Characterization of Bacteriocinogenic Lactobacilli from Fermented Idli Batter: Insights into Strain Diversity and Potential

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Abstract*— The study aimed to characterize bacteriocinogenic Lactobacilli isolated from fermented idli batter, with potential applications in biopreservation and biomedicine. From 22 isolates, eight promising strains were selected based on their broad-spectrum antimicrobial activity against lactic acid bacteria and pathogenic microorganisms. These isolates underwent comprehensive characterization using classical phenotypic, physiological, and biochemical tests, including carbohydrate utilization profiling. All strains were found to be homofermentative, catalase-negative, and gelatin-negative, indicating their suitability for fermentation processes. Molecular characterization was also performed to identify and confirm the genetic diversity of these strains. The findings highlight the potential of bacteriocinogenic Lactobacilli from idli batter as a source of natural antimicrobial agents. These strains not only contribute to the understanding of microbial diversity in traditional fermented foods but also hold promise for applications in enhancing food safety and developing functional probiotic formulations for biomedicine.

Indexed Terms- Antimicrobial Activity, Phenotypic Characterization, Molecular Characterization, Probiotics.

I. INTRODUCTION

Probiotics are defined as ‘live microorganisms that, when administered in sufficient amounts, confer health benefits on the host’, according to the Food and Agriculture Organization of the United Nations/World Health Organization. Mankind has exploited lactic acid bacteria (LAB) for thousands of years for the production of fermented foods because of their ability to produce desirable changes in taste, flavor and texture as well as inhibit pathogenic and spoilage microorganisms. Due to their participation in various food fermentations throughout millennia, it is presumed that most members of this group do not offer any health risks to humans and are classified as GRAS (generally recognized as safe) species. The LAB, often regarded as 'food grade' organisms, have significant potential for selection and use as protective cultures. Numerous potential uses of protective cultures exist across diverse food systems. These organisms have been extracted from grains, dairy and meat products, fermented vegetables, and the mucosal surfaces of animals. Various antimicrobials, including lactic acid, acetic acid, hydrogen peroxide, carbon dioxide, and bacteriocins generated by these bacteria, may suppress pathogenic and spoilage microorganisms, hence prolonging shelf-life and improving food safety.

Lactic acid bacteria (LAB) are regarded as the principal category of probiotic bacteria, with Lactobacillus, Lactococcus, Carnobacterium, Enterococcus, Streptococcus, Pediococcus, Propionibacterium, and Leuconostoc being the predominant species used. Their consumption as probiotics has been recognized to provide various health advantages, including the restoration of disrupted gut microbiota, mitigation of several intestinal disorders, reduction of heart disease risk through decreased blood cholesterol levels, enhancement of immune function, and prevention of infectious diseases, among others. "Prior research indicated that probiotic bacteria may cling to and persist in the gastrointestinal (GI) tract to provide health advantages, influencing the stability and maintenance of this ecosystem." The majority of lactic acid bacteria and probiotics are acquired from the intake of fermented foods, including dairy products, meat, vegetables, and others. In addition to serving as bio preservatives, these microorganisms help improve the texture and flavor of such items.

Lactic acid bacteria are Gram-positive, non-spore-forming, non-respiring but aerotolerant organisms that generate lactic acid as a principal fermentation product via carbohydrate utilization during fermentation. These bacteria generate lactic acid as a byproduct of carbohydrate catabolism and synthesize organic compounds that enhance the flavor, texture, and scent, resulting in distinctive organoleptic properties. Orla Jensen (1919) wrote a seminal monograph that established the framework for the classification of lactic acid bacteria. This categorization system was associated with certain parameters, including glucose fermentation characteristics, cell shape, sugar utilization capability, and optimal growth temperature range. This categorization system recognized just four genera of lactic acid bacteria: Lactobacillus, Pediococcus, Leuconostoc, and Streptococcus.

