RESEARCH PAPER

Isolation & Evaluation of Potential Probiotics Bacteria from Coconut Milk Infused with Curd and Their Functional Properties

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Abstract
The increased demand for plant-based non-dairy functional foods for people with lactose intolerance and for vegans, leads researchers to look more into the fermentation of plant-based foods as good vehicles for the probiotic microorganisms. The Coconut milk is rich in several nutrients, as well as has a suitable physical and chemical character, to be considered as an excellent candidate for probiotic fermentation. In this work the bacteria were isolated, characterized and identified from fortified coconut milk with curd. The goal was to isolate the most promising bacteria with good probiotic characters, test its functional character using the traditional methods as well as some analysis.
All isolates were characterized using both phenotypic and physiological traits. Using the Gram staining, Capsule staining and Spore staining technique the structure of the bacteria, their Gram character and the presence of their defensive cellular constituents (Spore, Capsule) was tested. The Catalase activity, resistance of the isolates towards pH and salt tolerance tests, were used for their physiological and biochemical characterization. This property is very important for their endurance of acidic environmental factor (gastric environment) and the osmotic stress in the gut. Antimicrobial characters against a series of common pathogenic organisms were tested. Presence of antimicrobial characters would mean the isolate is capable of producing useful substances.
All bacterial isolates were identified using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) which is the most modern technique, enabling to identify the bacterium rapidly and accurately based on their mass spectral protein profile. Combination of conventional microbiology and mass spectrometry made it easy to find the potential probiotics.
In conclusion, this work suggests a modern method combined with classical approaches for identification and selection of useful probiotics in fermented coconut milk. This is the very promising potential of coconut milk as a food vehicle and could potentially be used in formulating new plant-based probiotic functional foods, nutritional supplements and possibly as therapeutic agent.

Keywords: Probiotics, Coconut milk, antimicrobial activity, pH tolerance, NaCl tolerance

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1. Introduction

Probiotics are live microorganisms that have health benefits to the host primarily through Adjusting the gut microbiome by providing sufficient doses [1]. These helpful bacteria, including strains from *Lactobacillus* and *Bifidobacterium genera*, help restore microbial balance disturbed by causes like antibiotics or poor nutrition [2]. Originally used in the 1950s to differentiate from antibiotics, the term "Probiotic" comes from Greek roots meaning "for life" [3]. Emphasizing viability and dosage, the Food and Agriculture Organization (FAO)and World Health Organization (WHO) formalized the definition in 2001 as live microorganisms offering health advantages when consumed in sufficient amounts. Common strains include lactic acid bacteria, which naturally occur in fermented foods [4].Through competitive exclusion of pathogens, an increase in intestinal barrier activity,
immune modulation, and the production of endogenous useful metabolites such as short- chain fatty acids, probiotics have effects [5]. By affecting cytokine production, they adhere to gut epithelial cells, prevent dangerous bacterial adherence, and modulate immune responses [6]. These behaviors help in general microbial balance in the gut [7]. Probiotics aid digestion by alleviating lactose intolerance and reducing cases of diarrhea, including antibiotic-associated cases. They are promising in treating ailments such as irritable bowel syndrome. [8]. They improve immune functioning, bowel syndrome, hypertension, and hypercholesterolemia and possibly prevent the risk of respiratory infection [9]. Natural fermented is made by the cultivation of live beneficial bacteria during the fermentation process and probiotic sources of foods. The bacteriophages are lactic acid and are primarily *Lactobacillus* [10]. These species are the *Bifidobacterium*, which can withstand the gastric pH, and can nest in the intestines to facilitate microbial balance [11].
Previous literature shows that fermented foods and lactic

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acid bacteria are effective probiotics, as they help to enhance host immunity against pathogens and gastrointestinal health in the absence of antibiotics [12]. The fact that these strains have been able to survive in various food matrices highlights their importance in the routine consumption of food to sustain healthy benefits [13]. Coconut milk has become a potential non-dairy source of probiotic fermentation in the wake of the growing need for vegan and lactose-free milk products [13]. The presence of large amounts of lipid compounds, especially the fatty acids of medium chain like lauric acid, promotes the growth of the probiotics and increases the stability of the product [14]. Some strains of probiotics, such as *Lactobacillus reuteri,* have been shown to survive in coconut milk [15].

Growing non-dairy probiotic substrates is driven by widespread lactose intolerance, which affects up to 68% of the world's population, and rising veganism motivated by ethical, environmental, and health issues [16]. In certain situations, such as high cholesterol or allergies to dairy it is not ideal to use a dairy-based product for probiotics and instead a plant-based one needs to be implemented which can still effectively deliver viable probiotics [17]. Probiotics produced with coconut milk showed very good resistance to acidity at pH 2.0, maintaining viability for up to four hours. This demonstrates that they are capable of surviving passage through the gastro-intestinal tract and may contribute to inhibitions of pathogenic bacteria. Probiotic survival within a non-dairy medium may be attributed to adapted survival strategies such as alteration of proton pump activity and modification of cell membrane fatty acid composition [17][18].

