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*Research Article*

# Characterization of Lactic Acid Bacteria for Production and Utilization of Probiotic

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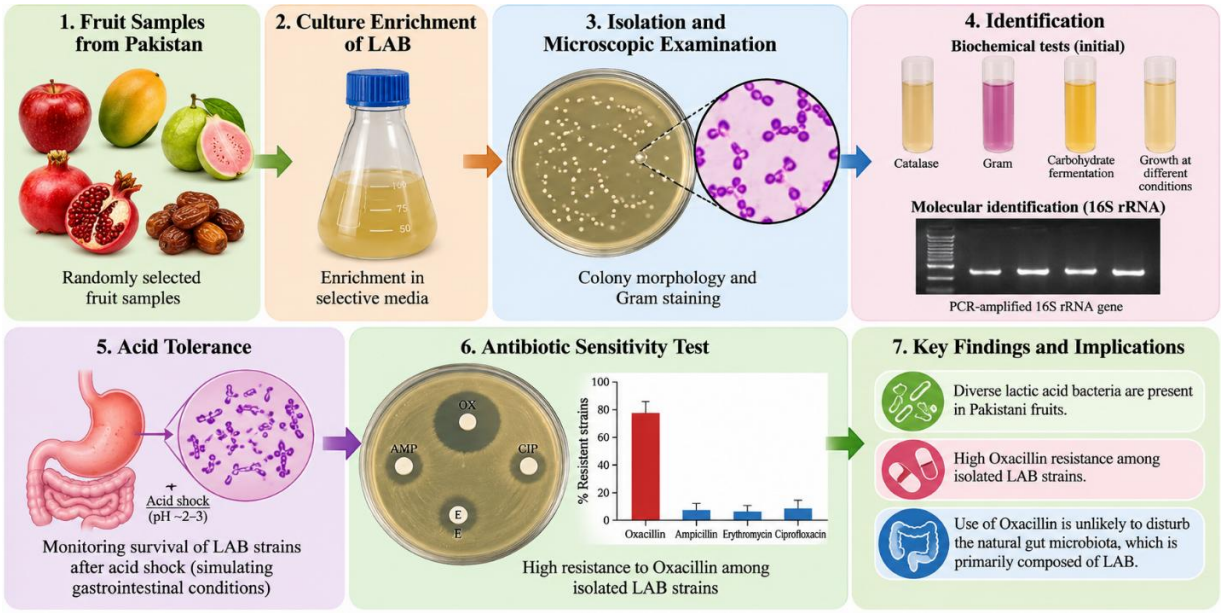
## Abstract

Lactic acid bacteria, or LAB, have long been known to exist in dairy products, but less attention has been paid to their prevalence in Pakistani fruits. Thus, microscopic examination were used to study various lactic acid bacteria that are typically present in fruits grown in Pakistan. Fruit samples chosen at random were examined after the culture enrichment process. Conventional morphological and biochemical examination served as the basis for the initial identification, The final variation and identification of lactic acid bacteria will be done by using molecular approaches, polymerase chain reaction-amplified 16S rRNA-specific DNA that are considered validated method and more reliable to any biochemical test. Before making any alterations, the growing conditions for each strain were carefully controlled. Monitoring all strains after an acid shock that replicates the harsh, acidic environment seen in the gastrointestinal tracts. The findings of the antibiotic sensitivity test revealed an unusually high rate of Oxacillin resistance among isolated LAB strains. As a result, it is expected that using these antibiotics against infections will not disturb the gastrointestinal tract's natural microbiota, which is primarily composed of lactic acid bacteria.

**Keywords**: Bacteria, Human Health, Lactic acid ,Probiotics, molecular approaches

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## Graphical Abstract




## 1: Introduction

Presently consumers are well aware of functional food production. Demand for probiotic-based functional foods expanded quickly as a result of consumer awareness, and probiotic cultures are used in the majority of dairy industries to formulate functional meals. Indeed, probiotics in the dairy industry represent for 33% of market for functional food. The nutritional components of food itself, which include live bacteria (probiotics), are added in foods to provide health benefits. The market for functional foods can contain up to 70% probiotic foods. According to predictions made in 2020, the market in 2027 for functional foods would expand at a compound annual growth rate of 7.5% to reach $309 billion (Aspri et al., 2020). This escalation in the market is due to the probiotics association with health benefit and innovative technological intervention.

