitivity test

Yasmin *et al.*, (2020) presented the disc diffusion method to test the isolates for antibiotic sensitivity. A 0.2 µl of overnight culture of the LAB in MRS broth was inoculated on agar plate and evenly spreads with the swab across the entire surface of the agar plate in multiple directions (lawn culture) to ensure a uniform distribution of bacteria. The standard antibiotic discs of chloramphenicol, tetracycline, Gentamycin, penicillin G and cephadoxamine were laid on the inoculated agar plates and incubated at 37°C for 18-24 hours.

Bacteriocin Production and optimization

The inoculum is usually prepared in MRS broth, the log phase of bacterial cell growth has been used for estimating the bacteriocin production. After a specific incubation period (12–48 hours), the centrifugation of probiotic culture has been done at 10,000 rpm for 15-20 minutes to collect the supernatant for estimation of bacteriocin (Keerthini *et al.* 2021).

Effect of carbon sources on production of bacteriocin

A method of Yuliana *et al.*, (2023) was engaged to assess the role of various carbohydrates on bacteriocin synthesis. For this analysis, the cultured inoculum was enumerated with 1ml of 2% glucose, fructose, sucrose, lactose, starch and incubated at 37°C for 48 hrs. After incubation bacteriocin was estimated in the supernatant by Folin-Lowry's method (Alzahrani 2021).

Impact of salt on the synthesis of bacteriocin

The influence of NaCl on bacteriocin formation was determined by substituting the various amount of NaCl (1% to 4%) to the 10 ml of broth and inoculated with 0.2 ml of probiotic bacteria. After that, each tube was incubated for 48 hours at 37°C. Then, the supernatant was pooled for protein estimation by Folin-Lowry method (Yuliana et al., 2023).

Effects of pH on the synthesis of bacteriocin

The pH has an effect on bacteriocin formation, it is evaluated by the inoculation of probiotic culture in a MRS broth varied with different pH values of 5, 6, 7, and 8. The culture was allowed to grow for about 48 hours in room temperature. The amount of the protein bacteriocin produced by the probiotic bacteria in a supernatant of culture was estimated Folin-Lowry method (John et al., 2022).

Impact of bile salt content on the synthesis of bacteriocin

10 ml of cultured broth (pH 6.5) enriched with bile salt (1-4%) was used to inoculate the 0.2 ml probiotic culture. After that, each tube was incubated for 48 hours at 37°C. The supernatant was collected to determine the protein concentration by Folin-Lowry's method.

Probiotic curd formation

A cell pellet of 24 to 48 hrs probiotic culture was made into a cell suspension in the normal saline and 1.5 ml of this cell suspension was added to 50 ml of sterile milk and then incubated 18 to 20 hours at 37°C. The quality of curd and pH has been investigated.

Total titratable acidity of curd

Using a 0.11 N NaOH solution, the acid contained in 10g of the curd samples was neutralized to calculate the titratable acidity. Few drops of phenolphthalein indicator throughout the titration process, until a pink endpoint was attained.

STATISTICAL ANALYSIS

All experiments were conducted in triplicate The mean and standard error of the data was calculated using One-way ANOVA followed by multiple comparisons using Dunnett's test is a multiple comparison. Statistical significance is denoted by an asterisk (*) when p values are *p < 0.03 to 0.05, **p < 0.001, ***p < 0.0001 and ****p < 0.00001.

RESULTS

Fruit and vegetable peels are often discarded as waste, but they are rich sources of bioactive compounds, including phytochemicals and antioxidants. These compounds contribute to various health benefits and are increasingly studied for their potential applications in food, pharmaceuticals, and nutraceuticals.

Phytochemicals and Antioxidant Properties of Fruit and vegetable peel extract

A summary of the three FPP's and VPP extract polyphenolic compositions and antioxidant potential is provided. The findings showed that FPP and VPP extracts had a different level of phytochemical and antioxidant activities. OPP showed the greatest TPC concentrations (15.49 mg GAE/g dw), and VPP showed the lowest TPC values (1.21 mg GAE/g dw). Among the evaluated APP, a evidential deviation in TPC was found (p ≤ 0.05) (Table 1). Conversely, APP (2.88 mg QE/g dw) had the advanced (p < 0.05) flavonoid concentrations, proceeded by VPP (1.09 mg QE/g dw) and APP (2.88mg QE/g dw). The results published by Suleria et al. are fairly similar to the TPC values of OPP and the TFC values of APP and VPP. Additionally, they discovered a comparable pattern of rising.