Probiotic strains isolated from other non-dairy fermented food products showed similar salt (Sodium Chloride) tolerance of 4-6% as many dairy-based probiotic strains allowing them to be incorporated into salt fermented products or to survive during the preservation process. These strains remained viable at 37C, therefore would be suitable for culture and growth within a human-body-like environment [18]. It appears probiotics grown with coconut milk were more resistant to saline conditions; this may be attributed to the medium chain lipids present within the cell membrane, enhancing the membrane's stability [18].

Probiotics isolated from fermented non-dairy product sources also produce organic acids and other antibacterial compounds capable of inhibiting pathogenic microbes including *E. Coli* and *S. Aureus.* High inhibition zone of up to 20mm was reported for some of these, and in general there appears to be a high potential for growth inhibition of these pathogenic species [18]. In combination with lauric acid, extracts from fermented coconut curd could be shown to successfully inhibit the growth of L. Species by up to log 3 CFU/mL [19].

These functional properties together demonstrate how probiotics from plant-based sources like coconut milk can be effectively included within vegan-based food products. Coconut milk supplemented with curd was used in this study as a natural source of probiotic bacteria [20]. The samples were serially diluted and then grown on selective media in order to get specific bacterial colonies [21]. The identification of the isolates was initially done by Gram, capsule and spore staining to analyze their cell morphology [22]. The physiological and biochemical properties of the isolates were analyzed through the analysis of catalase activity, tolerance to different pH levels and sodium chloride levels [23]. In addition, the functional efficacy of the isolates was established through the analysis of their antimicrobial action against the selected pathogenic strains [24].

This research is expected to identify probiotic bacterial strains with high physiological and antimicrobial capabilities. They can be useful in the preparation of therapeutic interventions and functional food products, and some of these isolates hold potential [25].

2. Materials and methods

2.1Material and Instruments

Selective cultivation of lactic acid bacteria was done using MRS (de Man, Rogosa, Sharpe) agar and broth; nutrient agar supported general bacterial development; and Mueller-Hinton agar helped with antimicrobial susceptibility testing [26]. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) allowed exact pH changes, copper sulphate ($CuSO_4$) was employed for oxidase assays, malachite green for endospore staining, and a Gram staining kit crystal violet, Gram's iodine, safranin, acetone-alcohol) for cell wall characterization [27]. Catalase assays were done with hydrogen peroxide (H_2O_2); everything was done with distilled water in terms of dilutions and preparations [27]. Media and glassware sterilized at 121^0C 15 psi at 15 minutes in an autoclave; centrifuge using 15 mL tubes to harvest cells at 5000rpm. Calibration of a pH meter to 6.2 -6.5 was instrumented, as well as 20 minutes of pH meter, and media [28]. Incubators were maintained at 37^0C 24-48hours; transfers were accurate and of sterile tip using micropipettes(10-1000µL); a laminar airflow cabinet provided aseptic conditions during streaking and handling [29]. Short-term inoculated plates and stock cultures were stored in a 4^0 C refrigerator; a MALDI-TOF mass spectrometer was used to identify bacteria in a fast manner as per the manufacturer guidelines [30].

2.2 Sample collection and preparation

Aseptic preparation of fresh coconut milk was performed and used as the fermentation substrate. The coconut milk inoculum was also added with fresh curd, which initiated the process of fermentation; it was a natural source of lactic acid bacteria. The mixture was then thoroughly mixed and left to ferment at room temperature or even in a warm environment. A sufficient duration of the fermenting process was performed to stimulate the growth and enrichment of beneficial bacteria [21], [31].

2.3Isolation of bacteria

After fermentation, bacterial isolation was done with the help of the serial dilution technique to minimize the microbial load and to get well colonies. Proper dilutions

were transferred to de Man, Rogosa and Sharpe (MRS) agar medium, which is a selective medium for the growth of lactic acid bacteria. The plates that were inoculated were then incubated at 37^0 C over the next 24-48 hours under conditions that were appropriate. Following incubation, individual colonies were picked due to the differences in size, shape, color, margin, and surface features to conduct a further analysis [23], [32].

2.4Screening of potential probiotic isolates
A preliminary probability screen of the bacterial isolates was performed using phenotypic and microscopic properties. Morphology of colonies was strictly monitored, and Gram staining was done to establish Gram reaction and cellular structure. The morphology of the cells was observed with the help of a microscope, and rod-shaped or cocci-shaped bacteria were determined. Moreover, isolates were also evaluated on spore formation and only bacteria that did not form spores were taken into consideration in further probiotic characterization [30], [33].

2.5 Staining techniques
2.5.1. Gram staining:
Gram staining is a differential procedure, which is used to differentiate Gram-positive and Gram-negative bacteria on the basis of the difference in cell wall structure [34]. The technique is sequential staining (crystal violet) followed by Iodine, alcohol, decolorization, and counterstaining (safranin), and Gram-positive bacteria hold the complex of crystal violet and iodine due to the presence of the thick peptidoglycan layer and look purple when viewed under a microscope [34]. Conversely, Gram-negative bacteria are decolorized by alcohol and then absorb safranin, producing a pink or red color [35].