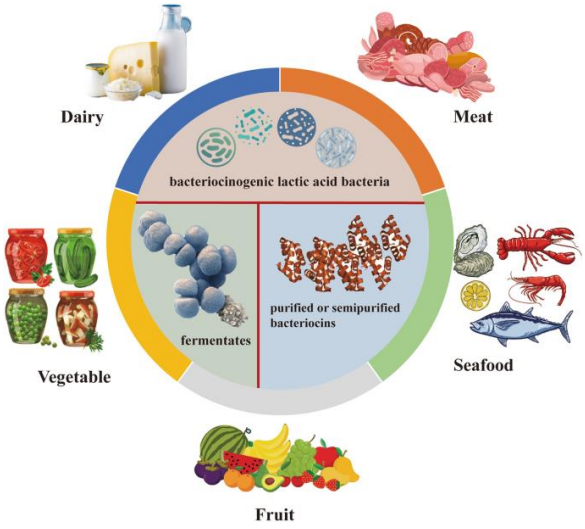

Figure 1: Applications of Bacteriocinogenic Lactic Acid Bacteria in Food Preservation Across Various Food Categories

Lactic acid bacteria has also been classified into different genera/species based on their acid production characteristics by fermenting sugars and its growth at specific temperatures. Another way to categorize lactic acid bacteria is by their carbohydrate fermentation capabilities; some are homofermentative, while others are heterofermentative. There are two types of lactic acid bacteria: those that ferment glucose into lactate (homofermentative) and those that ferment glucose into ethanol and carbon dioxide (heterofermentative). Homofermentative bacteria include Streptococcus and Lactococcus, while heterofermentative bacteria include Leuconostoc, Wiessella, and certain lactobacilli. Classification of lactic acid bacteria used to be focused on biochemical and physiological traits; however, molecular characterisation has emerged as a powerful tool for bacterial identification and classification in recent years. Multiplex PCR assays employing particular recA derived primers, soluble protein patterns, 16S rRNA gene sequencing, PCR-based fingerprinting, and random amplified polymorphic DNA profiling are all part of molecular characterisation.

You can't overstate the importance of lactic acid bacteria and all the ways they help with food fermentation and preservation. Using lactic acid bacteria has improved product attributes and imparted specific qualities that increase customer acceptability and appeal, and it has been used to make many traditional cuisines. In addition to being essential for a healthy digestive system, the majority of goods made with lactic acid bacteria also provide excellent consumer health advantages. Kefir, cheese, butter, yogurt, sauerkraut, buttermilk, brined veggies, sourdough, soya curd, koumiss, idly batter, uttapam, fermented meat, and drinks are among the fermented foods and drinks made by lactic acid bacteria. The doses of probiotics that have been shown to have a positive impact in clinical studies vary between 1-10 billion Colony Forming Units (CFU)/dose, which falls within the suggested effective dosage range of over 100 million CFU/dose. Food items that may include probiotics must also adhere to the standards set forth by FAO/WHO [1]. In general, for commercial purposes, and depending on the product, potential probiotics that are species- or strain-dependent should also meet a number of requirements, including (i) safety: isolation from suitable habitats, screening and selection of probiotics in terms of phenotype and genotype pathogenicity, correct identification and antimicrobial susceptibility; (ii) functional: probiotics should be tolerant to the GI environment and possess intestinal epithelial adhesion properties; (iii) beneficial: lactic acid production and antagonism against pathogens; (iv) technology: tests for genetically stable strains are required for large-scale production and (v) physiological: immunomodulation, cholesterol metabolism, antimutagenic and anticarcinogenic properties are required assays.

The microbial habitat in idli batter is very diverse, with lactic acid bacteria (LAB) flourishing in the ideal circumstances to aid in fermentation, flavor development, and storage. In example, lactobacilli play an important role because of the fermentation advantages they provide and the bacteriocins they produce, which might have uses in the food and pharmaceutical sectors. Lactobacilli isolated from idli batter have little known strain variety and bacteriogenic capability, despite fermented foods being widely consumed.
In order to fill this informational void, this study will isolate and characterize bacteriocin-producing Lactobacilli strains from idli batter. The research investigates the variety of strains, tests their antibacterial capabilities, and looks at possible uses for them. Functional probiotics, bio preservation, and the long-term use of microbiomes from fermented foods are all areas that this study adds to.

