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Random UV-Mutagenesis of Lactobacillus Species for the Generation of a Mutant with Better Probiotic Potential

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ABSTRACT

Antibiotic resistance has reduced the value of synthetic drugs, offering up possibilities for the development of new substitutes with significant potential to benefit their host. Probiotics are living microbes that restore the microbiome in the gut. The main purpose of this research is to explore the probiotic potential of Lactic Acid Bacteria and its mutant strains. LAB was isolated from a variety of fermented foods. They were first examined for cultural, microscopic, and biochemical properties. The four probiotic LAB isolates were identified to the strain level using 16S rRNA gene sequence comparisons. The tolerance of these strains to pH, NaCl, and phenol was used to establish their safety. In vitro testing for probiotic potential comprised survival under simulated GI tract conditions and antimicrobial activity. Lastly exposure to U.V. for varied periods for strain improvement. The isolates were screened as *Limosilactobacillus fermentum* (LAB01), *Lactobacillus acidophilus* (LAB02), *Lactobacillus paracasei* (LAB03) and Lactobacillus delbrueckii subsp bulgaricus (LAB04). Mutated strains were resistant to a wide spectrum of antibiotics, whereas most strains were only tolerant to limited antibiotics. The correlation between gut flora and health is exciting since it opens new avenues for research. Based on these findings, it can be concluded that LAB is a potent probiotic.

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Keywords: Antibiotic, Probiotic, Lactic Acid Bacteria, Fermented Food, 16S rRNA, Antimicrobial, Resilience, Mutated

Abbreviations

LAB: Lactic Acid Bacteria, GI: Gastro Intestinal, WHO: World Health Organization, ORS: Oral Rehydration Therapy, PBS: Phosphate Buffer Saline, LSB: Lactobacillus Selection Base, OD: Optical Density, UV: Ultra Violet, PCR: Polymerase Chain Reaction, DNA: Deoxyribonucleic Acid, BLAST: Basic Local Alignment Search Tool, NCBI: National Center for Biotechnology Information, CTAB: Cetyl Trimethyl Ammonium Bromide.

Introduction

The necessity for probiotics has skyrocketed in recent years on account that consumers are more conscious of the impact of microbial flora in the intestinal environment, its influence on the body's immune response, and increased demand for animal-based products. This has led to the market flooding with probiotic-fortified food [1]. This increase in market value is most likely attributable to the fact that probiotic foods are more concerned with warding off infection without ingesting the actual antibiotic. According to WHO a prerequisite for live microbes to be contemplated as a probiotic when taken confer a health benefit (Food and Agriculture Organization of the United Nations. & World Health [2]. Generally, these live microbes partaken for their prospective health ascendancy are the Lactic Acid Bacteria [3]. LAB are heterogeneous and predominant in niches of dairy, meat, and vegetable, as well as the gastrointestinal, mucosal cavities, and urogenital tracts of humans and animals, along with soil and water [4]. These Gram-positive, catalase-negative microbes produce Lactic acid, in addition, to being beneficial as inoculum in the food sector where they play a substantial role in fermentation activity as a part of food preservation [5, 6].

There is mounting evidence to support the assertions that probiotics have positive effects such as improved gut health, immune

response augmentation hindering perspective pathogenic strains cancer prevention of antibiotic-associated diarrhea and lactose metabolism improvement [7-11]. Data shows that dietary items containing probiotic bacteria may help to prevent coronary heart disease by lowering serum cholesterol levels and controlling blood pressure [12, 13]. Due to their metabolic adaptability, they are accompanied by ORS for the treatment of acute infectious diarrhea [14]. Previously, researchers have worked with Lactobacillus mutants that can accumulate long-chain polyphosphate, which improves the function of the intestinal barrier [15].

The incentives of probiotics are limitless ergo it would be of great assistance if we locate more. The purpose of this study is to explore the different probable sources for the isolation of LAB. These isolates were characterized for identification up to the species level and they were further assessed for their probiotic potential. Several criteria must be satisfied by a bacterium to be selected as a probiotic strain, a few of them are tolerance to pH, NaCl, phenol, gastric fluid, antibiotic susceptibility, and antimicrobial activity with safety being of the greatest significance This study aims to perform a thorough screening of Lactobacillus and its mutant strains and to assess their probiotic potential.