2.5.2. Spore staining:
A heat-fixed smear is stained and then steamed (about five minutes to allow the dye to enter the coats of the spores) to obtain spores stained green [35]. The smear is then washed in water to decolorize the vegetative cells, after which it can be counterstained with safranin- 1-2 minutes, resulting in pink-stained vegetative cells [36]

2.5.3Capsule staining:
The polysaccharide capsule is stained to appear in capsule staining; this staining is not able to tell the difference between Gram-negative and Gram-positive. Under the Anthony technique, a smear is air-dried without heating it, stained with crystal violet (1-2 minutes), then rinsed slightly using 20% copper sulfate, which removes stain on the capsule and leaves the bacterial cells purple [37]. Bacterial cells in the India ink method are prepared and stained on a slide with ink and allowed to dry in the air, and then counterstained with safranin, leaving visible capsules as transparent stars around the pink cells on a dark ground [38].

2.5.4Catalasetest:
The catalase test determines the presence of catalase enzyme by subjecting the bacterial colonies to 3 per cent hydrogen peroxide, whereby the immediate generation of bubbles signifies the positive reaction owing to the liberation of oxygen [27]. It is a test that does not require staining and is conducted as follows: $H_2O_2$is applied directly to a slide or a bacterial colony, and effervescence is observed [26].

3. Physiological tolerance test
3.1. NaCl tolerance:
Salt tolerance of isolates was tested at 2%, 4%, 6%, and 8% NaCl concentrations. Isolates were inoculated in MRS medium with various NaCl concentrations and incubated between 24-48 hours. Qualitative measurements of growth (±) were done by optical density or turbidity. The halo-tolerance and resiliency of the strains are measured in this study under the presence of osmotic stress.[39].

3.2. pH tolerance:
PH tolerance was assessed at pH 2, 4, 6 and 8 by adding HCl or NaOH to adjust MRS broth to these pH levels. Seeding of isolates took place within such conditions and viability was checked by optical density reading or plate. This test measures acid and alkaline resistance that simulates gastrointestinal tract conditions.[40].

4. Acquired and MALDI-TOF pathogens.
The pathogenic strains of bacteria used in this study were gathered in accordance with ethical considerations and seeking the necessary institutional approval. Formal consent was given by a written request sent to the Head of the Department for the gathering of clinical pathogens from Parul Sevashram Hospital for a dissertation-related study. To guarantee safe lab handling, all isolates were collected, processed, and preserved in accordance with approved biosafety procedures. For fast and exact identification of pathogenic and probiotic bacterial isolates, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was employed, Authorization for MALDI-TOF analysis was given by the Gujarat Biotechnology Research Centre (GBRC), Gandhinagar [41].

5. Preparation of cell-free supernatant for antimicrobial activity
For 48 h, probiotic cultures were incubated; after that, the broth was centrifuged at 6000 rpm for 15 min. The supernatant served as the antimicrobial agent. An identical procedure was used for a control comprised of an MRS broth blank [24].
5.1 Antimicrobial activity (cup-borer method)

Test pathogens (e.g., *Escherichia coli, Salmonella typhi, Staphylococcus aureus*) were evenly seeded onto nutrient agar plates [27]. Using a sterile cup borer, wells were aseptically prepared, and the collected cell-free supernatant was dispensed into them. After 24 hours of incubation at 37 °C, the zones of inhibition were measured in millimeters (mm) [42].

6. Results and Discussion

6.1Morphology analysis

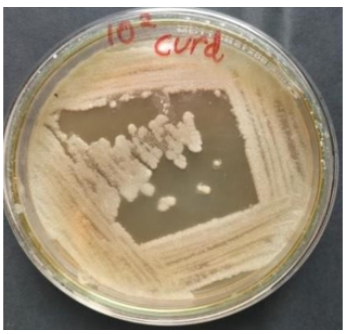
Table:1 Growth of pathogenic test organisms used for antimicrobial activity evaluation.

Sample no	Shape	Size	Color	Texture	Elevation	Margin
10^1	Coccus/ cocci	Large, spreading colonies with consistent thickness along streak lines	Creamy white	dry	Flat to slightly	Entire (smooth edge) along streaking lines
10^2	coccus	Small to medium streaking lines	Creamy white	dry	Slightly raised	Entire(smooth)
10^3	coccus	small	Off white /creamy white	Smooth with a slightly matt surface	Slightly raised	Entire (smooth, even edges)
10^4	coccus	small	white	smooth	Low convex to flat	Entire (smooth , well-defined edges)
10^5	coccus	small	Creamy white	Smooth and uniform	Flat to slightly raised	Entire (smooth and even edges)

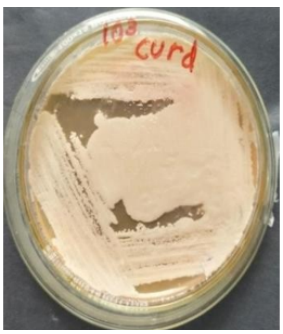
6.2 Isolation and screening

The curd sample was repeatedly diluted in a series of plates (10^1 -10^5), and as the dilution was reduced, the sample grew thick with colonies forming well isolated on higher dilutions. Colonies diminished in value as the dilution was increased, and made it possible to obtain effective isolation and screening of morphologically discrete strains of bacteria.