Table 1 Phytoconstituents of fruit and vegetable peel extract

S.no	Parameters	OPP	APP	VPP
1.	Total phenol content (mg GAE/gdw)	15.49± 0.34	5.85± 0.24	1.21± 0.24
2.	Total flavonoid content (QE/gdw)	2.14± 0.28	2.88± 0.34	1.09± 0.24
3.	DPPH (mg AAE/gdw)	5.54± 0.14	1.90± 1.24	0.83± 0.24
4.	ABTS (mg AAE/gdw)	11.54± 0.44	8.57± 0.26	2.45± 0.24

Fruit by-products are potentially more significant to stop biomolecules from oxidation and determine their expected uses in the nutrient and medicine industries. Given this, DPPH and ABTS tests have been employed to assess the free radical scavenging capacity of FPP and VPP. In comparison between APP, VPP and OPP's ethanolic extracts, the OPP extracts show higher radical scavenging of DPPH and ABTS (5.54 mg AAE/g dw) and (11.54mg AAE/g dw) radicals respectively. Fruit & vegetable peel extracts, rich in phytochemicals, antioxidants, and dietary fibers, hold significant potential as prebiotics. Their ability to modulate microflora and reduced oxidative stress could improve overall gut health which makes them promising candidates for promoting digestive health and preventing chronic diseases. By incorporating these extracts into functional foods or supplements, they can serve as a natural and sustainable prebiotics for producing probiotics by means of improving human health through better gut microbiome management.

Probiotic bacterial isolation and identification

The Probiotic bacterial samples were isolated from fruit and vegetable peel slurries including apple, bananas, orange peels. The slurry was prepared by blending the peels and fermenting them in sterile conditions for 48-72 hours. After inoculation of fermented peel slurry in MRS at 37°C for 48 hours, the colonies with probiotic characteristics like round, creamy white or slightly translucent, and had smooth edges were observed. Gram staining results for isolated strains showed Gram-positive rod- or coccus-shaped morphology, which is a characteristic of LAB (Fig. 1). All isolates are typically negative for the catalase reaction, confirming their identity as LAB. These two isolates underwent additional assessments for several physiological and biochemical characteristics. The carbohydrate chemical action confirms the isolates can fermented carbohydrates only but did not ferment mannitol. This pattern of identification is consistent only with taxonomic category related to the *Lactobacillus* and *Pediococcus*.

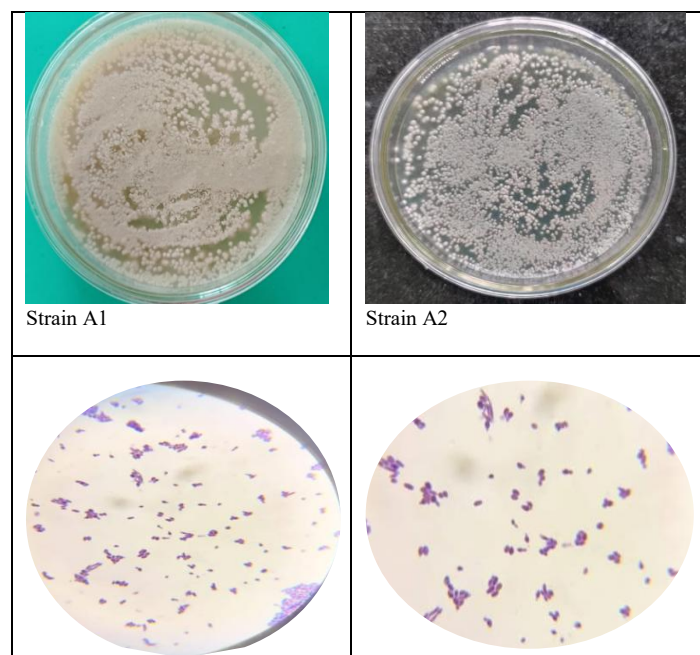


Figure 1 Plating different isolates on MRS media b. Gram stain microscopic view of selected A1 and A2 strains

The metabolic properties of bacteria that produce lactic acid

Biochemical properties of identified LAB explored that two isolates namely A1 and A2 were negative for catalase, oxidase, vagus proskauer, citrate, casein, lipid hydrolysis, starch hydrolysis and positive for methyl red and Indole tests (chowdery et al., 2012). Urease activity helps the probiotic bacteria counter stress during fermentation.