Material & Method

Fermentation, Isolation, and Maintenance of Bacterial Isolates

In the study, traditional fermented foods were used as source material. Four samples of fermented food products green pea (*Pisum sativum*), white pea (*Pisum sativum*), chickpea (Cicer arientinum), and dragon fruit (*Selenicereus undatus*) offered by a local market were cleansed with tap water before being soaked for roughly 6 hours, ground, and fermented for 12 hours[16]. All samples were diluted in sterile PBS and allowed to settle to remove any remaining food particles. The suspension was employed to isolate bacteria by pour plating into a nutritive medium. The most visible bacteria colonies on a plate were purified using the streak plate technique on nutritional agar. Numerous bacteria isolated from various sources were selected for their ability to produce lactic acid bacteria on specialized media, LSB broth. Bacterial cultures that had been purified were kept. Pure strains were injected into sterile LSB broth and cultured according to the manufacturer's instructions. Glycerol stocks were made by combining equimolar quantities of cultures with 60 percent sterile glycerol after the cultures reached an optical density of 1.0 (600 nm) (60 mL glycerol in 100 mL distilled water). The glycerol vials were initially placed at 4°C, then 0°C, and finally -20°C. Vials were labeled with the culture name, initials, date, and vial number for identification.

Characterization of Isolates

All four isolates were subjected to biochemical analysis such as catalase, oxidase, indole, and H_2S generation and 16S rRNA identification technique. Initially, the isolates were grown in LSB medium for 24 hours. Gram staining of the isolates was used to assess the gram nature and colony features.

Strain Improvement Using UV-Mutagenesis

To achieve UV-mutagenesis of the relevant strains, the 'Direct Plate Irradiation' method was used [11]. For the series of direct-plate kill tests, sub-cultures were created by inoculating sterile LSB broth (50mL final volume) with overnights and raising the density to roughly 1 x 10^8 cells per mL, around 0.7 to 0.8 OD at 600nm. These sub-cultures were grown in flasks shaken in an orbit-shaker at 37°C. When the sub-culture reached the desired optical density, a series of dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^5) were made. Spreading 0.1 mL of the diluted sub-cultures over multiple nutrient LSB agar plates resulted in the final plates for UV irradiation. A total of 5 plates of each dilution were plated for each isolate, and the plates were housed in a black box to avoid photoreactivation. All UV irradiation was done in a custom-built, glass-fronted UV chamber. Later, one plate from each treatment set was randomly placed in the chamber's center and irradiated for the predefined period. The UV power switch light was manually regulated for each UV exposure time point. The irradiation periods in the technique were 5 seconds, 10 seconds, 20 seconds, and 30 seconds, with the last plate remaining unexposed, and the plates were immediately placed inside the black box following the exposure. All plates were grown at 37°C for 24 to 36 hours before counting the colonies. As with the pure isolates, they were submitted to Agarose Gel Electrophoresis and PCR amplification methods.

Identification using 16S rRNA Sequencing: (Extraction of gDNA, PCR Amplification of 16S rRNA Sequence, and DNA Sequencing)

Initially, genomic DNA isolation of the desired cell culture was carried out in the procedure, using a CTAB lysis buffer. Subsequently, the isolated genomic DNA was run on 0.8 % agarose with the tracking dye ethidium bromide in the gel electrophoresis apparatus then extracted DNA samples were kept at -20°C for subsequent application. A pair of primers 8F and 907R was used for amplification of the fragment of nine hundred base pairs in size. The polymerase chain reaction was performed in a 50 μL reaction volume containing 2.5 units of Taq DNA polymerase, 2.5 mM $MgCl_2$, 0.5 μM of each primer, and 0.2 mM of dNTPs, 1X PCR buffer, and 100 ng of genomic DNA. The amplification was carried out in the Gene amp 9700 PCR system and the condition was as follows: Initial denaturation at 96°C for 5 minutes followed by 35 cycles of denaturation at 96°C for 30 seconds, annealing at 55°C for 30 seconds, elongation at 72°C for 1 minute, and 5 minutes elongation at 72°C. PCR products were analysed on 1.5% agarose gel, visualized by the Wilburg gel documentation system after staining with Ethidium bromide. Nucleotide sequencing (forward and reverse) was done with a commercial sequencing service (Eurofins, India). After the qualitative analysis of the extracted DNA using agarose gel electrophoresis, the 16S rRNA gene was amplified using Polymerase Chain Reaction in PCR Thermocycler. The isolates were then identified by 16S rRNA gene sequencing with minor modifications. The phylogenetic tree was constructed in MEGA 11 using the Maximum Likelihood (ML) method along with the Bootstrap Phylogeny test with number of bootstrap replications set at 1000 and the Tamura-Nei model.