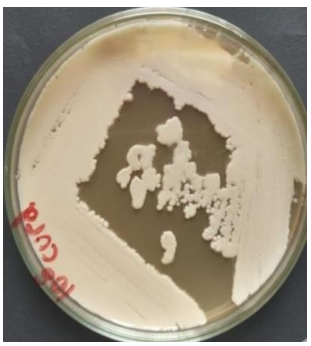
(a)10^1 dilution



(b)10^2dilution



(c)10^3dilution



(d)10^4dilution **(e)10^5dilution**

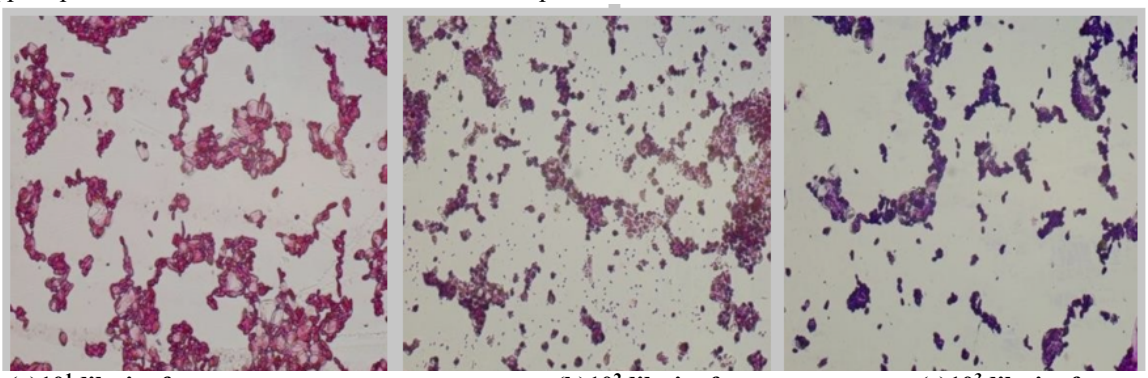
Figure 1: Serial dilution streaking of the curd sample showing bacterial growth at different dilutions.

6.3 Staining results

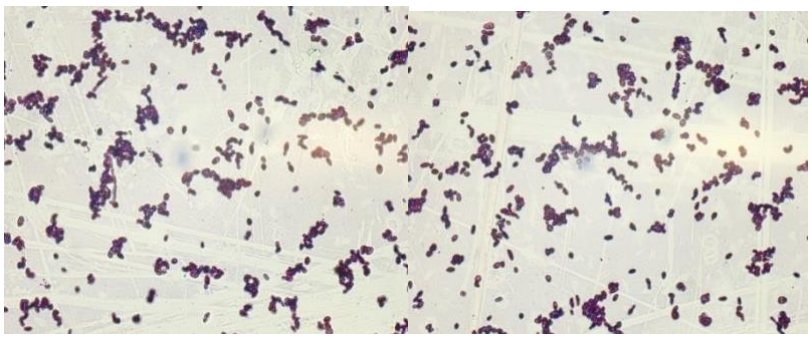
6.4.1Gramstaining

Gram staining of the bacterial isolates identified both Gram-positive and Gram-negative bacterial isolates, which had different staining properties. Uptake of the crystal violet-iodine

The complex caused the gram-positive cells to appear purple, whereas uptake of safranin counterstain made gram-negative cells appear pink and structural variation in cell wall composition was confirmed.



(a)10^1dilutionfactor **(b)10^2dilutionfactor** **(c)10^3dilutionfactor**

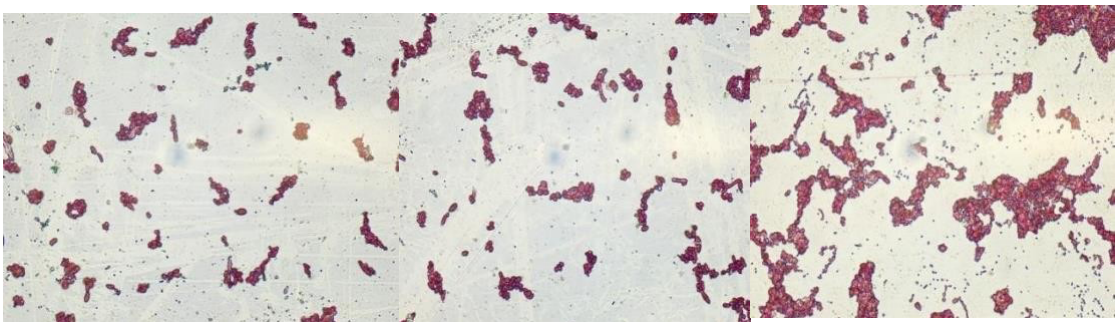


(d)10^4dilutionfactor **(e)10^5dilutionfactor**

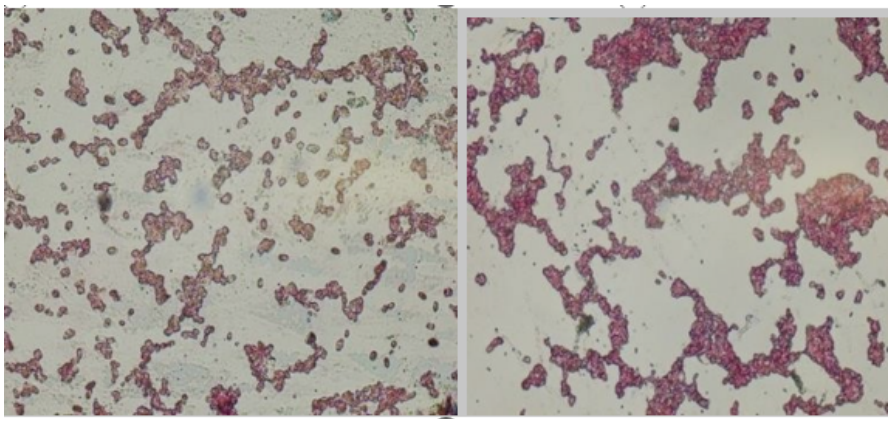
Figure2: Gram staining of bacterial isolates obtained from serial dilutions of curd sample.

