Isolation, Characterization, and Biological Activity of Indigenous Probiotics Derived from Traditional Fermented Dairy and Non-dairy Products

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Abstract: This research intended to examine the health-promoting potential of probiotics isolated and preserved from local dairy and non-dairy products. Eight isolates were identified, with four selected for further analysis based on colony morphology and genotypic identification. The detected isolates were found gram-positive, catalase-negative, and had distinct colony morphologies. Their growing conditions in MRS broth were optimized. All of the bacterial species showed robust growth at a temperature of 37°C, with the exception of *Vagococcus fluvialis*, which displayed optimal growth at 45°C. *Lactobacillus furfuricola* exhibited anti-acne potential against *Propionibacterium acnes*, resulting in a 17.4 mm growth inhibition zone. Yogurt formation varied with bacterial inoculation and milk type. Additionally, antibiotic susceptibility patterns also varied across the isolates. *Enterococcus faecium* and *L. furfuricola* exhibited the highest proteolytic activity, yielding casein hydrolysis zones up to 34.66 mm and 40.50 mm, respectively, in their cell-free culture fluids. *L. furfuricola* showed the greatest survivability in simulated salivary fluid (SSF) at 0 and 2 minutes, while *V. fluvialis* showed the greatest survivability in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) at 0 minutes and 2 hours. Inoculum size optimization for fruit peel extracts showed variable growth preferences; all the isolates also grew well in the extracts of different fruits and dry fruit peels. The probiotics also expressed free radical-scavenging activity in MRS as well as in biowaste-based media. These findings can be exploited for the potential of probiotic strains from fruit peels in enhancing health-promoting properties.

Keywords: Antioxidant; Anti *Propionibacterium acnes*; Fruit peel extracts: GIT simulation; Probiotics.

I. INTRODUCTION

The prevalence of a symbiotic interaction between microbial communities and the human body underlines the possibility of a mutually beneficial association. The initial recognition of a favorable link between microorganisms and the human body was attributed to Doderlein in 1892. Metchnikoff (1908) subsequently documented the presence of microorganisms derived from fermented milk products, which had beneficial effects on human health. Significant breakthroughs in the science of microbiology have been achieved in the past century, primarily due to technological innovation, particularly in the domain of genetic analysis (Pires *et al.*, 2016). The term "microbiome" was introduced by Joshua Lederberg in 2001. It encompasses the whole genetic succession of all living microorganisms within the human body, including various systems such as the gastrointestinal, cutaneous, respiratory, and urogenital systems. A more appropriate designation for this microbial population is "microbiota," denoting the distinct and unique makeup (genera/species) of this microbial population that exhibits variability across individuals and exerts influence on human well-being (Santacroce *et al.*, 2021).

Probiotics refer to living microorganisms that have beneficial effects on host, which have emerged as a focal point in the quest for improved health and wellbeing. These beneficial bacteria are introduced into the GIT of the host can modulate the composition and function of microbiota, exerting a positive influence on the host's physiology (Cheng *et al.*, 2019). The impact of food components on the enteric microbiota and their positive benefits on animal health are also responsible for eliciting pragmatic impacts on probiotics (Sánchez *et al.*, 2017). The use of probiotics in animal meals and liquids has the potential to improve overall health and well-being. A diverse array of beneficial bacteria can be naturally present in many food and beverage sources, as well as introduced into food and beverage products (Marsh *et al.*, 2014). In the dairy sector, probiotic microorganisms have been employed

to enhance the production of innovative probiotic functional dairy products. Yogurt, milk, and cheese are frequently linked to the consumption of probiotics (Tamime *et al.*, 2005). In addition to dairy or milk-based probiotic products, many non-dairy probiotic products have also developed. Furthermore, a vast number of human investigations have demonstrated that consumption of specific strains of lactic acid bacteria might enhance humoral immune responses to infections (Gill & Guarner, 2004).

The *Lactobacillus* and *Bifidobacterium* genera are the most often described sources of probiotic bacteria. However, some other genera that produce lactic acid such as *Enterococcus*, *Pediococcus*, *Lactococcus* and *Streptococcus* are also reported as prospective probiotics. (Hanchi *et al.*, 2018). In addition, *Akkermansia muciniphila*, several *Bacillus spp*., and *Propionibacterium freudenreichi* are classified as GRAS bacteria, and yeasts of the genus *Saccharomyces* have also been documented to possess probiotic properties (Kvakova *et al.*, 2021). Despite many other health issues, antimicrobial resistance is a serious problem faced by the world (Vilacoba *et al.*, 2016). People are replacing antibiotics with natural chemicals and probiotics. It is usually believed that natural compounds may treat more ailments than synthetic drugs and antibiotics (Suez & Elinav, 2017).

Beyond the conventional provenances, many non-dairy sources are also being screened for potential probiotics. These include non-fermented foods such as vegetables and fruit juices, as well as a variety of non-dairy conventional fermented foods (Kumar *et al.*, 2022). In addition to human breast milk, human excrement from healthy adults, as well as newborns who are breastfed, have all been shown to be possible sources of probiotic microbes (Sornplang & Piyadeatsoontorn, 2016).