The Food and Agriculture Organization/World Health Organizations defined probiotics as "live microorganism which when administrated adequate amount ($10^7$ CFU/g) they confer health benefits on the host" (FAO/WHO, 2002). Normally probiotics are isolated from dairy sources but, nondairy sources have become increasingly popular during the last two decades that also have a potential of probiotics (Silva et al., 2014). Mostly probiotics are incorporated naturally from non-dairy sources like fruits and vegetables that are used for large number of people who are lactose intolerant and therefore, need lactose free diet (Riaz et al., 2017). To satisfy costumer growing concern over food origin the extraction of probiotics from nondairy sources is also being performed such as from fruits like apples, cherries, papaya, orange, mango, pineapple, grapes and strawberry.

Probiotic bacteria become popular as a result of scientific evidence pointing to their positive impact on human health (Zitouni et al., 2021). Health benefit of probiotic include, anti-mutagenic, anti-carcinogenic agents and ameliorating diseases effects, such as antibiotic- associated acute diarrhea and antibiotic-associated allergy-related condition, constipation, diabetes, bowel disease, and

Hypertension. Probiotic are members of the (LAB) group and able to produce antimicrobial compounds, such as "bacteriocins" which is used against food borne pathogens as natural bio-protector. Lactic acid bacteria (LAB) also produce lactic acid, anti-fungal peptide (and other organic acid) that prevent pathogenic bacteria, food spoilage, and are helpful for lowering the pH of different food product. Lactic acid bacteria (LAB) that is heterogenous which play important role in process of fermentation (Viecosaiz et al., 2022). LAB

have main application is as starter cultures, with variety of fermented dairy products (i.e., fermented milks yogurt, cheese) vegetable and cereal products, meat, fish, fruits. Besides, all these it also involve in nutritional value, flavour, and texture of the fermented food (Zampieri et al., 2022). LAB are used as adjunct cultures (Rokana et al., 2018). Numerous probiotic culture have been found to be effective on food-borne pathogens in both human gut and salivary secretions (Bazireh et al., 2020).

Probiotic are members of the (LAB) group and able to produce antimicrobial compounds, such as "bacteriocins" which is used against food borne pathogens as natural bio-protector.Numerous probiotic organisms found in human saliva and the stomach have shown promise in the fight against food-borne pathogen-caused illness. It also helps to prevent the lethal diseases (Terai et al., 2015). Competition for adhesion sites and food sources is one of the modes of action, as is the creation of antimicrobial substances, intestinal barrier function enhancement.Fruit become more popular due to growing understanding of their nutritional worth (Silva et al., 2010). By creating better value uses for its byproducts, since fruit include high levels of minerals, vitamins, and secondary phytochemical substances, there has been an increase in consumer demand for them (Rufino et al., 2010).

Given the importance of probiotics to understanding regarding their presence on regional fruit research to identify organisms with probiotic potential is essential (Benavides et al., 2016) There are different methods that are used for isolation and identification of lactic acid bacteria. In previous studies isolation and identification of bacterial strain were based on cultivation on MRS/M-17 media but that was lacked validated method of identification. Molecular methods, particularly 16S rRNA gene sequencing and polymerase chain reaction (PCR)-based validated methods, are often employed to identify LAB species from fruit (Bhattarai et al., 2021). Furthermore other properties of lactic acid bacteria strain that will be used to characterize the probiotics to survive under gastrointestinal (GI) tract, auto aggregation, antimicrobial activity and antibiotic susceptibility. Probiotic bacteria isolated from nondairy source like fruits can be characterized and identified to increase the capacities of various microbiological resources for the production of nondairy sources. Future possibilities include the use of probiotic products through functional foods, which is a good strategy (Evivie et al., 2017). The market for functional foods can contain up to 70% probiotic foods (James & Wang, 2019). Demand for probiotic-based functional foods expanded quickly as a result of consumer awareness, and probiotic cultures are used in the majority of dairy industries to formulate functional meals. Yogurt, different kinds of cheese, fermented milk, sour cream, and a long list of other dietary items have all lately been investigated for probiotic