Determination of Probiotic Potential

Endurance to NaCl

Tolerance to NaCl is seen as a need for probiotic potential. The ability of isolates to withstand varying NaCl concentrations was investigated using the approach described by with minimal alterations [17]. Each chosen isolate's tolerance to sodium chloride salt was tested using a 96-well microtiter plate assay. Isolates were cultured in LSB broth at 37°C for 48 hours. Separately, a concentrated NaCl salt solution was produced and autoclaved. The growth was evaluated hourly at 37°C incubation temperature for 3 hours, viable cell colonies were counted by spreading 100μL of cultures onto LSB agar plates.

Phenol Tolerance

To assess phenol tolerance tests were carried out in the manner described by, with minor adjustments [18]. Each isolate's resistance to phenol was evaluated using a 96-well microtiter plate assay. Isolates were cultured in LSB broth for 48 hours at 37°C. Each

isolate's 1 % culture was introduced to 10 mL of fresh LSB broth and adjusted to concentrations of 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 1.0%, 3.0%, and 5.0% using sterile concentrated phenol solution. To determine viable cells, aliquots of 0.1 mL at varying intervals were spread on LSB agar plates.

Resilience Simulated Gastric Fluid Tolerance

For growth and adherence to the gastrointestinal tract, Lactic acid bacteria must tolerate the presence of digestive enzymes from the stomach and small intestine. Resistance to Gastric fluid was evaluated as suggested, with slight variation [19]. In sum, the simulated gastric fluid was made by dissolving 2.0g sodium chloride, 3.2g pepsin, and 7.0mL HCl in one liter of distilled water, with a pH of 1.2. Gastric acid tolerance was evaluated on the chosen isolates. Isolates were cultured in LSB broth for 48 hours at 37°C. Each isolate's 1 percent culture was cultured for 48 hours before being injected into 10 mL of simulated stomach juice. Temperatures of 37°C were used for growth incubation for 0, 1, 2, 3, and 6 hours. At 600 nm optical density, the culture growth was examined.

Susceptibility to Different pH

Resistance to different pHs is a prerequisite for probiotics as the pH of the alimentary canal varies. The 1 % culture of isolate developed for 48 hours was inoculated into fresh LSB broth adjusted to pH 1.0, 2.0, 3.0, 4.0, 6.0, and 8.0 using 5N HCl and 1N NaOH. The ability of isolates to survive at different pH was tested by the following method described by, with minor modifications The culture growth was hourly monitored at an optical density of 600 nm for 3 hours against the control [20].

Resistance to Antibiotics

The LAB isolates were investigated for susceptibility/resistance to antibiotics as demonstrated by, with a few more antibiotics for example Amikacin (10µg), Ciprofloxacin (30µg), Trimethoprim (30µg), Levofloxacin (5µg), Nalidixic acid (30µg), Ceftriaxone, Cefoperazone, Ofloxacin [21]. The antibiotic resistance and susceptibility of selected isolates were assessed using the antibiotic discs diffusion method. The plates were prepared by spreading 0.1 mL of culture on LSB agar.

Results and Discussion

Isolation, Microscopic Evaluation, and Biochemical Analysis of Lactic Acid-producing Isolates

Four isolates were obtained from fermented food products: Green pea (*Pisum sativum*), White pea (*Pisum sativum*), Chickpea (*Cicer arientinum*), and Dragon fruit (*Selenicereus undatus*). They were acquired at a local market in Mumbai, India. The capacity to thrive on Lactobacillus selection base medium (LSB) was used to select lactic acid-generating bacterial isolates. Tables 1 and 2 show the sources of Lactic acid bacteria isolation and morphological characterization, respectively. Bacterial isolates were investigated based on Gram nature, and morphological and biochemical characteristics. The biochemical characteristics of bacterial isolates were determined to be conducive to growth at various temperatures (15°C, 45°C) and in the presence of bile salt. On either hand, none of the lactic acid-producing isolates tested positive for oxidase, catalase, indole, H2S generation, or gelatin liquefaction.

Table 1: Sources for Isolation of LAB Isolates and Sequence Analysis

Isolated from	Strain identified	Accession-ID
Chickpeas (LAB01)	*Limosilactobacillus fermentum*	ON624361
Green peas (LAB02)	*Lactobacillus acidophilus*	ON624358
Dragon fruit (LAB03)	*Lactobacillus paracasei*	ON624359
White peas (LAB04)	*Lactobacillus delbrueckii subsp bulgaricus*	ON624360