The survivability of probiotics in the supplement determines its efficacy. The viability of a microbe impacts its capacity to colonize new environments (Wilkinson, 2018). It is well known that antimicrobial and other beneficial metabolites cannot be developed through non-viable cells (Lahtinen *et al.*, 2005). The immobilization of cells is a crucial factor in ensuring the stability, viability, substrate recycling, and tolerance to various environmental challenges, including temperature and pH (Tsen *et al.*, 2007). Commercial starter cultures were initially delivered in liquid form prior to the invention of technologies for creating and conserving condensed starter cultures. Biotechnological advancements have played a crucial role in improving the stability and effectiveness of probiotics, leading to the introduction of concentrated starter cultures in frozen and freeze-dried forms in food formulations (Desmond *et al.*, 2001).

The present study isolated and characterized probiotic bacteria from dairy and non-dairy items from Martinpur village, Nankana Sahib. Eight probiotic strains from four species were identified for their probiotic potential after extensive characterization. Due to their "Generally Regarded as Safe" status and selective antibiotic resistance, these isolates can be used as probiotics during antibiotic treatment. Probiotics are stored at the conservatory of the Microbial Biotechnology Laboratory, Institute of Zoology, University of Punjab, Lahore and we aim to facilitate further study into their probiotic properties and therapeutic potential, advancing our understanding of indigenous probiotics and their role in promoting human health.

II. Material and Methods

A. *Sample Collection and Bacterial Isolation*

Six samples from Nankana Sahib, Pakistan, were collected for probiotic bacteria separation. Samples included dairy waste-influenced soil at various depths, homemade pickles, raw cheese, and yogurt. Martinpur village soil samples were gathered from a residence. The test area had an earthen floor that had been stained by household dairy waste and washings for years. Samples were obtained from 5, 10, and 15 cm of soil excavated with a sterile spatula. A hole was dug and marked with a sterile scale at the desired depths, and soil samples were obtained using a tiny sterile spade. Homemade pickles and raw cheese were tested using an autoclaved spatula, while yogurt was syringed. All samples were immediately placed in autoclaved vails and delivered to the laboratory for analysis.

De Man, Rogosa, and Sharpe (MRS) broth was used to grow each sample. Each sample was put into a loop that held 15 ml of sterile MRS broth. For four days, the vails were kept at 37 °C. The growth was then streaked onto the MRS agar plates which were then incubated anaerobically at 37 °C for 4 days. Pure culturing was performed on colonies obtained from MRS agar plates. The soil sample collected at a depth of 15 cm exhibited three unique colony kinds, namely 3A, 3B, and 3C. The quadrat technique was employed to streak each colony individually on a nutrient agar plate. The pickle and raw cheese samples were categorized into four and three unique kinds of colonies, denoted as 4A, 4C1, 4C2, and 4C3, and 5B, 5C, and 5D, respectively. The various bacterial colonies were streaked onto nutrient agar plates, as previously described, and thereafter incubated at a temperature of 37 °C for a duration of 4 days. The bacterial colonies of each unique type were carefully isolated from the nutrient agar plates and subsequently re-streaked onto MRS agar plates. The MRS agar plates were then cultured under anaerobic conditions. Following that, the pure cultures were conserved using the process of streaking on MRS agar slants.

B. *Colony Morphology of the Isolates on MRS and Nutrient Agar media*

The colony morphology of all the isolates were observed following their growth of isolated colonies on MRS and nutrient agar plates. Different features like margin, elevation, configuration, texture and color were recorded (Dejene *et al.*, 2021).

C. *Phenotypic and Genotypic Characterization of the Bacterial Isolates*

For phenotypic characterization of the bacterial isolate's gram staining, catalase, citrate, gelatinase hydrolysis and carbohydrate

fermentation tests were performed according to (Dejene *et al.*, 2021; Dela Cruz & Torres, 2012; Maria, 2009; Reiner, 2010; Reiner, 2012), respectively. For molecular level identification of the bacterial isolates their 16s rRNA genes were got sequenced commercially.

D. Optimization of Growth Conditions

For 48 hours at 37°C, 9 ml of sterile MRS broth and 1 ml 48-hour-old bacterial culture were incubated to optimize temperature, pH, and inoculum Un-inoculated MRS broth was adjusted to acidic (6), neutral (7), and basic (8) for pH optimization. Different concentrations of 48 bacterial cultures were injected in autoclaved MRS broth to optimize inoculum size. Optical density (OD) of bacterial cultures was obtained at 600nm in triplicates for each growth condition after 48 hours.

E. Yogurt formation test

Locally purchased processed (Olper's) milk was used to evaluate yogurt formation. The milk was taken to the lab and diluted by mixing 5 and 2.5 ml with 5 and 7.5 ml of autoclaved distilled water. Out of 10 ml of non-diluted and diluted milk samples, 0.1 ml was taken out. To make 10 ml cultures, 48-hour-old bacteria were injected. Incubated vails at 37°C for 48 hours were checked for yogurt formation. A similar process was used with raw cow milk

F. Microbial Inhibitory Potential

The antimicrobial efficacy of 4C2 was examined in a test using P. acnes through the application of the well diffusion method. A 100 µl culture of acne bacterium was placed onto the BHI agar plate. Agar was prepared by using a sterilized yellow tip to create a well. In a sterile environment, 4C2 culture was transferred to the wells. The plate was examined for a zone of growth inhibition after 48 hours of incubation.