6.4.2 Spore staining

Spore staining showed endospores in some bacterial isolates, which turned green with malachite green staining, and the vegetative cells stained to absorb the pink safranin counterstain. This sharp distinction enabled the separation of spores and vegetative cells, which proved the existence of sporulating bacteria.



(a)Isolatefrom10^1dilution **(b) Isolate from10^2dilution** **(c)Isolatefrom10^3dilution**

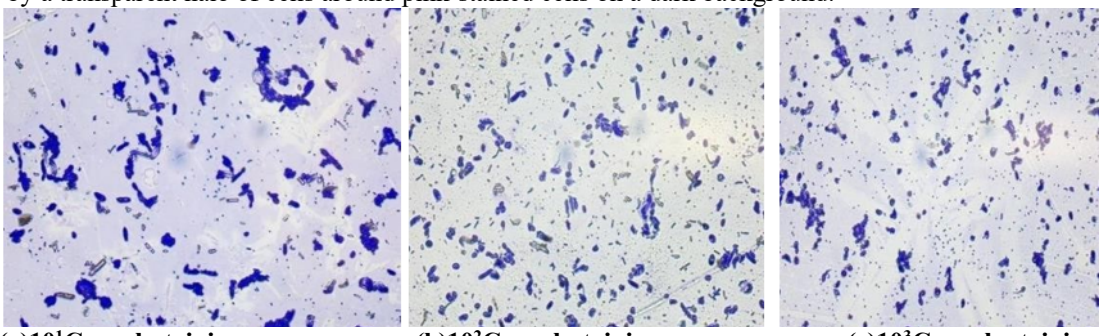


(d)Isolate from10^4dilution **(e)Isolate from10^5dilution**

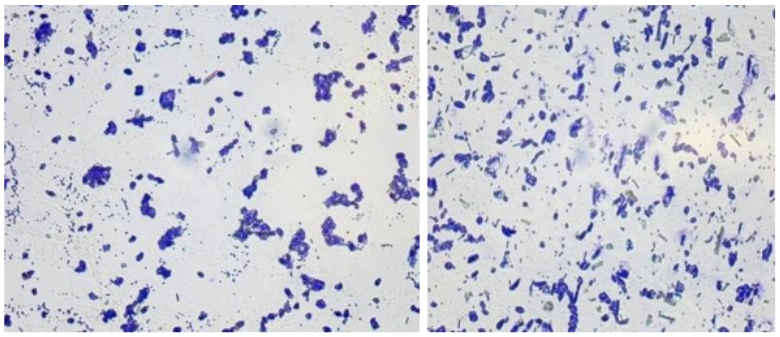
Figure 3: Spore staining of bacterial isolates obtained from serial dilutions of the sample.

1.6.3Capsulestaining

The staining of capsules revealed the bacterial cells with a polysaccharide capsule. With the Anthony method, the cells were stained in purple with clear, unstained halos indicating the capsule, but with the India ink method, the capsules were indicated by a transparent halo of cells around pink-stained cells on a dark background.



(a)10^1Capsulestaining **(b)10^2Capsulestaining** **(c)10^3Capsule staining**

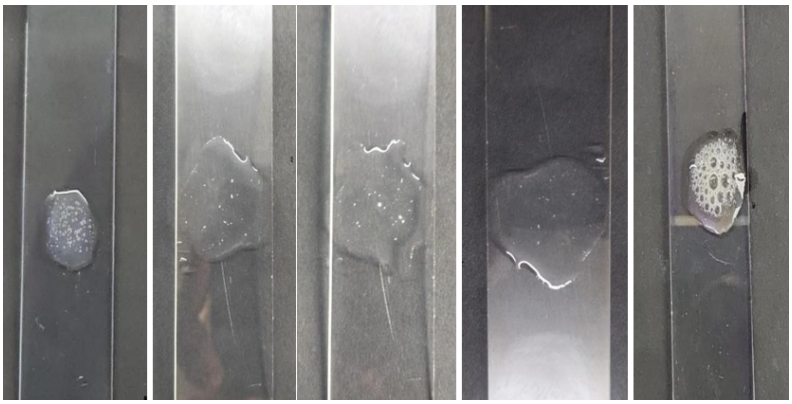


(d)10^4Capsulestaining **(e)10^5Capsulestaining**

Figure 4: Capsule staining of bacterial isolates obtained from serial dilutions of sample.

1.6.4 Catalase test

The catalase test was performed by placing the colonies of bacteria in the 3per cent hydrogen peroxide solution, which resulted in the instant formation of bubbles, which is a positive result for the presence of the catalase enzyme. Bubbles that were not produced were catalase- negative.

