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CHARACTERIZATION, PROBIOTIC POTENTIAL AND SAFETY ASSESSMENT OF LACTIC ACID BACTERIA ISOLATED FROM GUAVA FRUITS

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ABSTRACT

There is a necessity to find additional lactic acid bacteria (LAB) from a variety of sources that can be used as probiotics in various food products, attention has not been focused on nondairy sources of LAB, hence, the need to undertake this study. A total of forty guava fruits were screened for presence of LAB using de-Man Rogosa Sharpe media. Morphological, biochemical, and physiological assessments were carried out for preliminary identification. Probiotic potentials of the isolates were assessed using antagonistic activity, bile tolerance, salt and temperature tolerance while safety attributes were evaluated using antibiotics susceptibility, mucin degradation and gelatinase activity. Molecular identification was carried out on two exceptional probiotic isolates using polymerase chain reaction and 16S rRNA. The LAB isolates obtained in this study included *Lactobacillus, Leuconostoc* and *Lactococcus*. Only six isolates showed significant antagonistic activity against the test isolates, tolerated 0.1 to 0.3% bile salts with optimum growth obtained at 37°C. None of the isolates exhibited degradation of mucin, hemolytic or gelatinase activities. The six isolates exhibited probiotic properties and were safe to be incorporated into food. This study revealed that guava-derived LAB strains represent valuable candidates for the development of probiotic-enriched foods and therapeutic applications.

1. INTRODUCTION

Lactic acid bacteria (LAB), recognized for their vital function in food fermentation, form a varied range of beneficial microorganisms (Ruiz-Rodriguez *et al*., 2016). They have gained a lot of interest lately because of their probiotic characteristics, which are classified as live bacteria that enhance the host's health, even at low concentrations (Tufail & Schmitz, 2024).

Fruits, generally have natural characteristics that allows microorganisms, such as lactic acid bacteria (LAB) to survive. According to Marchesi *et al*. (2015) and Zoumpopoulou *et al*. (2016), LAB is a type of bacteria that can colonize intestinal epithelial cells and withstand harsh conditions in the human body, including low pH, pancreatic juice, and salivary enzymes. Numerous bacteria in this class are known to have probiotic qualities, and increasing research suggests that eating a balanced diet high in fruit may help prevent prolonged diseases, increase immunity and reduce the expression of incendiary biomarkers (Hosseini *et al*, 2018).

A wide variety of fruits can enhance food preservation by supplying nutrients, fiber, minerals, bioactive compounds, and the ability to metabolize carbs to lactic acid (Ansari *et al*., 2022). LAB helps preserve perishable foods by creating conditions that prolong their shelf life, particularly when paired with fruit-based substrates like guava (Venkatesan & Muniyan, 2024). Their capacity to modify the immune system and produce bioactive compounds that inhibit pathogenic microorganisms, like antimicrobial peptides, accounts for their probiotic potential (Indira *et al*., 2019).

The tropical fruit guava (*Psidium guajava*), which is consumed as a fresh snack or in making meals, gives a portion of the global population a substantial supply of fiber all year long (Verma, 2023). They are generally high in nutrients, including probiotic-acting phytoactive substances (Kumar *et al*., 2022).

Despite its numerous nutritional, culinary and health benefits, few studies have reported the occurrence of lactic acid bacteria from guava fruits; Tam *et al*. (2025) isolated LAB from guava and utilized them in yoghurt production, *Lactiplantibacillus plantarum* have also been isolated from pickled guava (Mohamad *et al*., 2022) and *Lactobacillus brevis* was isolated and characterized as a probiotic strain from guava fruit (Anitha & Manivannan, 2022).

Understanding the probiotic potential, molecular characteristics, and safety profiles of lactic acid bacteria present in guava will advance food microbiology while also promoting the creation of functional foods from guava that augment health. Thus, this study investigated the isolation, identification, probiotic and safety attributes of LAB from fresh guava fruits in Ibadan, Nigeria.

Materials and Methods.

Sample Collection and Isolation of Lactic Acid Bacteria

Forty samples of guava (*Psidium guajava*) void of dirt were collected from a farm; fruits were washed with sterile distilled water, peeled and diced to remove surface contaminants; 10g from each guava sample was homogenized in 100mLof sterile distilled water, and then serially diluted. Using the pour plate method, 1 mL of the 10^3dilution was inoculated into the de-Man Rogosa Sharpe (MRS) and incubated for 48 hours at 37°C. Pure cultures were then frozen in MRS broth with 20 % glycerol till further investigation (Linares-Morales *et al*., 2020).