Invitro attributes of Probiotic LAB exposed to gastric conditions (acid, bile, salt and temperature)

The probiotic Isolates were optimized at various pH, carbon sources and bile salt concentration as shown in results for about 2 hours. All isolates exhibited good tolerance to acidic and bile environments, with a survival rate of above 80%, suggesting their potential as prebiotics. The Probiotic bacteria can survive at acidic pH 1.5 to 2.0 as they enter into gastrointestinal tract and need to stay for about 4hrs or more. Due to the pH of gastric acid, the optimization of growth of probiotic at periventricular and ventricular area of intestine at lower pH is considered as a first factor for authentication of isolates as prebiotics (Table-2 &3). Therefore, having

tolerance in the acidic environment is a significant standard to consider that the isolated colonies are probiotics with their viability and functionality (Dunne *et al* 2001).

Because the colon and small intestine contain high amounts of bile salt, which is poisonous to cells, being resistant to bile salt is an important characteristic of a possible probiotic. The isolates A1 and A2 are the majority of LAB isolates who showed tolerance to bile salt at greater than 75% survival. Two isolates were chosen for identification out of the four due to their greater tolerances to gastrointestinal tract conditions.

Table 2 Acid resistance of LAB isolated from fruit and vegetable pulp

LAB isolates	Survival of LAB in pH after 6h (OD at 620 nm)			
	control	pH 4	pH 3	pH 1.5
A1	1.93±0.01	1.55±0.02	0.88±0.05	0.45±0.07
A2	1.86±0.11	1.58±0.13	0.56±0.10	0.37±0.12
A3	1.98±0.08	1.91±0.05	0.83±0.15	0.59±0.06
A4	1.86±0.03	1.87±0.04	0.56±0.09	0.20±0.00

LAB isolates	Survival of LAB in bile after 6h (OD at 620 nm)					
	Control	0.2%	0.5%	1.0%	1.5%	2.0%
A1	1.93±0.02	1.55±0.01	1.88±0.03	0.55±0.01	0.68±0.02	0.48±0.01
A2	1.86±0.01	1.46±0.01	0.76±0.05	0.47±0.01	0.27±0.02	0.10±0.02
A3	1.98±0.02	1.85±0.01	0.93±0.04	0.99±0.01	0.54±0.03	0.12±0.03
A4	1.86±0.01	1.34±0.01	0.76±0.01	0.20±0.01	0.09±0.04	0.03±0.04

Antibiotic sensitivity test:

Clear zones of inhibition were observed around the antibiotic discs for some LAB isolates, indicating sensitivity to specific antibiotics, while no inhibition zones around other discs indicated resistance (Table 4), with diameters of the inhibition zones in millimeters for each LAB isolate against the tested antibiotics. LAB strain exhibited varying sensitivity to different antibiotics. All strains were sensitive to Chloramphenicol, Tetracycline, Gentamycin, Penicillin-G, Cefpodoxime with inhibition zones larger than 15 mm, indicating high susceptibility. The isolated LAB strain's resistance to Tetracycline, Penicillin-G, Cefpodoxime, as demonstrated by the small or absent inhibition zones.