G. Antibiotic Susceptibility Test

The spread plate method was utilized to conduct the antimicrobial susceptibility test (Lalitha, 2004). A total of fifteen different antibiotics were used. A 100 µl fresh culture of the test bacteria was uniformly distributed over the MRS agar plate. Four antibiotic disks were put at a distance from each plate. Anaerobic conditions were maintained for two days at 37 °C while the plates were incubated. Subsequently, they were examined to determine their susceptibility to antibiotics.

H. Survivability of the Probiotic Isolates under GIT Simulation

The simulation of the GIT was conducted utilizing several simulatory fluids, including SSF, SGF, and SIF. The method outlined by Minekus *et al.* (2014) was used to simulate gastrointestinal digestion utilizing several probiotic species, with slight adjustments. After all, three simulation stages, MRS agar plates were incubated anaerobically at 37 °C for 48 hours, and outcomes were measured in units of C.F.U./ml of the specimen. Fig. 1 shows the overview of different phases of in-vitro digestion.

I. Enzyme Production Test

The bacterial isolates were assessed for the production of amylase, protease, cellulose and lipase production following their growth on respective agar media plates (Khalil, 2011).

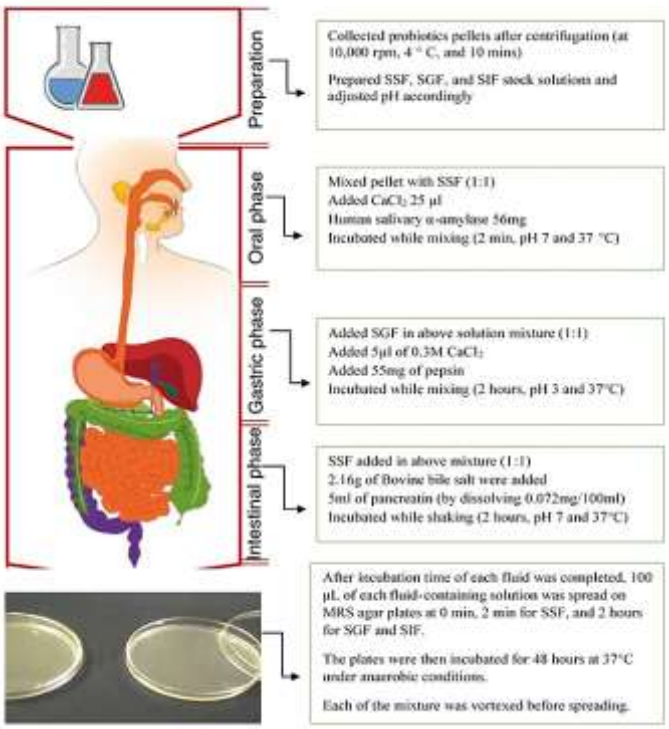

Figure 1. Overview of different phases of in-vitro digestion protocol followed in this study.

J. Optimizing the Inocula sizes of the Probiotic Isolates in Extracts of Fruits and Dry Fruit Peels

Peels of apples, oranges, pomegranates and almonds were suspended in 5% concentration and autoclaved. Then 48 h. grown culture of a bacterium was inoculated as of total volume of 5 ml. The inoculated extracts were then incubated at a temperature of 37°C for 24 h and 48 h and OD was measured at 600 nm (Balaban *et al.*, 2021).

K. Antioxidant Properties of Probiotics and Fruit Peel Extracts

1.5 ml of 0.1mM solution DPPH solution was added to 3.5 ml of a bacterial culture. The solution was softly agitated and placed in an incubator for 30 minutes at a temperature of 37 °C. Following the incubation period, the absorbance was measured. The control consisted of a mixture of 1.5 ml of DPPH solution and 3.5 ml of methanol solution. A blank solution of 3.5 ml of methanol was used. Every individual sample was assigned a distinct blank, and the entire experiment was conducted in triplicate. The same procedure was done for 5% extracts of fruits and dry fruit peels by Gupta *et al.* (2020). Calculate the antioxidant activity by:

$$\text{DPPH RSA (\%)} = ((\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}}) \times 100$$

Percent increase or decrease in its antioxidant activity was calculated for a respective bacterium whole growing in standard MRS and a respective fruit peel's extract.

L. Statistical analysis

The data were analyzed utilizing a one-way ANOVA, which was then followed by the utilization of post-hoc testing followed Şahin and Aybek (2019), by assisted SPSS version 26.

III. RESULTS

Colony Morphology and Phenotypic & Genotypic Characterization of the Isolates

Eight isolates from the samples processed on MRS selective medium underwent commercial sequencing for the 16S r-RNA gene. Out of the eight species, four were processed for further research purposes. Colony morphology remained the same on MRS agar and nutrient agar media. Table 1 provides details on the identification and colony morphology of probiotic isolates.