Characterization of LAB

The LAB isolates were preliminarily identified based on morphological and biochemical characteristics while two isolates were further identified using molecular tools following the methods of Ruiz Rudriguez *et al*. (2019) and Mohamad *et al*. (2022),

Antimicrobial Activity.

The assay outlined by Gunajit *et al*. (2017) was used to assess the antimicrobial activity using agar well diffusion technique. Cell free supernatants of the isolates obtained from centrifuging 48 hours broth culture of the each isolated LAB at 10,000rpm were tested against two strains each of *Escherichia coli* and *Staphylococcus aureus*.

Screening for Probiotic Potential of LAB Isolates.

Bile Tolerance was analyzed by culturing the LAB in MRS broth incorporated with 0.1 to 0.3 % of bile salt (Oxoid), the control was prepared without bile salt. After 24 hours incubation, the optical density (OD) at 600nm were measured and compared to the control culture (Rajyalakshmi *et al*., 2022). Temperature Sensitivity was

Safety Assessment of the LAB Isolates:

Antibiotic Susceptibility Testing

Agar diffusion assay was employed to determine the susceptibility of the LAB isolates to clinically used antibiotics using the method of James and John (2015); Hemolytic activity was done by streaking LAB isolates on blood agar plates and Gelatinase activity was done by plating isolates on nutrient-gelatin media as described by Pooja *et al*. (2015) and Mucin degradation test was carried out with the procedure of Megur *et al*. (2023).Freshly cultured isolates were used for these assays and cultures were incubated at 37^0C for 24 hours.

Data Analysis

The quantitative data were analyzed using SPSS software's One-way Analysis of Variance (ANOVA). The means from three replicates of the assays were separated and compared using Duncan Multiple Range Tests with significant value set at $P < 0.05$.

Results

The guava fruits sampled in this study harbored sixteen LAB isolates with spherical and smooth edges were obtained .The isolates were gram-positive with rod and cocci shapes and negative for catalase, oxidase, and indole tests as shown in Table 1. The isolates were mainly from the genera *Lactobacillus, Lactococcus,* and *Leuconostoc*. *Lactococcus garvieae* and *Leuconostoc mesenteroides*, were identified at the species level by BLAST analysis of sequence fragments.

Cell-free supernatants from LAB inhibited *Escherichia coli* and *Staphylococcus aureus* with zones of inhibition ranging from 6.00 to 17.00 mm; *L. plantarum* and *L. pentosus* produced the highest zone of inhibition against the test bacteria while *L. brevis* produced the lowest zone of inhibition as shown in Table 2. Table 3 illustrates the impact of bile salts on the development of LAB isolates.

There were notable differences between the cultures' increase in broth containing bile salt ($P<0.05$). LAB isolates' growth was inhibited with increasing bile salt concentrations (from 0.0% to 0.3% w/v), suggesting decreased viability at higher bile levels. Higher bile tolerance is shown by isolates GS4 and GS10's greater growth across concentrations

All of the guava LAB isolates reached their maximum optical density (OD) values at 37 °C, where they also showed optimal development. At 25 °C, growth is noticeably slower for all isolates, indicating that bacterial metabolism is slowed down by the lower temperature. Growth also decreased at 40 °C as presented in Table 4. Table 5 shows the effects of varying NaCl concentrations on the growth of the preferred LAB isolates. The optimal growth conditions for all LAB isolates are at 0% NaCl; growth slows down as NaCl concentration rises, suggesting a decreased ability to withstand high salt concentrations. The exception, GS4, exhibits comparatively steady growth up to 4% NaCl, indicating a greater resistance to salt than the other isolates.

The susceptibility of the selected LAB isolates to antibiotics is shown in Table 6. All the lactic acid bacteria isolated in this study were resistant to Vancomycin, highest zone of inhibition was recorded for Ampicillin and Nitrofurantoin, only *Leuconostoc mesenteroides* was resistant to Ampicillin. Table 7 displays the hemolytic activity, gelatinase activity, and mucin degradation of the isolates. The isolates showed no hemolytic activity on blood agar, and were tested negative for gelatin hydrolysis and breakdown of mucin.