Table 3 Bile tolerance of isolated lactic acid bacteria

Table 4 Diameters of the inhibition zones (in millimeters) for each LAB isolate against the tested antibiotics

LAB Strains	Chloramphenicol (30 µg)	Tetracycline (30 µg)	Gentamycin (30 µg)	Penicillin-G (30 µg)	Cefpodoxime (30 µg)
A1	(Sensitive) 20.7 mm	(Sensitive) 18 mm	(Sensitive) 20.5 mm	Resistant 10.6 mm	(Sensitive) 20.8 mm
A2	(Sensitive) 20 mm	Resistant 10.9 mm	(Sensitive) 20.2 mm	Resistant 10.9 mm	(Sensitive) 20.1 mm
A3	(Sensitive) 18.5 mm	Resistant 10.8 mm	Resistant 15 mm	Resistant 10 mm	Resistant 10.4 mm
A4	(Sensitive) 20.6 mm	Resistant 8.6 mm	Resistant 14 mm	Resistant 9.5 mm	Resistant 9 mm

Bacteriocin Production and optimization

The isolated LAB strains were optimized for bacteriocin production with various salt, acid (pH), bile salts concentration and different carbon sources. The amount of bacteriocin produced under various conditions was estimated by the Folin-Lowary method. According to results, Among the four strains two strains A1 & A2 are bacteriocin producers. A1 and A2 strains produce 1.026 and 0.974 mg/ml of bacteriocin (Table 5).

Table 5 Mean of OD at A1 and A2

Strains	Concentration mg/ml	Mean of concentration
A1 strain		
S1	0.986	1.026
S2	1.067	
A2 strain		
S1	0.941	0.974
S2	1.007	

Optimization of Carbon source on the production of bacteriocin:

A1 produced a significant number of bacteriocin in medium containing glucose, whereas the A2 strain implanted in sucrose-containing medium produced a high amount of bacteriocin. Stricter action was seen with fructose and starch (Fig. 2a & 3b). Carbon sources significantly affected bacteriocin production on both strains. Glucose yielded the highest bacteriocin activity (2.4 mg/mL) in A1 strain followed by Sucrose (1.6 mg/mL), Lactose (1.4 mg/mL) and fructose (1.3mg/mL). In A2 strain sucrose is the sole source followed by glucose for bacteriocin production yielded (2.1mg/ml).

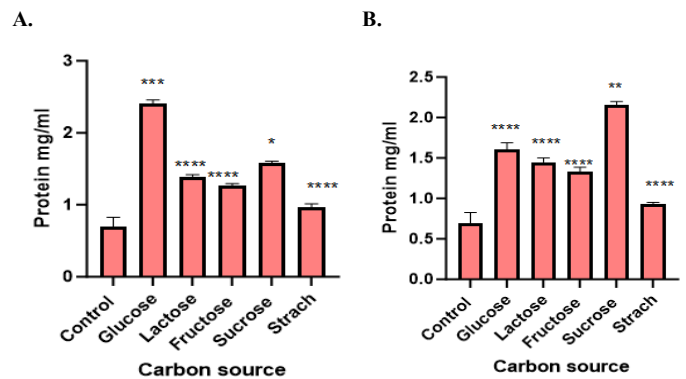


Figure 2a & b Effect of different carbon sources on bacteriocin production by A1 strain and A2 strain.

Different carbon sources affect the metabolic pathways of bacteria and also influence their growth rate and secondary metabolites production like bacteriocin. In A1 strain glucose promotes increased bacteriocin synthesis, it can also result in catabolic repression (CCR), a condition in which too much glucose suppresses secondary metabolism. The results of A2 strain showed the low bacteriocin production in presence of glucose and demonstrate elevated bacteriocin synthesis in presence of sucrose as a result of the presence of two fermentable sugars.

NaCl concentration's impact on bacteriocin synthesis

For the A1 strain, the results (Fig. 3a & 4b) indicated that the highest protein content was earned at 1% NaCl and the lowest bacteriocin production was at 5% NaCl. The A2 strain produced the least quantity of protein bacteriocin when exposed to 3% NaCl, whereas the largest amount was seen when exposed to 5% NaCl.

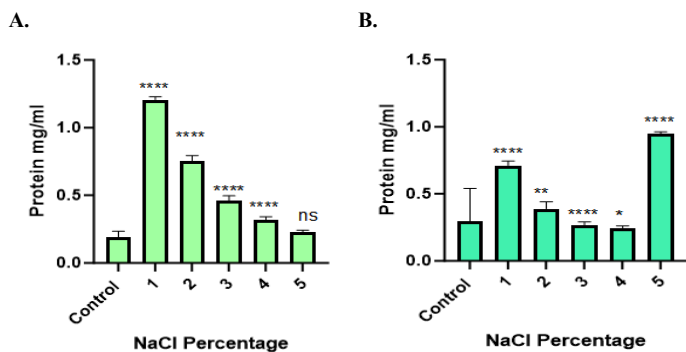


Figure 3a & 3b Effect of NaCl percentage on bacteriocin production by A1 strain and A2 strain.