II. REVIEW OF RELATED STUDIES

Sircar, Bijayanta& Mandal, Shyamapada. (2023). Lactic acid bacteria (LAB) from various sources are significant as probiotics, and several researchers worldwide have identified LAB isolated from different fermented foods as possible antibacterial agents. This research investigated the antibacterial activity and probiotic properties of lactic acid bacteria isolates from idli batter, for the first time in Malda, West Bengal, India. Outcomes The LAB obtained from fresh and fermented idli batter samples exhibited antibacterial activity against pathogenic and food-borne bacteria, with inhibition zone diameters of 16, 18, and 23 mm at concentrations of 25, 50, and 75 µl/well, respectively, as assessed by the agar-well diffusion technique. The identification of isolated LAB was conducted using biochemical assays, 16S rRNA gene sequencing, and phylogenetic analysis. The LAB isolates from fresh idli batter, LMEM1001 and LMEM1002, exhibited maximum similarities of 96.81% and 95.20%, respectively, with Lactiplantibacilluspentosus and Lactiplantibacillus plantarum. In contrast, the fermented idli batter isolates, LMEM1006 and LMEM1008, demonstrated maximum similarities of 96.11% and 98.40%, respectively, with Lactiplantibacillus plantarum and Limosilactobacillus fermentum. The safety profile of isolated LAB was conducted utilizing antibiogram, DNase, and gelatinase assays. Conclusions Lactobacilli generated from idli batter have been shown to be effective probiotics, exhibiting significant antibacterial activity against both clinical and food-borne bacteria. The idli batter isolates of LAB may serve as probiotics for human consumption and as biotherapeutics in addressing bacterial antibiotic resistance.

Shinde, Harshraj. (2015). The current work isolated bacteriocin-producing Lactobacillus species from idli, and bacteriocin was generated from Lactobacillus spp. by fermentation. Following the discovery of bacteriocin, its antibacterial efficacy was evaluated against five bacteria responsible for food deterioration and human pathogenicity: Bacillus licheniformis, Streptococcus thermophilus, Streptococcus acidophilus, Escherichia coli, and Zymomonas anaerobia. Bacteriocin demonstrated significant antibacterial activity in the experiment against all five bacterial strains, producing zones of inhibition of 18mm, 22mm, 19mm, 17mm, and 21mm, respectively. Erythromycin served as the positive control, while DMSO functioned as the negative control. This research demonstrated the potential of using bacteriocin as a biopreservative to mitigate food deterioration microorganisms.

Kawahara, Takeshi, et al. (2010). We evaluated the antibacterial efficacy of Lactobacillus curvatus strain Y108, isolated from the traditional Japanese pickle Nozawana-zuke, and partly identified the antibacterial compound produced by the strain. The Y108 strain demonstrated antibacterial efficacy against L. curvatus JCM 1096, Listeria monocytogenes JCM7671, Staphylococcus aureus subsp. aureus JCM20624, and Serratia marcescens JCM20012. The antibacterial activity was eliminated after treatment with several proteases and lipase, but not with catalase, and it exhibited reasonable stability against heat treatment for 2 hours at 100 degrees Celsius. The Y108 strain exhibited enhanced antibacterial activity at 20 degrees Celsius compared to 30 degrees Celsius, its optimum growing temperature. The SDS-PAGE examination of the pure culture supernatant identified two antibacterial peptide agents, F3-I and F3-II, with net molecular weights of 5.5 kDa and 4.5 kDa, respectively. The IN-terminal amino acid sequences of F3-I and F3-II exhibited homology with those of

lactocin 705 alpha and 705 beta, respectively. Nonetheless, the molecular weights and specific antibacterial activity of the two peptides significantly differed from those documented for lactocin 705. Duhan, Joginder et al. (2013). Probiotics are advantageous microorganisms that are native to a healthy digestive tract. These are living microorganisms supplied in sufficient quantities to provide health benefits to the host. They function by displacing harmful bacteria in the digestive tract and subsequently adhering to the intestinal wall, therefore augmenting the population of good bacteria that regulate and sustain equilibrium between advantageous and detrimental microorganisms. The practice of consuming fermented foods, including yogurt, sauerkraut, fermented milk, miso, and soy drinks, is primarily founded on the health advantages conferred by these microorganisms. "Numerous lactic acid bacteria (LAB) serve as probiotics, with the predominant species being Bifidobacteria (Bifidobacterium bifidum) and Lactobacteria (Lactobacillus acidophilus, Lactobacillus plantarum)." The advantages of probiotic microorganisms are widely established, although their mode of action remains ambiguous. Lactic acid bacteria (LAB) safeguard food against spoilage and harmful microbes by generating organic acids, hydrogen peroxide, diacetyl, antifungal agents such as fatty acids or phenyllactic acid, and bacteriocins. Bacteriocins are a diverse group of tiny, heat-stable peptides synthesized by several bacterial species, including numerous probiotic strains. Bacteriocins produced by lactic acid bacteria (LAB) are regarded as safe natural preservatives with significant antibacterial properties and antagonistic effects. Bacteriocins primarily suppress the proliferation of foodborne pathogenic bacteria, hence preventing food deterioration. They also safeguard the body from cancer and significantly enhance the immune system. These are categorized into many categories, with classes I (Lantibiotics) and II (Bacteriocins) being the most extensively researched. "This study focuses on the variety of bacteriocins, their expression systems, and their applications, which facilitate the use of lactic acid bacteria as probiotics."