Table 1: Morphology and Biochemical characteristics of Lactic Acid Bacteria from Guava

Isolate code	Gram reaction	Cellular morphology	Catalase test	Indole Test	Oxidase test	Probable identity
GS1	+	R	-	-	-	*L plantarum*
GS2	+	R	-	-	-	*L. plantarum*
GS3	+	C	-	-	-	*L.gravidae*
GS4	+	C	-	-	-	*L.mesenteroide*
GS5	+	R	-	-	-	*L. brevis*
GS6	+	R	-	-	-	*L. pentosus*
GS7	+	R	-	-	-	*L.acidophilus*
GS8	+	R	-	-	-	*L. rhamnosus*
GS9	+	C	-	-	-	*L. garvieae*
GS10	+	R	-	-	-	*L. plantarum*
GS11	+	R	-	-	-	*L. rhamnosus*
GS12	+	R	-	-	-	*L. pentosus*
GS13	+	R	-	-	-	*L. brevis*
GS14	+	R	-	-	-	*L. plantarum*
GS15	+	R	-	-	-	*L. rhamnosus*
GS16	+	C	-	-	-	*L. mesenteroide*

Keys: GS: Guava sample, + positive, - negative, C cocci, R Rod

Table 2: Antagonistic activity of selected LAB isolates against *Escherichia coli and Staphylococcus aureus.*

Isolates code	Zone of Inhibition (mm)			
	Escherichia coli	***Escherichia coli***	***Staphylococcus aureus***	***Staphylococcus aureus***
L. plantarum	10.00 ± 0.01	16.00 ± 0.01	17.00 ± 0.01	12.00 ± 0.01
L.mesenteroides	10.00 ± 0.00	06.00 ± 0.00	10.00 ± 0.01	06.00 ± 0.00
L. pentosus	10.00 ± 0.00	13.00 ± 0.01	17.00 ± 0.01	11.00 ± 0.01
L. garvieae	12.00 ± 0.01	12.00 ± 0.02	10.00 ± 0.01	10.00 ± 0.01
L.plantarum	12.00 ± 0.01	06.00 ± 0.00	12.00 ± 0.02	06.00 ± 0.00
L. brevis	06.00 ± 0.00	06.00 ± 0.00	10.00 ± 0.00	06.00 ± 0.00

Table 3: Effects of different concentrations of bile salt on the growth of selected isolates

Isolates	**Bile Salt % (w/v)/OD_{600nm}**			
	0.0	0.1	0.2	0.3
L. plantarum	0.124	0.112	0.089	0.061
L.mesenteroides	0.148	0.130	0.105	0.080
L.pentosus	0.135	0.115	0.097	0.070
L. garvieae	0.130	0.108	0.095	0.072
L. plantarum	0.140	0.120	0.078	0.065
L. brevis	0.122	0.095	0.083	0.060

Table 4: Effects of varying temperatures on the growth of selected LAB isolates

Isolates code	**Temperature//OD_{600nm}**		
	25°C	**37°C**	**40°C**
Lactiplantibacillus plantarum	0.080	0.145	0.130
Leuconostoc mesenteroides	0.076	0.155	0.128
Lactobacillus pentosus	0.080	0.148	0.127
Lactococcus garvieae	0.070	0.138	0.120
Lactiplantibacillus plantarum	0.085	0.150	0.125
Lactobacillus brevis	0.072	0.142	0.118

Table 5: Sodium Chloride (NaCl) Tolerance of the selected LAB isolates

Isolates code	**NaCl Concentration % (w/v)/ /OD_{600nm}**			
	0	**2**	**4**	**6**
L. plantarum	0.135	0.125	0.095	0.070
L.mesenteroides	0.150	0.140	0.135	0.078
L. pentosus	0.140	0.125	0.095	0.073
L.garvieae	0.130	0.118	0.085	0.065
L. plantarum	0.145	0.130	0.100	0.080
L. brevis	0.128	0.115	0.090	0.068