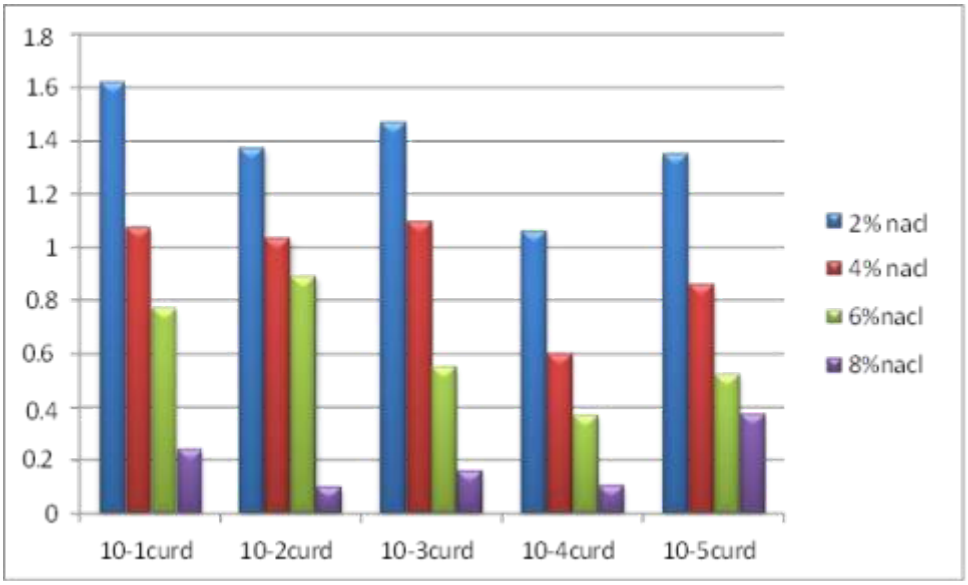


(a)10^1 (b)10^2 (c)10^3 (d) 10^4 (e)10^5

Figure5: Catalase test of bacterial isolates obtained from serial dilutions:(a) catalase-positive isolate**:**(10^1) (b) catalase negative isolate (10^2) (c)catalase negative isolate (10^3) (d)catalase negative isolate (10^4) (e)catalase positive isolate (10^5).

6.4 NaCl tolerance results

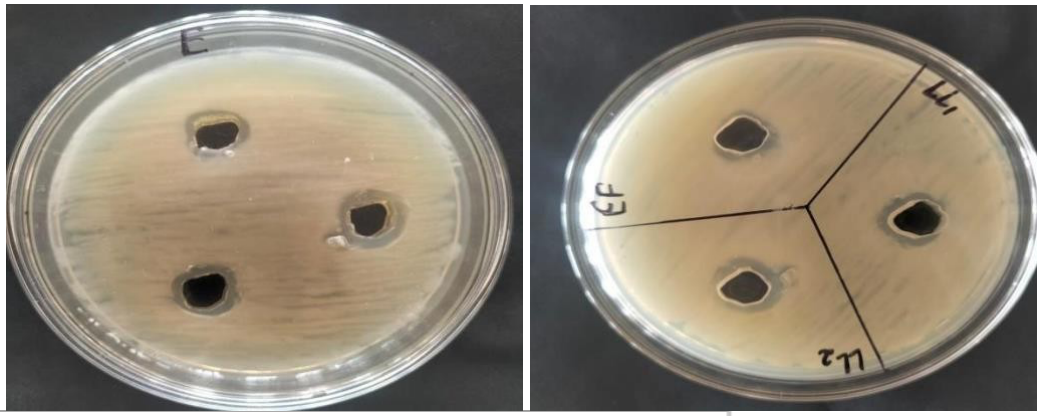
NaCl tolerance analysis revealed that bacterial growth was optimum in the 2% NaCl concentration in all serial dilutions. It was found that growth reduced graduallyat4%and6% NaCl and the growth was not much at 8% NaCl, which showed that the isolates are moderately salt-tolerant.

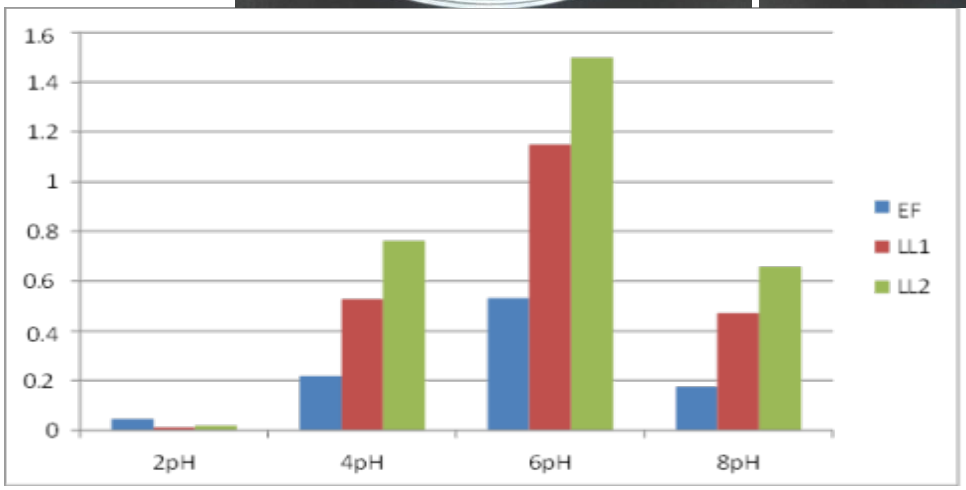


Graph 1:NaCL tolerance analysis showed maximum bacterial growth at 2% NaCl across all serial dilutions, with a gradual decline in growth observed at 4%,6% and minimal growth at 8% it, indicating moderate salt tolerance of the isolates.