Since low concentrations of NaCl promoted microbial growth without generating osmotic stress, probiotic strains such as A1 and A2 are likely to produce bacteriocin efficiently at these levels. A1 has moderate halo tolerance, it showed the best bacteriocin production at NaCl concentrations of 1% to 2%, but production was declined significantly beyond 3%. At low NaCl levels (1%), A1 might show efficient growth and bacteriocin synthesis. Up to 1-5 % NaCl, A2' strain production was lower a concentrations (0.5% to 4%) and production was high st 5% level.

pH variations effects on the synthesis of bacteriocin

The variations in the P^H have an exert influence on the bacteriocin production. pH 7.0 is the ideal for highest bacteriocin synthesis in A1 strain (2.12 mg/ml), while lower activity was detected at more acidic (pH 5.0) or alkaline (pH 9.0) conditions (Fig. 4a & 5b). Whereas in A2 strain (1.98 mg/ml) pH 6.0 is the optimum for standard bacteriocin production.

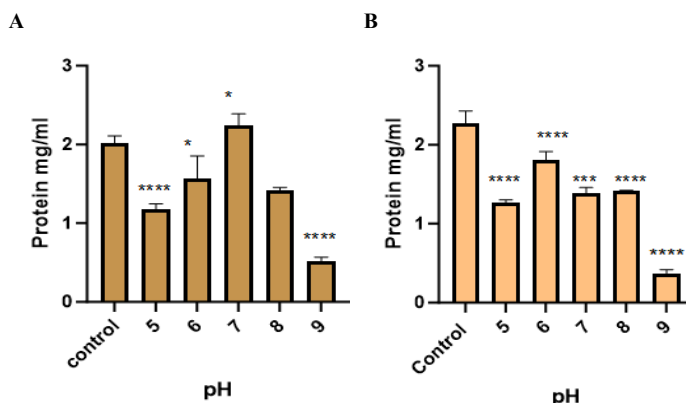


Figure 4a & 4b Effect of pH on production of Bacteriocin by A1 strain and A2 strain.

Usually, bacterial growth was optimum at neutral conditions (pH 6-7), probiotic strains were also active in this pH for bacteriocin production. The maximum metabolic activity and enhanced synthesis of secondary metabolites frequently occurred within this pH range.

Effect of bile concentration on Bacteriocin Production

The bile salts were also essential for survival and production of bacteriocin at intestinal level. The A1 and A2 probiotic strains were optimized by different concentrations of bile salts under physiological conditions. The maximum protein content was seen at 1.5% bile salt concentration and the lowest bacteriocin synthesis was seen at 3.8% bile salt concentration, respectively (Figure 5a & 5b).

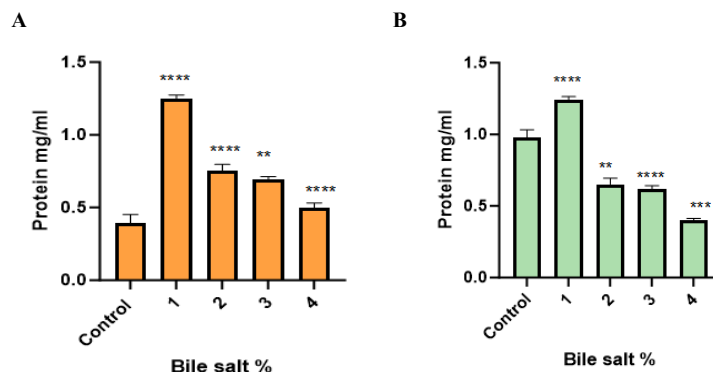


Figure 5a & 5b Effect of Bile salt concentration on production of bacteriocin by A1 and A2 strain.