Table 6: **Antibiotic Susceptibility Profile of the Selected LAB Isolates**

Isolate Code	Antibiotics/Zone of Inhibition								
	AMP	**CAZ**	**GEN**	**CRX**	**NIT**	**AUG**	**OFL**	**VAN**	**CPR**
Lactobacillus plantarum	22.0	15.0	14.0	16.0	20.0	19.0	13.0	0.0	18.0
Leuconostoc mesenteroides	0.0	10.0	0.0	15.0	9.0	0.0	10.0	0.0	12.0
Lactobacillus pentosus	21.0	20.0	17.0	19.0	21.0	23.0	18.0	0.0	22.0
Lactococcus garvieae	23.0	18.0	16.5	17.0	22.0	22.0	15.0	0.0	20.0
Lactobacillus plantarum	19.0	12.0	15.0	11.0	16.0	21.0	11.0	0.0	15.0
Lactobacillus brevis	16.0	14.0	0.0	12.0	0.0	10.0	11.0	0.0	11.0

Keys:

AMP: Ampicillin, CRX: Ciprofloxacin, CAZ: Ceftazidime, GEN: Gentamicin, AUG: Amoxicillin, OFL: Ofloxacin, VAN: Vancomycin, CPR: Cefpirome, NIT: Nitrofurantoin

Table 7: Hemolytic Activity, Gelatinase Activity, and Mucin Degradation of the Selected LAB .

Isolate Code	Hemolysis Type	Gelatinase Activity	Mucin degradation
Lactiplantibacillus plantarum	-	-	-
Leuconostoc mesenteroides	-	-	-
Lactobacillus pentosus	-	-	-
Lactococcus garvieae	-	-	-
Lactiplantibacillus plantarum	-	-	-
Lactobacillus brevis	-	-	-

Key: -: Negative

Discussion

The results of this research demonstrated the molecular diversity, probiotic potential and safety profiles of lactic acid bacteria (LAB) that were isolated from fresh guava fruits. Sixteen (16) isolates with characteristic lactic acid bacteria (LAB) morphology were obtained from guava after LAB was isolated and subjected to preliminary characterization. This morphological and biochemical identification is consistent with Fessard & Remize (2019) findings, which found that the most commonly isolated LAB genera from fresh fruits and vegetative samples are *Lactobacillus, Leuconostoc, Weissella, Enterococcus, and Pediococcus.* The occurrence of *Lactococcus* species and other LAB strains from Guava fruits is similar to what was reported by Tam *et al*. (2025), the LAB strains from their research had similar phenotypic traits and antibacterial potentials with what was observed in this study.

Antagonistic activity of lactic acid bacteria obtained in this study against the selected test isolates is a reflection of the production of metabolites produced by the isolates and it is accordance to what has been reported by Todorov *et al*. (2023) that characterized bacteriocin obtained from lactic acid bacteria present in Guava fruits, similar inhibitory properties of lactic acid bacteria obtained from Guava and other edible fruits against Gram-positive and Gram-negative bacteria.

.According to earlier research that corroborated what was obtained in our study, Krishnendra *et al*. (2013) and Arici *et al*. (2016) reported that LAB strains may have the capacity to suppress harmful microorganisms by generating organic acids and bacteriocins, which is a necessary characteristic for their probiotic and preservative uses.

The high bile tolerance seen in all isolates especially in *Leuconostoc mesenteroides*, is consistent with earlier research showing that LAB derived from plants frequently exhibits tolerance to gastrointestinal disorders (Schifano *et al*., 2021). At a lesser concentration (0.1%), the LAB isolates demonstrated a high tolerance to bile salt; however, as the bile concentration rose, their tolerance declined. This tolerance raises the possibility that these strains will live and colonize the gut, which is beneficial for possible probiotic application.an essential quality of successful probiotics (Schifano *et al*., 2021)."The lower survival rate can be explained by the fact that after bacteria were exposed to bile salt, cellular homeostasis was disrupted, which caused the lipid bilayer and integral protein of their cell membranes to dissociate, resulting in bacterial content leaks and ultimately cell death," according to Eurydice *et al*. (2016).

All of the isolates in the study grew between 25 and 40 °C, but they all grew best at 37 °C, which is a temperature that is typical of the human body. A minor inhibition at 40 °C indicated thermal sensitivity, while growth was decreased at 25 °C, most likely as a result of slowed bacterial metabolism. This study's findings are consistent with Pundir *et al*. (2013), who found that lactic acid bacteria could thrive in temperatures between 25 and 40 °C. Temperature has a significant impact on bacterial development and that the isolates would not have been able to survive in the human gut, which is crucial for probiotics, if they had not been able to grow within the temperature range.