## 2. Materials and Methods

### 2.1: Isolation of Probiotic Strain:

The samples was analyzed for total viable count (TVC) by streak plate method, using sterilized MRS media (De man, Rogosa and Sharpe). Stock culture after purification was preserved in glycerol stock solution at -20°C. Isolate colonies was verified by PCR amplification 16SrRNA gene. Universal Forward primers 9F(5'-GAGTTGATCCTGGCTCAG-3') and Universal reverse primer 151 OR(5'-GGCTACCTTGTTACGA-3') was used. The amplified PCR product was sequenced by MACROGEN sequencing, Kore (http://DNA.macrogen.com) (Zamanpour et al., 2023)

### 2.2: Confirmatory Test-(Biochemical test)

#### 2.2.1: Catalase test:

Prepare 3% hydrogen peroxide solution. Place small drop of hydrogen peroxide on a microscopic slide, used sterilized inoculating loop to pick up a colony from the plate and emulsify it in the drop of hydrogen peroxide. Result was observed by formation of bubbles occur surrounding the treated colony, so there is no bubble formation was observed around the treated colonies while treated with hydrogen peroxide it indicate the catalase negative test, otherwise it was catalase positive test.

#### 2.2.2: Gram staining:

For gram staining step one requires the preparation of bacterial smear. The main purpose of bacterial smear is to fix the bacteria to slide. Pick up a colony from the plate and placed on a micro-

scopic slide. Flood the entire slide with crystal violet and leave it for 60sec and then rinse with water for 5min. Again slide was flood with the iodine solution and let it stand for 60 seconds, before gently rinsing it with water for 5 seconds. Then drop wise, gently wash the slide with decolorizing ethanol and leave for 45 seconds. In last step was flood the slide with the safranine leave it for 60 seconds, then gently rinse with water for 5 seconds. Allow the slide to air dry. The color was examine under microscope. Color of bacteria was purple it indicate the gram positive bacteria. If color of bacteria was pink then it indicate the gram negative test (Rahmawati et al., 2021).

**2.2.3: Oxidase test:**

Streak plate methods was used for oxidase test for well-isolate colonies. Place 1 or 2 drops of 1% oxidase reagent on the culture. Do not invert or flood plate. Observe for color changes, colors was not changed to red it indicate the oxidase positive. Otherwise, if color was changed to red or blue then it was oxidase negative (Sultana et al., 2022).

**2.2.4: Proteolytic detection test**:

Milk agar plate was prepared 100ml distilled water and 2.5g of agar was used. After autoclave, 10ml sterilized skim milk was added in to agar plate and was streaked with agar and incubated 37℃ for 24-48h. The inhibition zone was observed around colonies and it was show positive result.

**2.2.5: Amylolytic detection test**:

The starch agar plates was used. One gram of starch and one gram of nutrient agar was added in 100ml distilled water, and then plates was incubated at 37℃ for 24 hours with streaked lined starch agar plates. After 24hrs Gram's iodine (1%) was swamped; clear zone was to be seen around the streaked lines. This was showed positive result.

**2.3: Screening of Lactic Acid Bacteria:**

**2.3.1: Simulated to gastrointestinal tract:**

Acid tolerance test of identified strain was to be determined by their growth in MRS broth heaving pH (1.0, 2.0, and 3.0). Culture plates was incubated at 37℃ for 20h aerobically. Triplets study was to be done (Zommiti et al., 2020). Concentration of bile salts (0.3%) was to be added into MRS broth. It was kept in an incubator at 37℃ for 10 hours. After every one hours, and 4hours, one ml of sample was to be drawn and plated on MRS media to check the probiotics viability. Triplet study was to be done, the triplet result and their mean was to be calculated (Kılıç et al., 2021).

**2.3.2: Antimicrobial activity of the identified strain:**

Antimicrobial activity was to identify by using agar disc diffusion method. Antimicrobial activity of probiotics was analyzed against the pathogens strain such as *Neomycin, Escherichia coli, Amoxicillin, Erythromycin and Tetracyline* (Gueimonde et al., 2013).