All the isolates were gram-positive, catalase-negative and citrate-negative except for *V. fluvialis*, which was citrate-positive. Except for *E. faecium* and *B. siamensis*, others had demonstrated negative gelatin hydrolysis. The carbohydrate utilization test showed that all the bacteria didn't utilize mannose except for *Lactobacillus furfuricola*. Whereas only *Bacillus siamensis* utilized maltose, while sucrose was utilized by *E. faecium* and *V. fluvialis*.

Table 1: Morphology and genotypic identification of pure cultures of bacterial colonies.

Colony Code	Morphology	Scientific Names (Accession number)
3A	White, dry with lobate margin, hilly elevation and irregular configuration	*Enterococcus faecium* (NR_114742.1)
4A	Sticky, off-white with smooth margin, raised elevation and round configuration	*Enterococcus faecium* (NR_114742.1)
4C1	Sticky, creamy (off-white) with wavy margin, umbonate elevation and irregular configuration	*Enterococcus faecium* (NR_114742.1)
4C2	Sticky, creamy with curled margin, umbonate elevation and concentric configuration	*Lactobacillus furfuricola* (NR_126193.1)
4C3	Sticky, creamy-white with wavy margin, raised elevation and round configuration	*Vagococcus fluvialis* (NR_026489.1)
5B	Shiny, creamy-white with smooth margin, raised elevation and round with raised margin configuration	*Enterococcus faecium* (NR_114742.1)
5C	Moist, off-white with smooth margin, drop like elevation and round configuration	*Bacillus siamensis* (KY643639.1)
5D	Shiny, white with smooth (entire) margin, flat elevation and L-form configuration	*Enterococcus faecium* (NR_114742.1)

Growth Conditions Optimization

Physical growth condition optimization revealed that *E. faecium* grew best at 37°C. *B. siamensis* and *L. furfuricola* grew best at 30°C. At 45°C, *V. fluvialis* had the maximum optical density illustrated in Fig. 2.

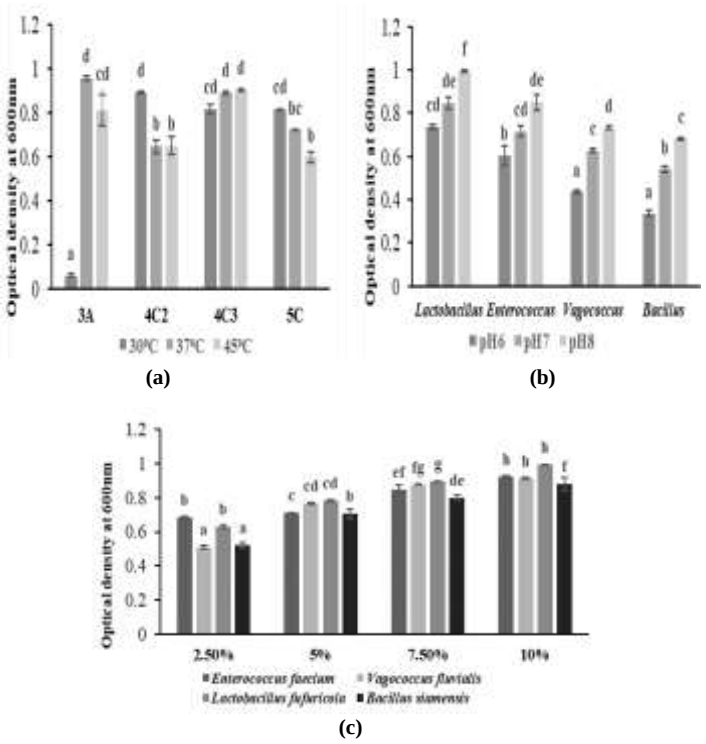

Figure 2. (a) Growth of the bacterial species at different temperature following 1ml of inoculation to the 9ml of MRS broth incubated for 48 hours, **(b)** Growth of the bacterial species at different pH following 1ml of inoculation to the 9ml of MRS broth incubated for 48 hours & **(c)** Growth of the bacterial species at different inoculum size following different concentrations of inoculation to the MRS broth incubated for 48 hours.

After being incubated at different temperatures, the outcomes were highly significant ($p \leq 0.05$) for identifying the optimal temperature for the specified probiotic strains. At alkaline pH (8), all four species exhibited maximum growth. Hence, the selected strains showed significant ($p < 0.05$) pH optimization following incubation at various pH. Each of the four species exhibited peak growth at a rate of 10%. As a result, the findings demonstrate a high level of statistical significance ($p < 0.05$) with the optimization of inoculum size for the probiotic strains after incubation at various levels.

Identification of Yogurt Formation

In Fig. 3, the experiment involved the observation of several bacterial inoculations in varying concentrations of both natural and processed milks. The inoculation of *E. faecium* and *B. siamensis* into both raw and processed milks resulted in higher water content in the yogurt production of cow milk compared to Olper's milk. In contrast, the introduction of *L. furfuricola* and *V. fluvialis* into both raw and processed milks resulted in a higher water content in the yogurt production of Olper's milk compared to cow milk.