Although isolates' tolerance to NaCl varied, all of them demonstrated optimal growth at 0% NaCl and could withstand 0% to 6%. Significantly, GS4 showed improved salt resistance, growing comparatively steadily up to 4% NaCl. This should increase its viability in situations with

high salt content, which is crucial for food applications. The results of this investigation are supported by those of Gunajit *et al*. (2017), who found that *L. plantarum* could withstand 6% NaCl, but the other isolates could only withstand 5% NaCl. Nandha & Shukla (2023) isolated LAB from *Theobroma cacoa* including the genera *Lactococcus,* and *Lactobacillus*, Ilesanmi & Eniola (2023) isolated probiotic LAB strains from pineapple and watermelon. The probiotic strain isolated from their studies showed no safety concerns fit for use in the food industry.

Antibiotic susceptibility profile of the LAB strain in this study was not similar to what was observed by certain researchers, Saif, (2016) reported that LAB from some fruits showed multidrug resistance, while only one out of 38 LAB isolates showed sensitivity to the antibiotics tested. LAB are believed to be resistant to certain antibiotics like Vancomycin. This indicates their potential effectiveness in therapeutic applications and probiotic formulations, which are likely to be inhibited by commonly used antibiotics.

The LAB's isolates sensitivity to cell wall and protein synthesis inhibitors has been reported by Gad *et al*. (2014). However, strain-specific modifications that may impact probiotic safety are suggested by isolates GS4 and GS13's resistance to a number of antibiotics, including ciprofloxacin and amoxicillin-clavulanic acid (Stefanska *et al*., 2021). Notably, every isolate is appropriate for use in probiotic applications as none recorded multidrug resistance.

The non-pathogenic nature of the LAB isolates was confirmed by safety evaluations that included gelatinase tests, hemolytic activity, and antibiotic susceptibility. Important markers of LAB's safety for usage with probiotics are their hemolytic and gelatinase activity.

All isolates exhibited gamma hemolysis (no hemolysis), which is favorable because it suggests non-pathogenicity and is consistent with the safe profile of many LAB strains. Hemolytic activity was evaluated to identify the pathogenic potential. According to Holzapfel & Todorov (2023) and Soleimani *et al*. (2023), LAB strains that exhibit γ-hemolysis do not possess virulence factors typically associated with pathogenic bacteria, which supports their classification as generally recognized as safe (GRAS) organisms. This study's findings are consistent with other studies that have reported the absence of invitro hemolytic activity by various probiotic LAB. Since the production of gelatinase can be linked to virulence in pathogenic bacteria, as these enzymes aid in the breakdown of host tissues and promote invasive infections, gelatinase activity testing, which assesses the production of proteolytic enzymes linked to tissue degradation, came back negative for all LAB isolates, confirming their safety.

To the best of our knowledge, the only study that isolated, identified and screened for probiotic potential of LAB from guava fruit was Anitha & Manivannan. (2022), *Lactobacillus brevis* was discovered as a promising probiotic candidate in their study, our study enumerated six promising probiotic culture that can be harnessed for production of functional foods and biopreservatives.

Conclusion

This study's findings demonstrate the potential of guava-derived LAB as exceptional probiotic candidates for food, feed, and pharmaceutical applications. These probiotics could function as novel cultures outside the dairy-derived LAB species, they can be added into fruits and fruit-based products to promote the organoleptic, nutritional and antioxidants qualities while lowering the anti-nutritive ingredients in food.

References

Anitha, S. & Manivannan, A. (2022). Isolation, molecular identification, and investigation of the probiotic potential of lactic acid bacteria isolated from Psidium guajava. *Research Square*.

Ansari, F., & Pourjafar, H. (2022). Influence of fermentation and germination on food bioactives. In *Retention of Bioactives in Food Processing*. Cham: Springer International Publishing, 277-308.

Eurydice, F., Ngongang, T., Tiencheu, B., Fossi, B.T, Achidi, A., Shiynyuy, D.M., Womeni, H. & Francois, Z. (2016). Isolation and Identification of Cholesterol-Lowering Probiotic Bacteria from Palm Wine (*Raffia mambillensis*). *Journal of Microbiology Research,* 6(5), 93-102.