**2.3.3: Antibiotic activity of identified strain:**

Antibiotic activity was identified by using antibiotic discs diffusion method. Broth cultures of LAB was prepare by using MRS media. LAB was calculated against different antibiotic discs like *Penicillin, Ampicillin, Streptomycin, Vanomycin, and Imipenem*. After incubation of 24h at 37℃ the diameter of inhibition zone was measured (Byakika et al., 2020).

**2.4: Hemolytic activity:**

To measure hemolytic activity, Isolates was incubated on the blood agar base medium containing 5% sheep blood at 37℃ for 48h. The result was considered positive because inhibition zone will be developed around the colonies or a change in color was observed in medium

**2.5: Statitical Analysis**

The assessment of data was done by using one-way &two-way ANOVA. Significance was determined using LSD test. The confidence interval was fixed at 95**%** therefore p-value of $<0.05$ was considered significant in the finding Software of statistix 8.1 was used by (Steel et al., 1997).

## 3. Result and discussion

### 3.1: Isolation of Probiotic Strain

The results of the current study reveal that most of the colonies on MRS agar plates were tiny and off-white in color as shown in (Figure 1) suggesting that these species were linked to lactic acid bacteria. Biochemical testing and colony/cell morphology were the main methods used to identify each isolate

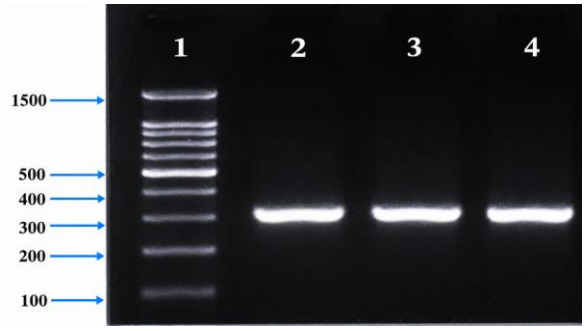



**Figure 1: Isolated Strains of probiotic bacteria in MRS-Agar tiny and off-white colonies**

### 3.2: Molecular Identification of Isolated Bacteria:

One of the most important applications of PCR is to identify isolates at the genus level. Phenotypic approaches are ineffective for identifying lactic acid bacteria. To ensure reliability, lactic acid bacteria from fruits should be identified using molecular identification methods. The 16S rRNA gene has been discovered to be a strong tool for understanding genetic variations among species. After amplification of the 16s rRNA gene using a PCR reaction for DNA taken from chosen isolates, the resulting amplification was purified and the final product was obtained on agarose gels. The electrophoresis result of the final product purified from 16s rRNA gene amplification using PCR. Confirmatory test was performed on selected fruits isolates and bacteria were found to be Gram-positive, catalase-negative, rod-shaped microorganisms that grew on MRS agar plates following a 24-hour incubation period at 37 °C. Several of the fruit samples under analysis produced colonies with catalase reactions that were negative. To facilitate additional study, all isolates that were gram positive and catalase negative were chosen. Gram staining form different fruits reveals the presence of Gram-positive LAB, which is consistent with their normal microbial composition. The thick peptidoglycan cell wall of bacteria makes them appear Gram-positive (purple/blue) under a microscope because it holds the crystal violet stain during the Gram staining procedure (Yelntty et al., 2014). In conclusion (Figure 4.3) shows the appearance of (purple/blue) stain that confirms the existence of Gram-positive LAB.

Therefore, during the current investigations, the genetic discrimination of the related species belonging to the Lactic acid bacteria group were obtained via PCR amplifications using 16S rRNA primers. Figure 2 shows the electrophoresis result of the final product purified from the 16s rRNA gene amplification using PCR. The length of the detected bands ranged between 320 and 400 bp, confirming the results of bacterium identification at the genus level




.**Figure 2: PCR product of 16s rRNA (320-400 bp) from 3 isolates that are reproduced using universal primers.**