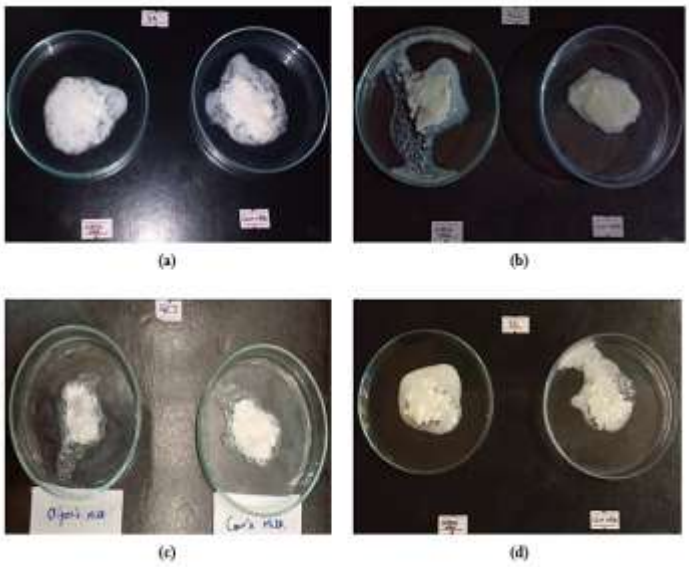

Figure 3. Yogurt formation when Olper's and cow milk were inoculated with different probiotic isolates **(a)** *Enterococcus faecium*, **(b)** *Lactobacillus furfuricola*, **(c)** *Vagococcus fluvialis* & **(d)** *Bacillus siamensis*.

Microbial Inhibitory Potential

In order to assess the efficacy of the probiotic *L. furfuricola* against the pathogenic and infectious bacteria *P. acnes*, a microbial inhibitory test was conducted. The zone of growth inhibition against tested bacteria was 17.4 mm, indicating this species' microbial inhibitory ability. Fifteen different antibiotics were used to examine the bacterial species and the results shown in Table 2.

Probiotic Isolates under GIT Simulation

The results for GIT simulations showed that when treated with SSF, *L. furfuricola* resulted more survivability at 0 and 2 min. When treated with SGF and SIF, *V. fluvialis* exhibited more survivability at 0 min and 2 hours in comparison to other bacterial strains. The evaluated data indicated in Fig. 4, that the results exhibit a high level of significance ($p \leq 0.05$).

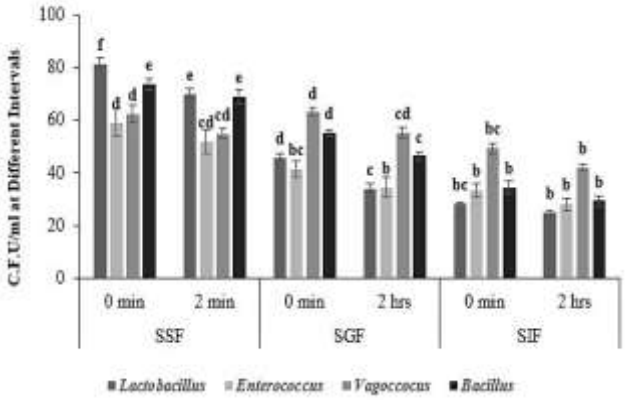

Figure 4: Survivability (C.F.U/ml) of the different probiotic species following their exposures to different levels of GIT simulation.

Table 2: Antibiotic susceptibility/resistance patterns of the bacterial isolates.

Name of Antibiotic	Conc.	Bacterial Species			
		E. faecium	*L. furfuricola*	*V. fluvialis*	*B. siamensis*
Rifampin	5 mcg	R	R	R	S
Norfloxacin	10 mcg	R	R	R	S
Trimethoprim	5 mcg	S	R	R	R
Metronidazole	5 mcg	S	R	R	R
Polymixin B	300 mcg	R	R	R	R
Chloramphenicol	30 mcg	R	S	R	R
Tobramycin	10 mcg	R	R	R	R
Erythromycin	15 mcg	S	S	R	S
Vancomycin	30 mcg	S	R	R	R
Augmentin	20/10 mcg	R	S	R	I
Tigecycline	15 µg	R	R	R	S
Cefepime	30 µg	R	R	R	R
Cefoperazone	30 µg	R	S	R	S
Clarithromycin	15 µg	S	R	R	S
Fusidic Acid	10 µg	R	R	R	R

R = the strain was resistant to the antibiotic where **S** = the strain was susceptible to the antibiotic and had diameter of growth inhibition zone, ranging from 8-13.5 mm.

Enzyme Production Activities

Among the four species, two species, *E. faecium* and *L. furfuricola*, showed high proteolytic activity with 34.66 and 40.5 mm diameter of zone of hydrolysis for enzyme production. However, it was shown that none of the species produced amylase, cellulase, or lipase, as determined by the presence of hydrolysis zones on the corresponding substrates upon solidification in agar gels. Table 3 displayed the results.

Table 3: Enzyme production activity of bacterial isolates

Bacterial Species	Diameter (mm) of zone of hydrolysis for enzyme production			
	Amylase	Protease	Cellulase	Lipase
Enterococcus faecium	-	34.66mm	-	-
Lactobacillus furfuricola	-	40.5mm	-	-
Vagococcus fluvialis	-	-	-	-
Bacillus siamensis	-	-	-	-

Blank space in table referred as No zone.