III. EXPERIMENTAL SET-UP

- Procedure of Isolation

The idli batter was composed of rice (Oryza sativa) and black gram (Phaseolus mungo), a leguminous plant. The components were cleaned, soaked, ground, and let to ferment overnight. The fermented idli batter was serially diluted with saline, inoculated onto De Man Rogosa Sharpe (MRS) agar, and incubated anaerobically at 37 °C for 24 to 48 hours. The colonies on MRS agar that were milky white, round, convex, raised, and non-pigmented were selected for subsequent subculturing. The colonies were inoculated on MRS agar to assess purity. The pure cultures were supplemented with glycerol and kept for further analysis.

- Isolate's Antimicrobial activity

Multiple indicator strains (Table 1) for the assessment of antibiotic activity were acquired from the Microbial Type Culture Collection (MTCC). The lactobacilli isolates were cultured in MRS broth, and the cell-free supernatant (CFS) was recovered from the 48-hour culture. The CFS was titrated to pH 5 using 3N NaOH, and the antimicrobial spectrum was assessed against several indicator LAB and pathogens via the agar well diffusion technique. The CFS, treated with protease (1 mg/mL) for 2 hours and adjusted to pH 5, was further assessed for antibacterial activity. The inhibition zone (in mm) was quantified for all indicator strains.

Table 1 - Antimicrobial activity against LAB and various pathogens Diameter of the well is 6 mm

	JJ18	JJ22	JJ24	JJ29	JJ30	JJ55	JJ58	JJ60
Lactobacillus plantarum (MTCC 6161)	10	10	10	10	10	10	10	10
Lactococcuslactissubsp.lactis(MTCC 3038)	12	14	14	17	15	16	12	17
Lactobacillus fermentum (MTCC 1745)	12	10	10	13	12	13	12	12
Lactococcuslactissubsp.lactis(MTCC	10	10	10	10	10	10	10	10

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Leuconostocmesenteroidessubsp.mesenteroides(MTCC107)	10	10	10	10	10	10	10	10
Lactococcuslactissubsp.chacetylactis (MTCC3042)	17	18	18	18	18	18	17	18
Lactobacillusrhamnosus(MTCC1408)	11	11	10	10	10	10	11	10
Brevibacterium casei (MTCC 1530)	12	11	11	12	11	11	11	11
Listeria monocytogenes (MTCC 657)	18	18	18	19	18	18	18	19
Staphylococcusaureussubsp.aureus(MTCC737)	18	18	18	19	19	19	18	19
Aeromonashydrophilasubsp.hydrophilia(MTCC 1739)	16	16	16	16	17	15	15	17
Pseudomonasaeruginosa(MTCC2295)	13	14	14	13	14	14	14	14
Micrococcus luteus (MTCC 106)	13	13	13	13	13	14	13	15
Bacillus cereus (MTCC 1272)	16	16	16	18	16	16	16	19
Vibrio parahaemolyticus (MTCC 451)	16	15	16	16	15	15	15	18
Bacillus subtilis (MTCC 619)	16	15	14	16	15	16	15	19

- Classical characterization of bacteriocinogenic isolates

The growth was measured in MRS broth at different temperatures (15, 37, and 45 °C), salt concentrations (4, 6.5, and 10% of NaCl), and pH levels (3.5, 4.5, 8.5, and 9.5). According to Pal et al. (2005), the isolates were tested for hydrolysis of gelatin, hydrolysis of starch, hydrolysis of acetoin, hydrolysis of ammonia, creation of carbon dioxide, formation of slime, and homo-hetero fermentation. The usage profile of carbohydrates was established by means of the HiCarbo kit. It was also found out what the optical properties of the lactate isomer are.