The homology analysis based on the 16S rRNA sequence comparison clearly demonstrated that

the majority of the strains clustered with Lactobacillus plantarum, which was the most frequently isolated species in this investigation. The molecular verification of this work were consistent with numerous previous studies. The presence of Leuconostoc mesenteroides on tomato surfaces and in tomato fruit puree has been repeatedly observed (Sajur et al., 2007). and (Nyanga et al,.2007) conducted a thorough investigation of the microbiology of masau fruit. Another study in 2022 employed molecular testing to isolate LAB from jackfruit and beetroot juice and found that the LAB identified were Lactiplantibacillus plantarum and Levilactobacillus brevis, respectively. Certain strains of Lactobacillus plantarum have been shown to have probiotic properties. As a result, the findings of this study are encouraging for the consumption of fruits as well as probiotic foods. Confirmatory test was performed on selected fruits isolates and bacteria were found to be Gram-positive, catalase-negative, rod-shaped microorganisms that grew on MRS agar plates following a 24-hour incubation period at 37 °C. Several of the fruit samples under analysis produced colonies with catalase reactions that were negative. To facilitate additional study, all isolates that were gram positive and catalase negative were chosen. Gram staining form different fruits reveals the presence of Gram-positive LAB, which is consistent with their normal microbial composition. The thick peptidoglycan cell wall of bacteria makes them appear Gram-positive (purple/blue) under a microscope because it holds the crystal violet stain during the Gram staining procedure (Yelntty et al., 2014). In conclusion (Figure 4.3) shows the appearance of (purple/blue) stain that confirms the existence of Gram-positive LAB.

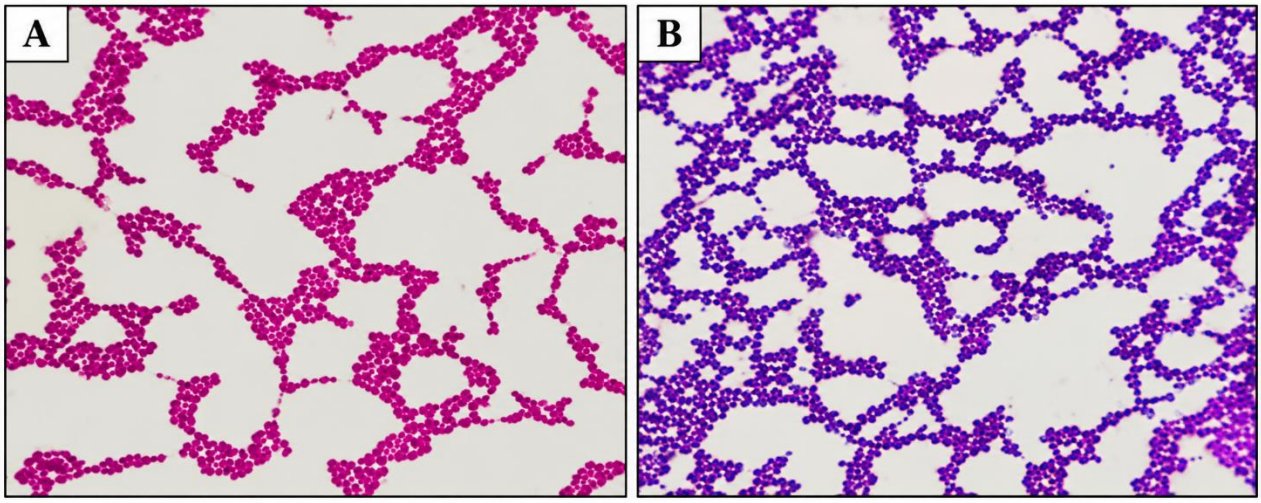



**Figure 3: Gram staining confirms the presence of Gram-positive LAB isolates by showing stains of (purple/blue) under a microscope.**

Catalase was not detected in any of fruits isolates. Hydrogen peroxide treatment of all fruits isolated colonies resulted in no bubble production, description of lactic acid bacteria. The enzyme catalase, which converts hydrogen peroxide into water and oxygen, is detected by the catalase test. In conclusion LAB do not make catalase, which is necessary for detoxifying hydrogen peroxide, hence they are typically catalase negative after treated with hydrogen peroxide and also oxidase-negative to have no color change. When the color turns purple in 60 to 90 seconds, the microorganisms are delayed oxidase positive. The cytochrome c oxidase enzyme is detected in bacteria by the oxidase test. Since LAB do not transfer electrons via the cytochrome system, they are usually oxidase negative. Proteolytic testing on banana cherry and olives in this investigation revealed positive results. On the protein-containing media, there is positive proteolytic activity indicated by the clear zones that encircle the colonies, measuring (8±0.67mm), (7±0.42mm), and (10±0.21mm), respectively. This suggests that protein hydrolyzing is possible for the LAB strain. Positive proteolytic activity was seen in the clear