Bacteriocin production usually decreased under high bile conditions, because the bacteria focus on survival rather than producing secondary metabolites. At this point, probiotics prioritize protecting themselves rather than synthesizing bacteriocin.

Making probiotic curd

Curd formation was observed after 6 hours of reaction time at 37°C using the LAB strains. The curd was firm, with no visible whey separation.

pH of curd

The pH of the milk dropped from 6.7 to 4.5 within 6 hours of fermentation with the LAB strains. The final pH was measured at 4.3 after 12 hours for A1 strain and 4.58 for the A2 strain. Comparatively speaking, the A1 strain generates more acid than A2 strain.

Titrateable Acidity of Probiotic Curd

Titrateable acidity of the curd increased steadily during fermentation. Initially, the acidity of the milk was 0.16% lactic acid. After 6 hours of fermentation with lactic acid bacteria (LAB), the titrateable acidity reached 0.65%. By the end of the fermentation (12 hours), the titrateable acidity had further increased to 0.85%." In the control group (without added LAB), the titrateable acidity increased slowly, reaching 0.25% after 12 hours.

Water holding capacity of curd: The probiotic curd showed a water holding capacity (WHC) of 63% for A1 strain and 71 % for A2 strain after 12 hours of fermentation with lactic acid bacteria (LAB) strains. This indicates the percentage of water retained in the curd after centrifugation.

DISCUSSION

The purpose of the current research is the isolation and characterization of probiotic bacteria from prebiotics such as fruit and vegetables peel mixture. For this isolation we have selected three fruit samples (orange, red apple and banana) and two vegetable samples (cucumber and cabbage). As the preliminary study we screened the phytochemical constituent in fruit peel extract and vegetable peel extract. Table 1 represents the peels chemical constituents and quantity of poly phenols and their antioxidant activity (Han et al., 2007). There have been numerous reports of phenolic compounds biological effects in humans, such as their antibacterial, anti-inflammatory, and antioxidant capabilities (Barber et al., 2020). Orange peel powder had highest amount of nutritional fiber as apple and vegetable peel. Similarly, samples of orange peel powder had larger levels of polyphenols and had twice as much antioxidant capacity. Orange peel is also a tremendous source of antioxidants because it includes a rich content of polyphenolic components, such as flavonoids and phenolic acids (Ballini et al., 2023). Prebiotics play a vital role in gut health by fostering the proliferation of beneficial microorganisms, which in turn can improve digestion, immune function, and overall health (Bhatt et al., 2012). Peels of fruits such as apples, bananas, and citrus are rich in insoluble fiber (cellulose, hemicellulose) and soluble fiber (pectin, inulin). Soluble fiber, in particular, serves as a substrate for gut bacteria, supporting their growth and enhancing the production of Short Chain Fatty Acids. The successful isolation of LAB from fruit and vegetable peels supports the central hypothesis that these commonly discarded by-products can serve as untapped reservoirs of probiotic bacteria (Bazireh et al., 2020). The high survival rates of several strains under acidic (pH 2.0) and bile salt (0.3%) conditions are particularly noteworthy, as these are key physiological barriers encountered during gastrointestinal transit. This acid-bile resistance is critical for colonization in the human gut, and the performance of some strains in this study rivals or exceeds that of established probiotic strains from commercial sources. The negative catalase test is a key feature for distinguishing LAB from other aerobic Gram-positive

bacteria. LAB are predominantly anaerobic or facultative anaerobic (Todorov et al., 2022).

The peels likely supply fermentable sugars, vitamins, and minerals, creating a favorable environment for LAB proliferation during fermentation. These findings confirm that the isolated microorganisms belong to the LAB group, which is known for their role in fermentation and probiotic functions.

According to Bhatt et al., (2012) Two of the four strains, A1 and A2, have demonstrated the predicted properties of putative probiotic bacteria. All of them have LAB's biological characteristics. The results from probiotic tests support the potential of certain LAB strains as probiotics because of their capacity to endure in hostile gastrointestinal conditions, inhibit pathogen growth, and promote gut colonization. The production of antimicrobial substances like bacteriocin and organic acids enhances their use in food preservation and as probiotics. Resistance to bile salt and simulated stomach juice were associated with the survivability of probiotic bacteria (Cleveland et al., 2001).