Fessard, A. & Remize, F. (2019). Genetic and Technology characterization of lactic acid bacteria isolated from tropically grown fruits and vegetables. *International Journal of Food Microbiology,* 301, 61-72.

Gad, G. F. M., Abdel-Hamid, A. M. & Farag, Z. S. H. (2014). Antibiotic resistance in lactic acid bacteria isolated from some pharmaceutical and dairy products. *Brazilian Journal of Microbiology,* 45(1), 25-33.

Gunajit, G., Bora, S. S., Parveen, A., R. & Barooah, M. (2017). Identification and Functional properties of dominant lactic acid bacteria isolated from Kahudi, a traditional rapeseed fermented food product of Assam, India. *Journal of Ethic Foods,* 4(3), 187-197.

Holzapfel, W. H. & Todorov, S. D. (2023). Special issue: Beneficial Properties and Safety of Lactic Acid Bacteria. *Microorganism,* 11(4), 871.

Hosseini, B., Berthon, B. S., Saedisomeolia, A., Starkey, M. R, Collison, A., Wark, P. A. B. & Wood, L.G. (2018). Effects of fruit and vegetable consumption on inflammatory biomarkers and immune cell populations: a systematic literature review and meta-analysis. *American Journal of Clinical Nutrition,* 108(1), 136-155.

Ilesanmi T.M. & Eniola K.I.T (2023). Isolation and antimicrobial activity of lactic acid bacteria isolated from pineapple and watermelon. *GSC Biological and Pharmaceutical sequences,* 23(02), 148-54.

Indira, M., Venkateswarulu, T. C., Peele, K. A, Bobby, M. N. & Krupanidhi, S. (2019). Bioactive molecules of probiotics bacteria and their mechanism of action: a review. *3 Biotech,* 9(8).

James, J. & John, T. (2015). Manual of Clinical Microbiology. In ASM Press eBooks.

Kodba, A., Pampangouda, Mahadevaswamy, Naik, N. M. & Huchchannavar, S. (2024). Isolation, characterization, and screening of Lactic Acid Bacteria (LAB) from fruits for the production of probiotic beverages. *International Journal of Advanced Biochemistry Research,* 8(4), 88-101.

Kumar, K., Ahmed, N., Rizvi, Q. H, Jan, S., Thakur, p., Chauhan, D. & Kaur, J. (2022). Guava Wastes and By-Products: Chemistry, Processing, and Utilization. *Handbook of Fruits Wastes and By-Products,* 99-10

Linares-Morales, J, R., Cuellar-Nevárez, G. E., Rivera-Chavira, B. E, Gutiérrez-Méndez, N., Pérez-Vega, S. B. & Nevárez-Moorillón, G. V. (2020). Selection of Lactic Acid Bacteria isolated from Fresh Fruits and Vegetables Based on Their Antimicrobial and Enzymatic Activities. *Foods,* 9(10), 1399.

Marchesi, J. R., Adams, D. H., Fava, F., Hermes, G. D. A., Hirschfield, G. M., Hold, G., Quraishi, M. N., Kinross, J., Smidt, H, Tuohy, k. M., Thomas, L. V., Zoetendal, E. G. & Hart, A. (2015). The gut microbiota and host health: a new clinical frontier. *Gut,* 65(2), 330-339.

Megur, A., Daliri, E. B., Balnionytė, T., Stankevičiūtė, J, Lastauskienė, E. & Burokas, A. (2023). In vitro screening and characterization of lactic acid bacteria from Lithuanian fermented food with potential probiotic properties. *Frontiers in Microbiology*, 14.

Mohamad, N.I., Manan, M.A, MOHD, M.I. & Abdullah Sani, N. (2022). Antibacterial activity and organic acids formation by *Lactobacillus* sp. Originated from pickled guava and papaya. *Journal of Sustainability Science and Management*, 17(2), 286-295.

Nandha, M.C. & Shukla, R.M. (2023). Exploration of probiotic attributed in lactic acid bacteria isolated from fermented *Theobroma cacao L*. fruit using in vitro techniques. *Frontiers in Microbiology*, 14, 1274636.

Pooja, T., Modi, H.A. & Prajapati, J.B. (2015). Isolation, characterization, and safety assessment of lactic acid bacterial isolates from fermented food products. *International Journal of Current Microbiology and Applied Sciences,* 4(4), 713-725.