zones surrounding the colonies, suggesting their possible application in protein degradation processes. When tested on skim milk agar, isolates of fruits showed good proteolytic activity. More than 6 mm of clear zones around the colonie indicating strong proteolytic activity. Same study observed by (Yelntty et al., 2014). proteolytic enzyme screening for the various fruits with a colony diameter of (2mm), isolates saw a clear zone of (15 mm, 12 mm, and 10 mm) after 24 hours. The outcome demonstrated that proteolytic enzymes could be produced by all samples. Comparable outcomes were documented by (Yelntty et al., 2014). Casein is a big molecule that forms colloids and is insoluble in water. A specific proteolytic enzyme is present in casein, which will cause it to hydrolyze into peptides and soluble amino acids. (Rosiana et al., 2009). The development of a clear zone surrounding bacterial colonies in skim milk medium indicates the elimination of casein particles. Proteolytic system contributes to the development of the organoleptic qualities of fermented milk products and is engaged in the consumption of casein within LAB cells (Yamina et al., 2013).

Fruits isoaltes shows amylolytic activity on agar medium with a clear zone diameter of about (1.20±0.21mm) for banana significantly different from those of the other isolates. LAB-OLV that show the diameter (0.55±0.1mm) and LAB-CH that have diameter (0.75±0.01mm) . The findings of this study indicate that cherries have a comparatively low starch content, which may translate into little amylolytic action when tested in this way. The starch in the medium could be used by the LAB to develop, and it would create a clean zone surrounding its growth site. (putri et al., 2012). According to (José et al., 2020) the same results were obtained while working with various fruits, the production media containing banana (1.54±0.05mm) and pineapple (2.0±0.78mm) byproduct had the highest lactic acid concentrations. The isolates of fruits also shows the resistance to different antibiotics. Cherry and banana exhibited the maximum inhibitory activity against *Ampicillin* (5.3±0.01mm) and (3.7±0.01mm), while LAB-CH lowest zone of inhibition was (2.0±0.02mm) against *Streptomycin* (Table 4.6). After a 24-hour incubation period, the isolate LAB-olives exhibited the maximum diameter inhibitory zone (20.1±0.01mm) against *Vanomycin* and the lowest zone (6.1±0.07mm) against Ampicillin. Regarding antibiotic resistance, tests were conducted on all three isolated bacterial strains to determine their susceptibility and resistance to five different antibiotics. Most of the strains exhibited sensitivity to 50% of the five antibiotics tested; however, *Vanomycin* showed the highest level of sensitivity. Since *Vancomycin* is one of the last antibiotics to be widely effective against clinical infections, resistance to it is a problem when it comes to antibiotic responses. Similar findings were noted by (Johnson et al., 1990), who found that isolate LAB-Gr for grapes had the greatest inhibitory action against *S. typhimurium* (9.7±0.2mm) and the lowest zone of inhibition (3.3±0.1mm) against *B. cereu*. Following a 24-hour incubation period, the isolate LAB-Bn2 exhibited the maximum diameter inhibitory zone (7.1±0.10mm) against *S. typhimurium* and the lowest zone (4.2±0.3mm) against B. cereus. Regarding antibiotic resistance, tests were conducted on all 15 isolated bacterial strains to determine their susceptibility and resistance to ten different antibiotics. Most of the strains exhibited sensitivity to 50% of the 10 antibiotics tested; however, *Oxacillin* and *kanamycin* showed the highest level of sensitivity of the reactions to antibiotics. In conclusion the presence or absence of bacterial growth around the antibiotic disks indicates susceptibility or resistance. The results based on the diameter of the zone inhibition around each antibiotic disk. A larger zone indicates susceptibility, while a smaller or absent zone suggests resistance.