- Characterization and Analytical procedures
- RAPD

Genomic DNA was isolated by the procedure as described by de Los Reyes-Gavilan et al. (1992). RAPD analysis was carried out using the primers R2 5'-GGCGACCACTAG 3' and M13 5' GAGGGTGGCGGTTCT-3'. The PCR cocktails (50 L) consisted of 50 pM of the primer, 50 ng of DNA, 1x Taq DNA polymerase buffer, 2 U of Taq polymerase, 0.4 mM of each dNTP, and 3 mM of MgCl2 (Genei, Bangalore, India). Amplification conditions were initial denaturation at 94 °C for 5 min, 40 cycles of 94 °C for 1 min, annealing at 38 °C for R2 and 40 °C for M13 for 45 s, and elongation at 72 °C for 1 min, followed by a final elongation at 72 °C for 10 min. The pattern was analyzed by running in 1.5% agarose gel electrophoresis with DNA ladder (Sigma, Saint Louis, USA).

- 16S rRNA gene analysis

Amplification of 16S rRNA gene was performed from genomic DNA of the isolates using universal primers fD1 (5'-GAGTTTGATCCTGGCTCA-3') and rP2 (5'-ACGGCTACCTTGTTACGACTT-3'), as described by Naik et al. (2008). PCR cocktails (50 L) contained 50 pM of primer, 50 ng of genomic DNA, 1x Taq DNA polymerase buffer, 1 U of Taq DNA polymerase, 0.2 mM of each dNTP, and 1.5 mM MgCl2. Amplification was performed in a DNA thermo cycler at 94 °C for 3 min, followed by 30 cycles of 10 s at 94 °C, 1 min at 56 °C and 30 s at 72 °C with an extension of 72 °C for 5 min. Purified PCR products were sequenced with automated DNA sequencer with specific primers using the facility at Macrogen In. Phylogenetic analysis for the isolates was performed for the isolates using MEGA software v5.05.

- 16S ARDRA

Restriction digestion of PCR amplified product was performed with the restriction enzyme AluI for overnight at 37 °C in 20 L volumes of incubation buffer containing 5 U of the restriction enzyme and adequate DNA. The pattern was analyzed by running in agarose gel electrophoresis with 100 bp DNA ladder.

- Multiplex PCR assay

A multiplex PCR assay was performed with the recA gene-based primers paraF (5'-GTC ACA GGC ATT ACG AAA AC-3'), pentF (5'-CAG TGG CGC GGT TGA TAT C-3'), planF (5'-CCG TTT ATGCGG AAC ACC TA-3'), and pREV (5'-TCG GGA TTA CCA AAC ATC AC-3'), as described by Torriani et al. PCR cocktails (50 L) contained 0.25 mM of primers, 50 ng of genomic DNA, 1x Taq DNA polymerase buffer, 1 U of Taq DNA polymerase, 0.2 mM of each dNTP, and 1.5 mM MgCl2. PCR were performed with initial denaturation at 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 10 s, and elongation at 72 °C for 30 s, and final extension at 72 °C for 5 min. The PCR products were visualized in agarose gel electrophoresis with 100 bp ladder.

IV. RESULTS OF THE STUDY

The molecular characterisation of bacteriocancinogenic LAB isolates from fermented idli batter is the focus of the current work. The overarching goal of this research is to establish a commercial starting culture from a homogeneous consortium of strains that exhibit a wide range of desirable characteristics. Eight of the twenty-two lactobacilli isolates (JJ 18, JJ 22, JJ 24, JJ 29, JJ 30, JJ 55, JJ 58, JJ 60) that exhibited the greatest zone of inhibition against other LAB and a variety of Gram positive and Gram negative pathogens were selected for the study (Table 1). Our isolates are likely bacteriocinogenic since their CFS may hinder the growth of other LAB species. Also, there was no zone of inhibition in the protease-treated CFS (Figure 2), suggesting that the action is mediated by a proteinaceous material. We found that our isolates effectively inhibited the growth of bacteria that have been shown to be prevalent contaminants in idli batter fermentation, such as Bacillus cereus and Staphylococcus aureus. Furthermore, the lactobacilli demonstrated strong suppression of the common food-borne pathogens Escherichia coli and Listeria monocytogenes. One study found that harmful bacteria including Staphylococcus aureus, Bacillus cereus, and Escherichia coli were killed when the bacteriocin plantaricin LP84 from Lactobacillus plantarum NCIM 2084 was added to idli batter. Results from this research indicate that native lactobacilli isolated from idli batter may be used as protective cultures in the food industry due to their strong antibacterial activity against the food pathogens listed above (Table 1).