Pundir, N. R. K., Rana, S, Kashyap, N. & Kaur, A. (2013). Probiotic potential of Lactic Acid Bacteria isolated from food samples: an in vitro study. *Journal of Applied Pharmaceutical Science,* 3(03), 085-093.

Rajyalakshmi, K, Priya, S.J. & Satya, A.K. (2022). Isolation, identification, and evaluation of probiotics from a nutritive fruit sample. *International Journal of Food Science and Nutrition,* 4(6), 06-09.

Ruiz Rodríguez, L.G., Mohamed, F., Bleckwedel, J., Medina, R, De Vuyst, L., Hebert, E.M. & Mozzi, F. (2019). Diversity and functional properties of Lactic Acid Bacteria isolated from wild fruits and flowers present in Northern Argentina. *Frontiers in microbiology*, 10:1091

Saif, F.A.A.A. (2016). Efficacy of Lactic Acid Bacteria isolated from some fruits and vegetables. *Egyptian Journal of Microbiology*, 51(1), 13-28

Schifano, E., Tomassini, A., Preziosi, A., Montes, J., Aureli, W, Mancini, P., Miccheli, A., & Uccelletti, D. (2021). *Leuconostoc mesenteroides* Strains Isolated from Carrots Show Probiotic Features. *Microorganisms*, 9(11), 2290.

Soleimani, H., Shokri, R., Nami, Y, Khandaghi, J. & Panahi, B. (2023). Potential probiotic characterization of Lactic Acid Bacteria (LAB) isolated from Duimaj, an Iranian traditional snack food, using biochemical, molecular, and computational approaches. *LWT*, 184, 115091.

Stefanska, I., Kwiecień, E., Jóźwiak-Piasecka, K, Garbowska, M., Binek, M., & Rzewuska, M. (2021). Antimicrobial susceptibility of Lactic Acid Bacteria strains of potential use as feed additives - the Basic Safety and Usefulness Criterion. *Frontiers in Veterinary Science*, 8, 687071

Tam, H. N. T., Nam, T. V. B. & Hau, T. H. (2025). Selection, identification, and application in yogurt fermentation of lactic acid bacteria isolated from guava (*Psidium guajava* L.) fruits. *CTU Journal of Innovation and Sustainable Development*, 17(3), 88-98.

Todorov, S.D., Holzapfel, W.H. & Tagg, J.R. (2023).

Bacteriocinogenic *Enterococcus casseliflavus* Isolated from Fresh Guava Fruit (*Psidium guajava*): Characterization of Bacteriocin ST192Gu and Some Aspects of Its Mode of Action on *Listeria* spp., and *Enterococcus* spp. *Fermentation*, 9(3), 226.

Tufail, M. A. & Schmitz, R. A. (2024). Exploring the Probiotic Potential of *Bacteroides* spp. Within One Health Paradigm. *Probiotics and Antimicrobial Proteins*, 17(2), 681–704.

Venkatesan, U. & Muniyan, R. (2024). Review on the extension of shelf life for fruits and vegetables using natural preservatives. *Food Science and Biotechnology*, 33(11), 2477–2496.

Verma, P. (2023). Guava: A Wonder Fruit. *Research & Reviews: A Journal of Pharmacognosy*, 10(2), 1–5.

Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J, Bai, X., Xie, J., Wang, Y. & Geng, W. (2021). Metabolism characteristics of Lactic Acid Bacteria and the expanding applications in the food industry. *Frontiers in Bioengineering and Biotechnology*, 9, 612285

Zoumpopoulou, G., Pot, B, Tsakalidou, E. & Papadimitriou, K. (2016). Dairy probiotics: Beyond the role of promoting gut and immune health. *International Dairy Journal*, 67, 46–60.

Supplementary File. Nucleotide Sequence of Lactic acid bacteria obtained in this study

Lactococcus garviae
CGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGCGGCGTTGC
TCGGTCAGGG

Leuconostoc mesenteroides
GAGCGCAGACGTTTATTAAGTCTGATGTGAAAGCCCGGAGCTCAACTCCGGAATGGCAT
TGGAAACTGGTTAACTTGAGTGCAGTAGAGGTAAGTGGAACTCCATGTGTAGCGGTGGA
ATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTTACTGGACTGCAACTGAC
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CACCATGGGAGTTTGTAATGCCCAAAG