All the data of the antibiotic test is represented in terms of mean and S.D. The study analyzed the effect of various treatments on antibiotic resistance in banana cherry and olives was examined by using an ANOVA test. The results indicated that the treatments had a statistically significant effect on resistance levels ($P < 0.001$). The treatment factor accounted for nearly all of the variation observed in the data, The study yielded differing results against antimicrobial activity, as indicates that isolates of cherry and olives exhibited the maximum inhibitory activity (11±0.032mm) and (20±0.01mm) against *E. coli*, while LAB-BA showed lowest zone of inhibition (6.1±0.32mm) against *Neomycin* and LAB-CH against *Tetramyicn.* After a 24-hour incubation period, the isolate olives inhibition zone with the largest diameter was seen against E. coli (20±0.01mm) and the intermediate zone (15±0.41mm) against *Amoxicillin*. All three of the isolated bacterial strains underwent tests to determine their susceptibility to and resistance to five different antimicrobial discs.

Furthermore LAB-cherry shows resistance against antimicrobial disc *Neomycin, Amoxicillin,* and *E. coli,* while same isolate remain intermediate against *Erythromycin* and *Tetracyline* for the isolates of LAB-olives shows resistance against all antimicrobial discs accept *E. coli* that are susceptible. LAB-BA shows that antimicrobial disc *Neomycin Amoxicillin* and *Erythromycin* show resistance and susceptible to the *E. coli.* In same study A 100 µl kiwi aqueous extract showed the highest antibacterial activity against K. *pneumonia* (14.83±0.16mm), followed by S. *aureus* (14.17±0.30mm), *P. aeruginosa* (12.33±0.33mm), *E. coli* (12.17±0.16mm), and *C. albicans* (10.33±0.21mm) (Nessma et al., 2015). This led to the conclusion that when it came to *S. aureus, K. pneumonia, and C. albicans,* kiwi extracts had the strongest antimicrobial properties. The current study's findings were consistent with those reported by (Mishra et al., 2010), who found that kiwi fruit extracts, both methanolic and aqueous, had the greatest inhibitory effect on *E. coli.*

All the data were represented in (Table 4.10) in terms of mean and standard deviation that shows the antimicrobial effects of *Neomycin, Amoxicillin, E. Coli, Erythromycin and tetracycline.* Antimicrobial test conducted against all fruits show by using ANOVA Test. that was performed to assess the effectiveness of different treatments. The analysis showed a highly significant effect of the treatments. The corresponding p-value was 0.001, indicating that the treatments had a statistically significant impact on antimicrobial activity. These results strongly suggest that the treatments applied were effective in inhibiting microbial growth

For bile salt test at 0hrs and 4hrs three sets of data were collected for this investigation. The LAB-BA showed (6.58 ± 0.07) CFU/ml and (5.18 ± 0.24) at 0 and 4 hours, respectively, while the LAB-CH demonstrated significantly higher viability with counts of (8.22 ± 0.3) CFU/ml and (8.41 ± 0.31) log CFU/ml at 0 and 4 hours, respectively. Furthermore, the colony cell count at 0 hours and 4 hours is (7.65± 0.71) and (8.66±2.05), respectively, according to LAB-OLV. Studies found that probiotic fruit isolates were significantly more resilient and viable at higher NaCl concentratin The small intestine secretes bile fluid, which damages bacterial cell membranes and impairs the permeability and viability of the cells. According to (Boke et al., 2010). The suggested concentration of bile salt for human use is between 0.15% and 0.3% for the selection of probiotic bacteria. The growth of the bacterial isolates at 0.3**%** concentrations of bile salts was used in this investigation to determine the ability of the isolates to tolerate bile. The effect of different concentration of NaCl on growth of bacterial isolates. The reduction in growth was significant at higher bile salt concentrations (0.3%). LAB from banana exhibited the lowest tolerance among the three sources, maintaining over 60% growth even at 0.3% bile salt concentration. In a study assessing the growth of various LAB strains with 0.3% bile salt concentration, the following results that were observed from ANOVA Test shows that all isolates of fruits strains also showed a significant treatment effect, P-value of 0.001, suggesting a notable effect of bile salts on their growth as well p-value less then 0.05, indicating that bile salts also influence their growth, Overall, the results demonstrate that bile salt concentration significantly affects the growth of all tested LAB strains, with varying degrees of impact.