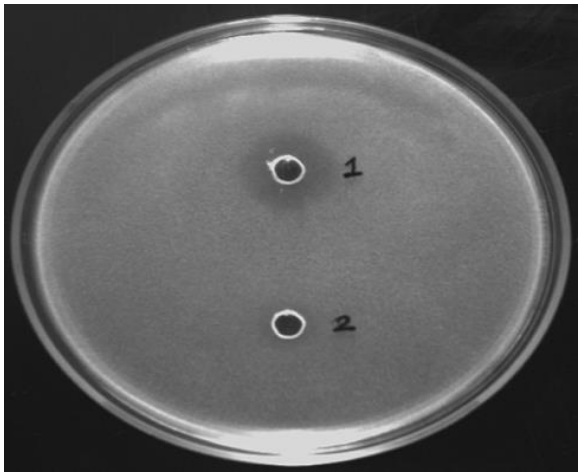

Figure 2 - Antimicrobial activity of JJ 18 against *Staphylococcus aureus*. (1) CFS adjusted to pH 5 (2) CFS treated with protease.

The inhibitory potential of these isolates against other pathogens like Aeromonas, Pseudomonas, Micrococcus, and Bacillus (Table 1) suggests its application in biopharmaceutical industry. Traditional techniques were used to identify the eight lactobacilli. There was no evidence of slime generation, and all of the isolates were Gram positive. There were a battery of physiological tests performed on the lactobacilli. The mesophilic nature of the 8 lactobacilli was shown by their robust growth at 15, 37, and 45 °C, and their lack of growth at 10 °C. The lactobacilli could withstand a 6.5% salt concentration, but a 10% concentration stunted their growth. The lactobacilli may thrive in environments with both acidic and alkaline pH levels. It is likely that the eight lactobacilli that tested comparable in morphological and physiological tests came from the same ecological niche. Each of the eight lactobacilli tested negative for biochemical characteristics including catalase synthesis, starch hydrolysis, gelatin, and arginine hydrolysis. The only strain that failed to produce acetoin was JJ60, although all the others tested negative. The homo-fermentative bacilli all had the DL lactic acid structure, as shown in Table 2. Traditional methods of identifying bacteria suggested that the samples could contain Lactobacillus plantarum. Nonetheless, the subspecies of

Lactobacillus plantarum cannot be distinguished based on this description alone. Consequently, the subspecies was identified using PCR-based molecular techniques.

RAPD analysis was performed initially to cluster the isolates using two different primers R2 and M13. JJ 18, JJ 22, JJ 29 and JJ 30 having similar pattern in the RAPD analysis belonged to a single group, while JJ 24, JJ 55, JJ 58, and JJ 60 having different patterns clustered in to different groups (Figure 3).

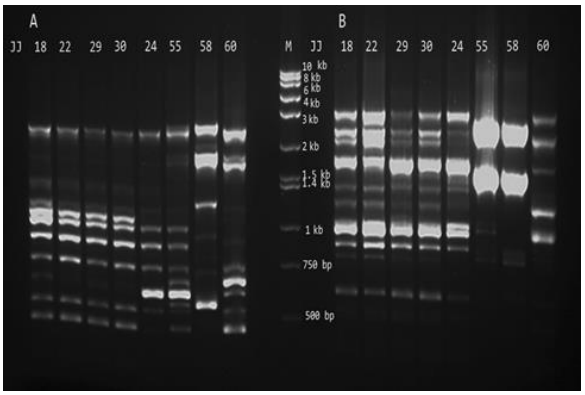

Figure 3- RAPD Analysis using the primer R2 (A) and primer M13 (B). M is the 500 bp marker.

Thus, five different clusters were clearly observed based on the RAPD analysis. The sugar utilization pattern was also different for all the five groups indicating strain level variation among these isolates (Table 2). The 16S rRNA were analyzed for the five different clusters of isolates. The PCR products were sequenced and were subjected to nucleotide BLAST. The isolates showed 99 to 100% homology towards Lactobacillus plantarum. Multiple sequence alignment was carried out by CLUSTAL W and later phylogenetic analysis was performed using software MEGA v5.05. All the isolates were phylogenetically closely related to Lactobacillus plantarum and Lactobacillus pentosus (Figure 4). Thus, other molecular methods were carried out to clearly identify the species.