6.5 pH tolerance results

The optimal growth of all three bacterial isolates (EF, LL1, and LL2) was at pH 6. Growth was significantly lowered at pH 2 and pH 8, and the activity of LL2 was always higher compared to EF and LL1 at all the various pH levels tested.





Graph 2:The data indicate that the optimal pH for the activity or growth of the tested samples(EF, LL1, LL2) is 6 pH, with LL2 consistently showing the highest performance across all measured pH values, particularly at the optimum.

6.6 Antimicrobial activity results

6.6.1 Antimicrobial activity was noted by clear zones of inhibition around wells that contained the LL1, LL2, and EF in seeded agar of *E. coli*. The largest zones of 13mm, 13mm, and 14mmwere present in EF, LL1, and LL2, respectively. An effective 9 mm well diameter was used to confirm that the 9 mm well diameter was effective, which was 4 mm (EF), 5mm (LL1), and 6mm (LL2), validating that the most effective isolate against *E. coli* was LL2.

Figure 7: Probiotics bacteria (LL1,112, EF) exhibited antimicrobial activity against *E.coli* pathogens, indicated by the zones of inhibition around the sample wells.

Table 2: Zone of inhibition against *E.coli*.

Escherichia coli	**EF**	**LL1**	**LL2**

Zone of inhibition in (mm)	13,14,13	13,14,15	15,14,16
Avg:	13.3–9mm(well)	14-9mm	15-9mm
Well diameter:	9mm	9mm	9mm
Zone :	4.3mm	5mm	6mm

6.6.2 Wells with LL1 and LL2 on *Salmonella typhi* seeded agar had clear inhibition zones signifying antimicrobial activity, but EF did not exhibit any inhibition. The zones produced byLL1were15, 13, and 12 mm, whereas the zones producedbyLL2were17, 18, and 16 mm, and this bears out the fact that LL2 is the most effective isolate in the battle against *S. typhi,* with an effective inhibition of about 4 mm

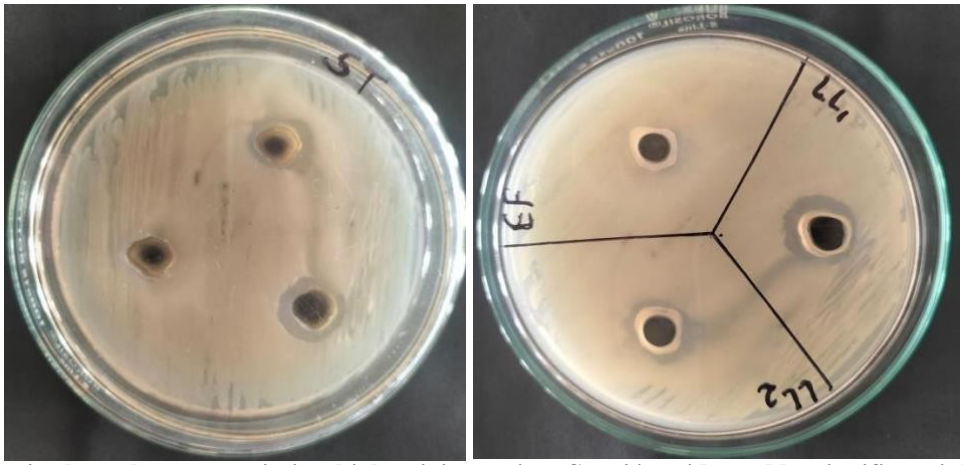


Figure 8: Bacteria showed strong antimicrobial activity against *S.typhi* , evidenced by significant inhibition zones compared to the control.

Table3:Zones of inhibition against *Salmonella typhi*

Salmonella typhi	**EF**	**LL1**	**LL2**
Zone of inhibition in (mm)	0	15,13,12	17,18,16
Avg:	-	13.3-9mm	17-9mm
Well diameter:	9mm	9mm	9mm
Zone :	Zone=-	4.3mm	8mm

6.6.3 Antimicrobial activity was found to produce a clear zone inhibitionaroundwellswithLL1 and LL2 on *Staphylococcus aureus* seeded agar, whereas EF did not. Zones of 15-16 mm and 15-18 mm were larger in LL1-produced zones and in LL2, respectively. At a 9 mm well diameter, effective inhibition was approximately 5 mm at LL1 and 8 mm at LL2, and this confirms that LL2 was the best isolate against *S. aureus*.