The threshold for tolerance was set at 75% survival rate of LAB after 2 hours of incubation in simulated gastric acid and bile salt. Bazireh et al., (2020) reported as a several probiotic strains of *Lactobacillus* and *Enterococcus* show sensitivity to different antibiotics. The antibiotic sensitivity results revealed that LAB strains from fruit and vegetable peel slurry generally exhibited sensitivity to Chloramphenicol, Pencillin, Tetracyclin, Gentamycin G, and Cefpodoxime. This sensitivity is in line with the fact that these antibiotics are effective against Gram-positive bacteria, including LAB (Jiménez-Moreno et al., 2023).

Resistance to *Pencillin*, *Tetracyclin*, *Cefpodoxime* was observed in all isolates, which is consistent with previous studies. LAB, particularly *Lactobacillus* species, are intrinsically resistant to Penicillin due to the structure of their cell walls and the absence of the target D-Ala-D-Ala in peptidoglycan synthesis.

When exposed to pH 1.5 to 2.0 (simulating the stomach environment), 80% of the LAB population survived after 2 hours of incubation. It indicates that LAB isolated from fruit and vegetable peel sources can withstand the at the acidic conditions present in the stomach. The high acid tolerance observed may be due to the adaptive mechanisms developed by these LAB strains from fruit and vegetable peels, such as producing protective exopolysaccharides (EPS) and cell surface proteins that enhance their resistance to acidic stress (Roy et al., 2024).

Bile tolerance is a critical feature for probiotics, as bile salts can disrupt bacterial cell membranes, leading to cell death. The LAB strains isolated from fruit and vegetable peels showed good bile tolerance, which suggests they are capable of surviving in the small intestine and contributing to gut health. This tolerance can be attributed to the activity of bile salt hydrolase (BSH), an enzyme produced by some LAB strains that detoxifies bile salts, allowing the bacteria to maintain their cell membrane integrity and survive in the intestinal environment (Kusada et al., 2021).

The optimization results indicate that the LAB strains produced the highest levels of bacteriocin under specific environmental conditions such as glucose, 2% NaCl, 1% bile salts, and pH 7.0. These findings align with previous studies on bacteriocin production, where LAB typically show optimal activity in slightly acidic environments (Leal-Sánchez, et al., 2002). The dependence on glucose suggests that efficient fermentation is crucial for bacteriocin production, as glucose is rapidly metabolized through the glycolytic pathway, supporting energy production and biosynthesis (Jiménez-Moreno et al., 2023).

The titratable acidity of the probiotic curd reached 0.85% lactic acid after 12 hours of fermentation and remained relatively stable (0.90%). The higher acidity compared to standard curd indicates the active fermentation by LAB, which is essential for producing the tangy flavor and ensuring probiotic viability in the curd (Cleveland et al., 2001).

The probiotic curd demonstrated a high-water holding capacity of 65% (Iqbal et al., 2021). The enhanced water retention in the probiotic curd is likely due to the production of exopolysaccharides by the LAB strains, which improve the curd structure and reduce syneresis. The probiotic curd outperformed both the control and standard curd in terms of WHC, indicating its superior texture and moisture retention (Putri et al., 2024).

CONCLUSION

This study demonstrates that fruit and vegetable peels—commonly regarded as waste—are valuable sources of lactic acid bacteria with promising probiotic potential. The isolated strains exhibited promising probiotic properties, including acid and bile salt tolerance, which are necessary for their survival and functionality in the gastrointestinal tract. These characteristics suggest their potential application in the development of functional foods, dietary supplements, and natural bio preservatives. The implications of these findings are twofold. First, they support a sustainable, low-cost approach to probiotic discovery by leveraging agri-waste materials in line with circular bio economy principles. Second, they underscore the need to diversify probiotic sources beyond conventional dairy matrices to meet the demands of vegan, lactose-intolerant, and health-conscious consumers. In conclusion, fruit and vegetable peels represent not only an environmental burden but also a biological opportunity. With further validation, peel-derived probiotics may contribute significantly to both gut health innovation and global sustainability efforts.

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