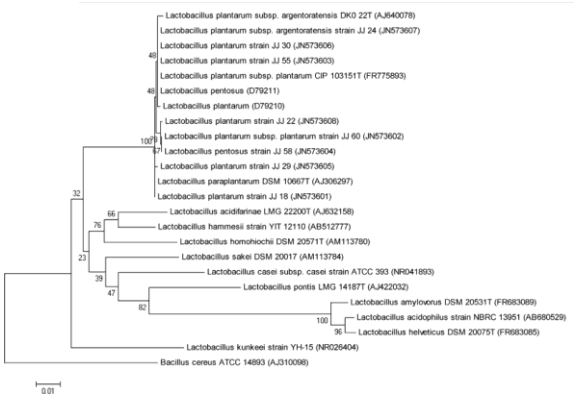

Figure 4 - Phylogenetic tree with the 16S rRNA gene using the MEGA v5.05 program by neighbor-joining (NJ) method.

The different clusters obtained as a result of RAPD indicated strain level variation among the isolates. As 16S ARDRA is a rapid and reliable tool for strain identification, the same was performed with AluI restriction enzyme. AluI is generally used in differentiating Lactobacillus species. The digestion pattern was similar for all the 8 lactobacilli (Figure 5).

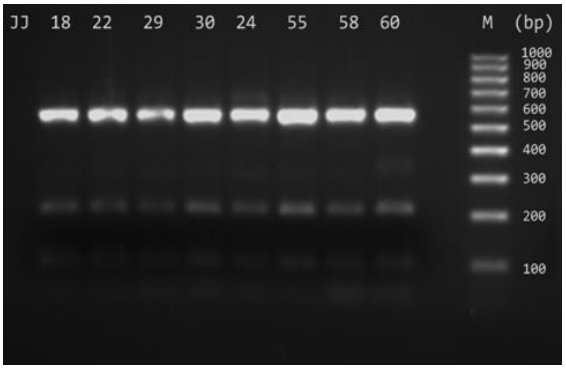

Figure 5 - 16S-ARDRA using AluI restriction enzyme for the isolates. M is the 100 bp marker.

The results showed high homology of the ribosomal genes. Generally, the Lactobacillus pentosus and Lactobacillus plantarum are genotypically closely related and show high homology in the 16S rRNA gene sequence. However, the differential utilization of carbohydrates by the isolates (Table 2) further prompted us to carry out sub-species level identification by other PCR method, using recA gene as it can also be used as a phylogenetic marker. The recA gene codes for a small protein (352 amino acids in Escherichia coli) implicated in homologous DNA recombination, SOS induction, and DNA damage-

induced mutagenesis. Multiplex PCR with recA gene-derived primers was carried out to identify the subspecies of the lactobacilli.

Table 2 - Phenotypic, physiological and biochemical characterization of the isolates.

	JJ 18	JJ 22	JJ 24	JJ 29	JJ 30	JJ 55	JJ 58	JJ 60
Cell form	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus
Gasfromglucose	-	-	-	-	-	-	-	-
Homo-hetero fermentation	Ho	Ho	Ho	Ho	Ho	Ho	Ho	Ho
Growthattemperature								
10 ℃	-	-	-	-	-	-	-	-
15 ℃	+	+	+	+	+	+	+	+
37 ℃	+	+	+	+	+	+	+	+
45 ℃	+	+	+	+	+	+	+	+
GrowthatpH								
3.5	+	+	+	+	+	+	+	+
4.5	+	+	+	+	+	+	+	+
8.5	+	+	+	+	+	+	+	+
9.5	+	+	+	+	+	+	+	+
Salt tolerance								
4%	+	+	+	+	+	+	+	+
6.5%	+	+	+	+	+	+	+	+
10%	-	-	-	-	-	-	-	-
Catalase production	-	-	-	-	-	-	-	-
Slime from sucrose	-	-	-	-	-	-	-	-
Acetoin production	-	-	-	-	-	-	-	+
Isomers of lactic acid	DL	DL	DL	DL	DL	DL	DL	DL
Arginine hydrolysis	-	-	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-	-	-
Gelatin liquefaction	-	-	-	-	-	-	-	-
Esculin hydrolysis	+	+	+	+	+	+	+	+
Carbohydrate utilization[a]								
Lactose	-	-	-	-	-	+	-	+
Galactose	-	±	±	-	±	±	-	+
Trehalose	-	+	-	-	-	+	+	+
Melibiose	+	-	-	-	-	±	+	-
L-Arabinose	-	-	-	-	-	-	-	±
Inositol	+	-	-	-	-	+	+	-
Sorbitol	+	-	-	-	-	+	+	-
Melezitose	-	-	-	-	-	+	+	+
α-methyl mannoside	-	-	-	-	-	-	±	-