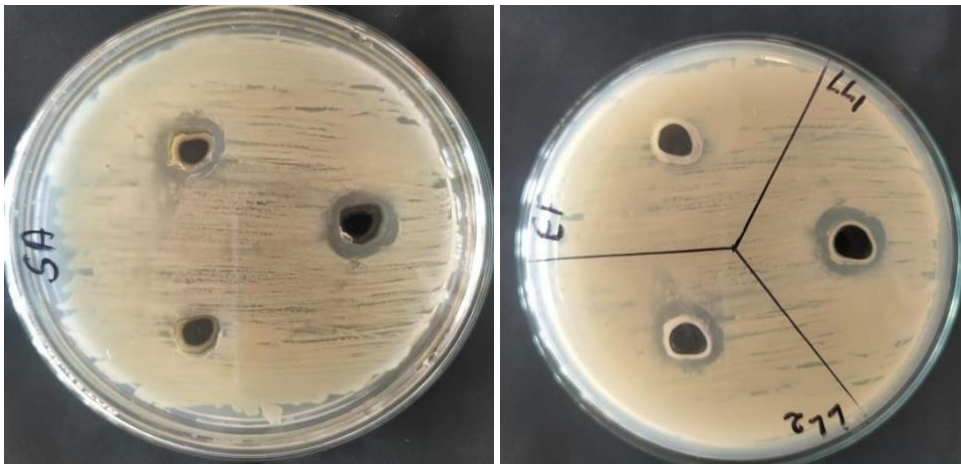


Figure 9: Bacteria demonstrated clear antimicrobial activity against *S. aureus*, indicated by distinct zones of inhibition around the sample wells.

Table 4: Zone of inhibition against *Staphylococcus aureus*

Staphylococcus aureus	**EF**	**LL1**	**LL2**
Zone of inhibition in (mm)	0	15,14,16	18,16,15
Avg:	0	15-9mm	6.3-9mm
Well diameter:	9mm	9mm	9mm
Zone :	0	6mm	7.3mm

6.7 MALDI-TOF

Among the five bacterial isolates that were analyzed using the MALDI-TOFMS, three of the isolates (EF, LL1, and LL2) were identified reliably with an acceptable score value. The other two isolates did not meet the confidence criteria to be identified definitively.

Table:5 MALDI-TOF MS Identification for the isolated bacteria

Sr.No	Sample Name	Sample ID	Organisms (best match)	Score value	Organisms (second-best match)	Score value
1.	E2 (-) (C)	Pathogen E (standard)	No organisms identification	1.53	No organisms identification	1.51
2.	E3 (+) (B)	Pathogen ST (standard)	*Salmonella sp*	1.84	*Salmonella sp*	1.82
3.	E4 (+++) (A)	Pathogen SA (standard)	*Staphylococcus aureus*	2.04	*Staphylococcus aureus*	2.02
4.	E5 (+) (B)	Curd 1 (standard)	*Pichia kudriavzevii*	1.90	*Pichia kudriavzevii*	1.70
5.	E6 (+) (B)	Curd 2 (standard)	*Enterococcus faecalis*	1.71	*No organisms identification*	1.64

6.	E7 (+++) (A)	Curd 3 (standard)	*Lactococcus lactis*	2.16	*Lactococcus lactis*	2.01
7.	E8 (+++) (A)	Curd 4 (standard)	*Lactococcus lactis*	2.14	*Lactococcus lactis*	2.01
8.	E9 (+) (B)	Curd 5 (standard)	*Candida tropicalis*	1.89	*Candida tropicalis*	1.75

7. Discussion

The combination of traditional phenotypic techniques and TOF-MS, which is an abbreviation of the mass spectrometry technique, made it possible to conduct a comprehensive characterization of the bacterial isolates, including their morphological, physiological, and antimicrobial properties. According to the result of Gram staining, capsule staining and spore staining tests, most of the selected strains are gram-positive cocci and rods, which is common in *Lactobacillus* and *Bifidobacterium* which are usually used for fermented products [43]. Physiologic results showed that some strains have positive catalytic activity, with a wide pH range from 3.0 to 9.0, and a good ability to tolerate high concentrations of salt (up to 8% NaCl). Those physiological characters exhibited high adaptation to the environment, which is typical for probiotic bacteria screened from non-dairy fermented foods [14].

Test of antimicrobial activity revealed that these strains can inhibit the growth of pathogen bacteria, like *S. Aureus*. This may reveal a possible therapy function of the selected strains. The findings are in accord with other reported probiotics strains that inhibit growth of food-borne pathogen and benefit to microbe safe [14]. By MALDI-TOF MS, rapid and specific species-level identification can be achieved with agreement 95% in general to conventional identification method [41].

Combined use of multiple analytical approaches was a useful method in probiotic research. Integrating phenotypic characterization with sophisticated mass spectrometry technique leads to a method with high performance to select and identify stable, functional and safe probiotic strains for use in food and health industries [14].

8. Conclusion

To characterize phenotypically and chemotaxonomically the selected strains of bacteria in relation to combining traditional microbiological methodology with the latest methods of analysis was the goal of this study. Cellular morphology and structural elements were examined through capsule staining, Gram staining, and spore staining techniques. Cellular physiologies were tested through catalase and examination of pH and sodium chloride tolerance. The antimicrobial capabilities were examined to isolate strains that may have therapeutic or probiotic use. The isolates were identified to the species level using MALDI-TOF MS, a technique that allows rapid, accurate identification of diagnostically relevant taxa based on species-specific mass spectral profiles. Bacterial pathogens were selected based on their distinguishable spectral profiles along with varying phenotypic characteristics. This combined methodical approach offers a robust method for microorganism characterization and subsequently advancements in functional probiotics and clinical diagnosis.

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