Probiotic Isolates in Extracts of Fruits and Dry Fruit Peels

After 24 and 48 hours, the optimization of inoculum size for peels of various fruits and dried fruit was attained. To achieve this, every bacterial species was cultured in an aqueous extract containing 5% bio-waste material. According to the evaluated data expressed in Fig. 5, the results exhibited a high level of significance ($p<0.05$).

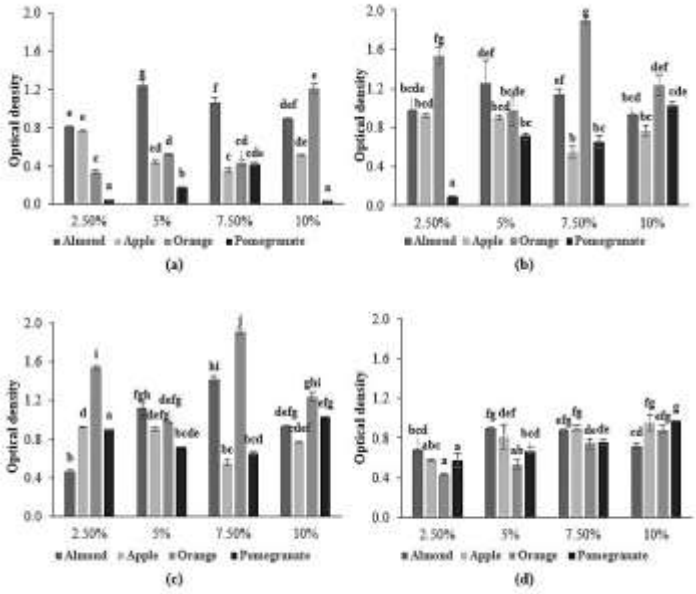

Figure 5: Growth of *Enterococcus faecium* **(a),** *Lactobacillus furfuricola* **(b),** *Vagococcus fluvialis* **(c)** & **(d)** *Bacillus siamensis in* the extract of different fruits and dry fruit peels with different inocula sizes after 24 hours of incubation.

Antioxidant Activity of Probiotics and Fruit Peels Extract

Bacillus siamensis was grown in 5% almond peel extract, which raised the RSA of the supernatant to 42.13% at a 2.5% inocula and 27.34% at a 5% inocula. However, %RSA declined to 38.46% and 72.65% at higher inoculum sizes of 7.5% and 10%, respectively. Similar trends were observed with apple peel extract which showed in Fig. 6, with % RSA peaking at 39.19%, 56.91%, and 68.10% at inocula sizes of 2.5%, 5%, and 7.5%, respectively, but dropping to 59.10% at a 10% inoculum size. In contrast, cultivation of *B. siamensis* in 5% orange peel extract significantly enhanced % RSA to 97.74% and 89.98% at 2.5% and 7.5% inocula, but decreased to 15.30% and 17.61% at 5% and 10% inocula, respectively. % RSA in pomegranate peel extract decreased to 58.60%, 36.03%, 75.25%, and 26.07% at corresponding inoculum sizes.

For *Lactobacillus furfuricola*, cultivation in 5% almond peel extract resulted in %RSA enhancement to 40.30% and 7.94% at 2.5% and 10% inocula, respectively, while dropping to 39.90% and 91.72% at 5% and 7.5% inocula, respectively. Whereas, apple peel extract showed variable effects, with %RSA decreasing to 85.17%, 36.39%, and 64.06% at 2.5%, 5%, and 7.5% inocula, respectively, but increasing to 101.9% at a 10% inoculum size. When the *L. furfuricola* was cultivated in 5% extract of orange and pomegranate peels, the %RSA of the supernatant went down up to 44.83%, 2.06%, 40.06% and 12.91% in orange peels and 80.02%, 76.98%, 60.40% and 61.50% in pomegranate peels at all the respective inocula sizes.

Growing *Enterococcus faecium* in 5% almond peel extract raised RSA to 87.05% at a 5% inoculum size, but it dropped to 67.60%, 19.44%, and 91.71% at 2.5%, 7.5%, and 10% inocula sizes, respectively. In apple peel extract, %RSA peaked at 20.98%, 95.61%, and 99.03% at 2.5%, 5%, and 7.5% inocula, respectively, but dropped to 60.73% at a 10% inoculum size. Orange and pomegranate peel extracts showed similar trends which illustrated in Fig. 6.

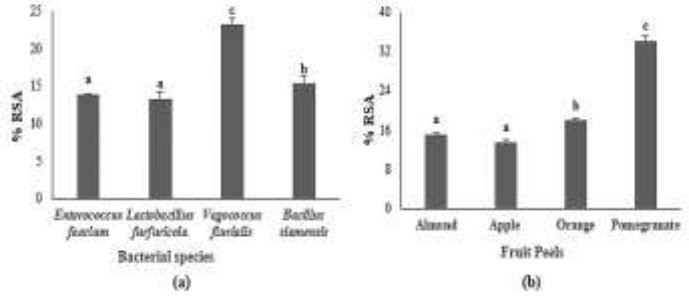

Figure 6: (a) Free radical scavenging activity (%) of bacterial species following their 48 hours growth in MRS medium, **(b)** Free radical scavenging activity (%RSA) of extracts of peels of different fruit wastes.