The isolate JJ 24 was identified as Lactobacillus plantarum subsp. argentoratensis based on its amplicon around 318 bp and 120 bp. Moreover, JJ 24

could not metabolize melizitose which is a key factor in identification of Lactobacillus plantarum subsp. argentoratensis. JJ 58 was identified as Lactobacillus pentosus based on its amplicon around 218 bp and JJ 60 as Lactobacillus plantarum subsp. plantarum based on its amplicon around 318 bp. JJ 55 had an additional amplicon around 200 bp in addition to 318 bp and 120 bp.

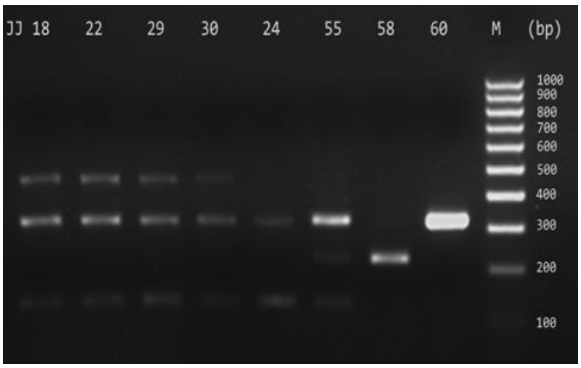

Figure 6- Results of Multiplex PCR using recA species specific primer.

The carbohydrate utilization profile of JJ 55 was almost similar to JJ 58, except for lactose utilization. Probably, JJ 55 must be closely related to Lactobacillus pentosus. Similarly, JJ 18, JJ 22, JJ 29, and JJ 30 had an additional amplicon above 400 bp in addition to 318 bp and 120 bp (Figure 6). Based on the sugar utilization profile, JJ 18, JJ 22, JJ 29, and JJ 30 were not able to metabolize melizitose which is a key character in identifying Lactobacillus plantarum subsp. argentoratensis. The sugar utilization profile of other sugars was also similar to that of JJ 24 (Table 2). Probably, the four isolates JJ 18, JJ 22, JJ 29 and JJ 30 were closely related to Lactobacillus plantarum subsp. argentoratensis. "Therefore, it is evident that in addition to various classical physiological, biochemical and sugar utilization profile, a combination of molecular methods can be used successfully for sub-species level identification of Lactobacillus isolates from fermented idli batter."

Because of their antibacterial action against food borne pathogens, bacteriocins generated by Lactobacillus plantarum are actively used for food preservation and are the topic of significant study. In order to manage harmful bacteria, the food industry may make use of LAB, which are natural food isolates. Fermentations of dairy products, meat, and vegetables are only a few of the many environments where the adaptable lactic acid bacteria Lactobacillus plantarum may be found.

V. CONCLUSION

This experiment successfully identified bacteriogenic Lactobacilli that were detected in fermented idli batter. This study's analysis of bacteriocancerogenic lactobacilli may aid in the development of idli batter that contains a consortium of lactobacilli as standard inoculums (starting cultures), which may have several beneficial effects. Because of their antibacterial activity against various illnesses and lactic acid bacteria, the eight strains selected for this study have the potential to cause cancer. Extensive phenotypic, physiological, and biochemical testing demonstrated their homofermentative nature and suitability for fermentation operations; molecular characterization highlighted their genetic diversity. These findings emphasize the importance of traditional fermented foods, such idli batter, as a source of beneficial bacteria that might have industrial and medicinal applications. The discovered strains have enormous potential for natural food preservation and the development of innovative probiotic treatments, both of which contribute to the safety of food and human health.

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