When exposed to stimulated in vitro gastric juice of different pH the survival of lab isolate showed significant viabilty ($p<0.05$). According to result all three strains of lab isolates showed growth at pH 0.1 0.2 and 0.3. The result of acid resistance test obtained shows that colonies from LAB-CH, OLV, and BA showed significantly greater viability at pH 2.0, with viable cell counts of 7.49±0.04 CFU/ml, 8.35±0.32, CFU/ml and 9.05±0.02 CFU/ml. In the current study, every strain exhibited satisfactory growth within the pH range of 2-3, with most of them growing at pH 1. Comparing all pH ranges, suggesting a strong acid tolerance that may be attributed to their innate ability to thrive in the acidic environment. Based on the study findings, there was no discernible variation in the pH of the samples over a 24-hour period at intervals of 0 minutes, 30 minutes, 60 minutes, 90 minutes, and 120 minutes. The current study's circumstances were set up to maximize the development of the bacterial isolates at all pH. The survival of different LAB isolate at different pH was statistically analyzed using an ANOVA test. The treatment factor was found to have a highly significant effect on the survival of the isolate, as indicated by the P-value of less then 0.05. These results suggest that the treatment had a profound impact on the survival rate of different isolates under pH 1 2 and 3 stress conditions. Similar study were found (Aiba et al., 1998).LAB-Gr2 and LAB-Bn2 was observed in MRS with 3% bile better than

growth without bile (Table 6). The PH-stressed LAB-Gr2 and LAB-Bn2 cells were able to tolerate PH 2.0 to 4.0 but grow better between pH 3 and 4. It was observed that LAB-Gr2 able to tolerate low pH stressed than LAB-Bn2.

All the selected bacterial isolates from cherry olives and banana were found to be positive for hemolytic activity, as there was change in the color of the medium surrounding the colony on blood agar plates, indicating that there was clear zone of hydrolysis on nutrient gelatin agar plates. Cherry and banana shows clear zone but olives shows partial hemolysis. A similar investigation found that bacillus species isolated from traditional brine pickles show hemolytic activity.The current study's findings were consistent with those reported by (Takkar et al., 2015). One of the most crucial aspects of using probiotic bacteria in food is determining their safety based on the FAO/WHO (2002) standards. One known virulence factor for pathogenic microorganisms is hemolysis. Therefore, the main need for the probiotic screening process is the lack of hemolytic activity (Padmavathi et al., 2018). The probiotic isolates are not pathogenic, as evidenced by their lack of gelatinase and hemolytic activities. According to (DeVuyst et al., 2003), hemolysis is the process that breaks down the cell's epithelium, while gelatinize activity damages the mucous lining. When analyzing the safety of probiotic hemolysis found on blood agar plates, it is necessary to evaluate the hemolytic activity of lactic acid bacteria. Alpha hemolysis causes greenish or brownish staning surrounding colonies. Cherry and banana shows clear zone but olives shows partial hemolysis.

## 4. Conclusion

The present study demonstrated that fruits such as banana, cherry, and olives harbor lactic acid bacteria (LAB) with promising probiotic characteristics. Preliminary identification based on colony morphology, Gram staining, catalase testing, and molecular analysis of the 16S rRNA gene supported the identification of LAB, with Lactobacillus plantarum reported as a predominant species. The isolates exhibited variable proteolytic and amylolytic activities, indicating their potential roles in the degradation of proteins and carbohydrates. Antimicrobial assays revealed differences in inhibitory activity among isolates and tested agents. Furthermore, the isolates demonstrated varying degrees of tolerance to acidic conditions and bile salts, suggesting potential for survival under gastrointestinal-like conditions. However, hemolytic activity was observed in the tested isolates, raising important safety concerns. Therefore, these strains cannot yet be considered suitable for probiotic applications. Further investigation, including accurate species-level identification, virulence assessment, antibiotic resistance characterization, and in vivo validation, is essential before their potential use in functional foods can be established.

**Author contribution**: Conceptualization MT, Methodology ST, Software FF, Formal analysis AS, Investigation ST, Data curation FF, Writing original draft MT, writing review and editing ST, FF and AS

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**Declaration:** it is to declare that AI is used to generate graphical abstract

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**Reference:**

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