When *Vagococcus fluvialis* was cultivated in a 5% extract of almond, the %RSA of the supernatant went down to 44.22%, 48.44%, 96.04% and 78.41% in all the respective inocula sizes. Furthermore, apple peel extract showed %RSA enhancement at 45.39% at 5% inoculum size but went down to 84.72%, 64.06% and 34.32% at inocula sizes of 2.5%, 7.5% and 10%, respectively. In orange peel extract, % RSA peaked at 96.45%, 50.37%, and 44.54% at 2.5%, 5%, and 10% inocula, respectively, but decreased to 3.18% at a 10% inoculum size. Pomegranate peel extract exhibited varied effects across inoculum sizes. The %RSA of the supernatant went down up to 46.45%, 53.39%, 98.26% and 39.06% at all the respective inocula sizes

IV. DISCUSSION

The current imperative lies in the exploration of probiotic strains with biotechnological and health-enhancing capabilities, which can serve as substances in food processing and contribute to the maintenance of human health. Four kinds of probiotics were identified in this investigation. The bacteria were gram-positive, catalase-negative, and three were citrate-negative, while only *V. fluvialis* was citrate-positive. Gelatin hydrolysis was positive for *E. faecium* and *B. siamensis*. MRS and nutrient agar media plates had sticky, lustrous white, off-white, and creamy colonies. Morphological and biochemical features indicated that all isolates were from distinct genera. The study identified and preserved potential probiotic isolates from fermented dairy and non-dairy foods in Nankana Sahib, Punjab, Pakistan, and tested their GIT survival for health benefits. The results showed that isolated strain distribution is sample-dependent. In 15 cm of dairy waste-impregnated soil, *Enterococcus* was found. *Lactobacillus* and *Vagococcus* were found in handmade pickles, however, *Bacillus* was abundant in buffalo milk cheese.

Several researchers have recorded the separation of potentially advantageous strains, specifically examining the probiotic capabilities of different *Lactobacillus* species (Bin Masalam *et al.*, 2018). The source material for the isolation came from dairy products, and each isolate was gram-positive and catalase-negative. Likewise, Bin Masalam *et al.* (2018) identified strains from dairy products and excrement from distinct biotopes. The isolated strains were characterized, probiotic potential was reported, and colony morphology was further examined using MRS medium.

The fermentation process of glucose, mannitol, maltose, and lactose in nutrient broth was previously recorded by McDade and Weaver (1959). In our work, we adhered to the Durham fermentation tube protocol, but with certain alterations. The isolates used in this study's fermentation in Durham showed signs of gas generation in *E. faecium* with sucrose, *L. furfuricola* with mannose, *V. fluvialis* with sucrose, and *B. siamensis* with maltose. Except for *V. fluvialis*, all isolates survived at 45°C but cultivated best at 37°C. The isolates' survival in the human gut, which is critical for establishing their probiotic benefits, would have been impossible if they could not withstand the temperature range selected. To complement their in-vitro analysis of potential food-origin probiotic isolates, Pundir *et al.* (2013) investigated the effect of temperature range on LAB isolates. The GIT simulation revealed a statistically significant difference in the total number of viable probiotics after the simulation, although there was a potential decline in the total number. The stimulation conditions will cause the bacterial burden to decrease, yet the probiotics will remain alive, albeit in smaller numbers.

The conventional fermentation of milk to produce yogurt involves verifying the probiotics used in both processed and raw milk. Physicochemical changes that occur during homogenization, pasteurization, and fermentation influence the taste and texture of the final product. Standardized processing procedures help yogurt produce appropriate texture and taste qualities (Deepa *et al.*, 2022). Yogurt that is abundant in probiotics may have advantageous effects on human health and the composition of intestinal bacteria. Any oligosaccharide can further enhance the effectiveness of probiotic-treated yogurt. Kamel *et al.* (2021) investigated that the incorporation of oligosaccharide as a prebiotic into probiotic-yogurt resulted in enhanced functional properties and an extended shelf-life of the yogurt. Sfakianakis and Tzia (2014) found that probiotic cultures and prebiotic additives boost dairy products' nutritional value while limiting sensory impacts. 0.1 ml inoculation of autoclaved milk samples produced homogenously fermented yogurt with nice textures from all isolates in this investigation. In this study, varied textures in terms of whey concentrations in different milk species allowed strain selection for greater yogurt yields from selected milk categories. Such efforts are anticipated to help the dairy business grow economically and biotechnologically.

In addition to their nutritional benefits, probiotics have been extensively studied for their potential in treating a range of illnesses. People who suffer from acne, a chronic inflammatory skin disease, recognize its severe negative impact on their quality of life. Since antibiotic resistance has grown, acne treatments with antibiotics have become less effective. Probiotics are living microorganisms added to diets to prevent infections and maintain gut and skin microbiome homeostasis (Goodarzi *et al.*, 2020). This isolate, *L. furfuricola*, eliminated *P. acnes*, making it a potential acne therapy. This potential probiotic strain against the pathogenic bacterium may replace antibiotics when taken orally or topically. Mottin and Suyenaga (2018) conducted a comprehensive analysis on the efficacy of probiotics in the treatment of acne and determined that probiotics have a substantial role in addressing skin conditions such as acne. It is imperative to obtain more probiotic isolates from nearby ecosystems in order to tackle the issues pertaining to that particular area. The current investigation used the well diffusion method protocol, with minor adjustments, to evaluate the antibacterial efficacy of LAB isolates, following the methodology described by (Khunajakr *et al.*, 2008).

The level of antibiotic resistance can differ among different strains or types of strains. The microflora of animals' intestines naturally contains bacteria that may develop antibiotic resistance. Bacteria that have developed resistance to antibiotics have the ability to transmit this resistance factor to other pathogenic bacteria via the interchange of genetic material. Hence, to address the issue that has emerged in probiotic investigations, it is imperative to verify the absence of transferable resistance genes in a potential probiotic strain (Bujnakova *et al.*, 2014; Mathur & Singh, 2005). The present study selected fifteen antibiotics due to their diverse action mechanisms, which also exhibited varying resistance profiles among different bacterial strains (Schirru *et al.*, 2012). In contrast, the antibiotic resistance characteristics exhibited by probiotic bacteria enable their survival within GIT, even when subjected to antibiotic treatment (Bin Masalam *et al.*, 2018). Therefore, these probiotics may also be advised in specific circumstances where the use of antibiotics is necessary. The *E. faecium* strain exhibited

susceptibility to Vancomycin at a dosage of 30 mcg by Bin Masalam *et al.* (2018) and Erythromycin at 15 mcg by Chahad *et al.* (2012). Haghshenas *et al.* (2016) found that all *Enterococci* isolates were vancomycin-resistant, claiming that this resistance is inherent. The isolated strain of *L. furfuricola* demonstrated sensitivity to Chloramphenicol at a concentration of 30 micrograms. The current investigation revealed that the bacterial strain of *V. fluvialis* showed resistance to all fifteen antibiotics tested, while *L. furfuricola* exhibited resistance to Norfloxacin at a dosage of 10 mcg. In contrast, the *B. siamensis* strain demonstrated intermediate zones of growth suppression when exposed to Augmentin at a dosage of 20/10 mcg. There is potential to use probiotic strain resistance to certain antibiotics to improve the management of intestinal infections in conjunction with antibiotic treatment, either as a preventive measure or as a therapeutic intervention.

The aforementioned study demonstrated that the anti-oxidative characteristics of probiotic bacteria, as assessed using established techniques exhibit strain-specific attributes for lactic acid bacteria (Kullisaar *et al.*, 2002; Lin & Yen, 1999; Mikelsaar & Zilmer, 2009; Mishra & Kovachich, 1984). The supernatants that were devoid of cells and the peels extracts that were inoculated showed antioxidant capabilities. Regardless of the methods used, we observed a wide dispersion of anti-oxidative parameter values within each microbial group, indicating the strain specificity of this trait (Amaretti *et al.*, 2013). The DPPH scavenging ability of cell-free extract of bacteria from four probiotic strains (*Lactobacillus, Enterococcus, Vagococcus*, and *Bacillus*) was used to assess antioxidant activity. In the cell-free supernatant, DPPH scavenging revealed that *V. fluvialis* had the highest antioxidant activity. Using different fruit peel extracts to treat these probiotics resulted in varying radical scavenging activities for each strain. Inoculating *Enterococcus faecium* led to a greater antioxidant activity in the overall pomegranate peel extract. The radical scavenging activity of the extract was enhanced upon treatment with orange peel extracts of *L. furfuricola*, *V. fluvialis*, and *B. siamensis*. Probiotics possess antioxidant properties that enhance the efficiency of metabolic processes inside the biological system (Afify *et al.*, 2012).

V. CONCLUSION

This study has effectively retained the variety of indigenous microorganisms. The isolates exhibit promising potential as probiotic alternatives for human health benefits due to their resistance to acidic conditions in the gastrointestinal tract (GIT) and their ability to inhibit the development of harmful acne bacteria. The significance of their proteolytic and anti-oxidant capabilities cannot be overstated. The use of antibiotic-resistant probiotic organisms in conjunction with antibiotics can be employed as necessary. It is anticipated that future endeavors and experiments may reveal additional probiotic characteristics of these isolates.

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AUTHOR CONTRIBUTIONS

Concept and design: AM, SA, MMJA, JIQ. Analysis and interpretation: AM, SA, MMJA, FL, MN. Data collection: AM, FL, R, MTM, Writing the article: AM, SA, MMJA. Critical revision of the article: AM, SA, MMJA, FL, JIQ, R, MTM, MN. Statistical analysis: MMJA, MN. Final approval of the article: all authors. Overall responsibility: AM.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DATA AVAILABILITY

Data presented in this study will be available on a fair request to the corresponding author.

ETHICS APPROVAL

Not applicable to this paper.

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