Table 2: Morphological Characterization of Lactic Acid Bacteria Isolates

Isolates	Size mm	Shape	Colour	Margin	Elevation	Opacity	Consistency	Gram Nature
LAB01	5 mm	Filamentous	White	Undulated	Flat	Opaque	Dry	Positive
LAB02	Pinpoint	Circular	White	Entire	Convex	Opaque	Moist	Positive
LAB03	3 mm	Circular	White	Entire	Convex	Opaque	Viscid	Positive
LAB04	2 mm	Circular	White	Entire	Convex	Opaque	Viscid	Positive

Table 3: Biochemical Characterization of Lactic Acid Bacteria Isolates

Biochemical tests	LAB01	LAB02	LAB03	LAB04
Growth at 45°C	+	+	+	+
Growth at 15°C	+	+	+	+
Catalase	-	-	-	-
Oxidase	-	-	-	-
Indole	-	-	-	-
H_2S production	-	-	-	-
Gelatin Liquefaction	-	-	-	-
Bile salt	+	+	+	+
Key: (-) Negative, (+) Positive				

Molecular Identification of Selected Lactic Acid Bacteria

For the specified LAB isolates, genomic DNA was extracted and 16S rRNA sequencing was undertaken. All of the isolated bacteria' 16S rRNA genes were amplified. The resulting nucleotide sequences obtained by Sanger's sequencing were checked for sequence homology with known sequences in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST). As an outcome, the isolates were screened as *Limosilactobacillus fermentum* (LAB01), *Lactobacillus acidophilus* (LAB02), *Lactobacillus paracasei* (LAB03) and *Lactobacillus delbrueckii subsp bulgaricus* (LAB04).

Identification of Lactobacillus Strains

The evolutionary history was inferred by using the Maximum Likelihood method and the Tamura-Nei model [22]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. This analysis involved 4 nucleotide sequences. There were a total of 1472 positions in the final dataset [23]. The result of the isolated *Lactobacillus* strains obtained by next-generation 16S rRNA sequencing analysis depicted that the LAB01 strain exhibited the highest similarity (95%) against the LAB03 strain, whereas the LAB02 strain was found to have 91% similarity with that of the LAB04 strain (Figure). The neighbour-join phylogenetic tree of all the *Lactobacillus* strains was generated by MEGA 11 and indicated that the isolated strains belonged to the genus *Lactobacillus*. All the 16S rRNA gene sequences were submitted to NCBI GenBank with their respective accession numbers.

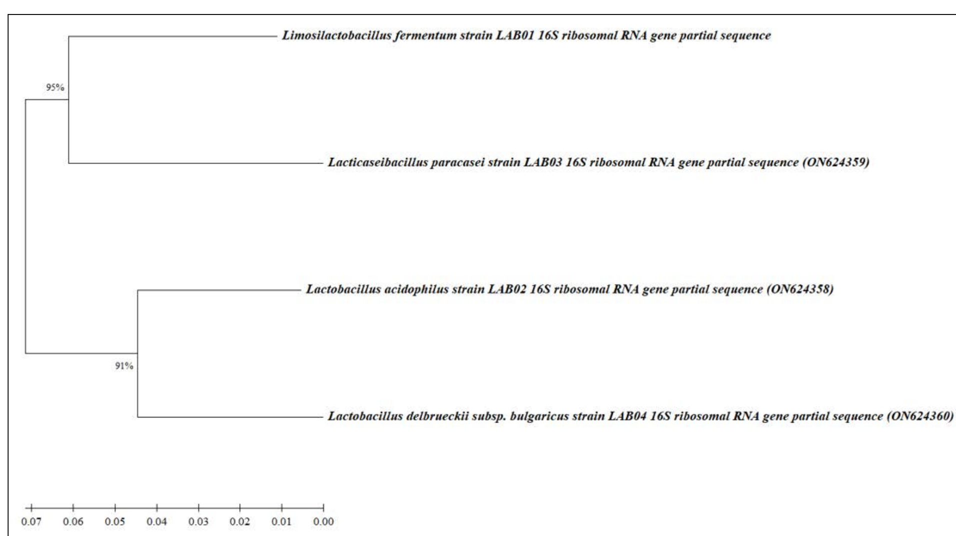

Figure 1: Phylogenetic Tree of All Lactobacillus Strains Obtained by Maximum Likelihood Method (ML) Method Using Mega 11 Software. The Branch Node Number Shows Percent Bootstrap Support. The Accession Numbers of the Organisms are Included in Parentheses and the Bar Scale Value 0.01 Indicate the Nucleotide Substitutions per Site

Strain Improvement Using UV-Mutagenesis

Given isolated were irradiated with UV for different intervals for strain improvement. The graph below represents the number of colonies after a varied time of exposure. From the data, it can be concluded that isolates have a higher survivability rate [24].

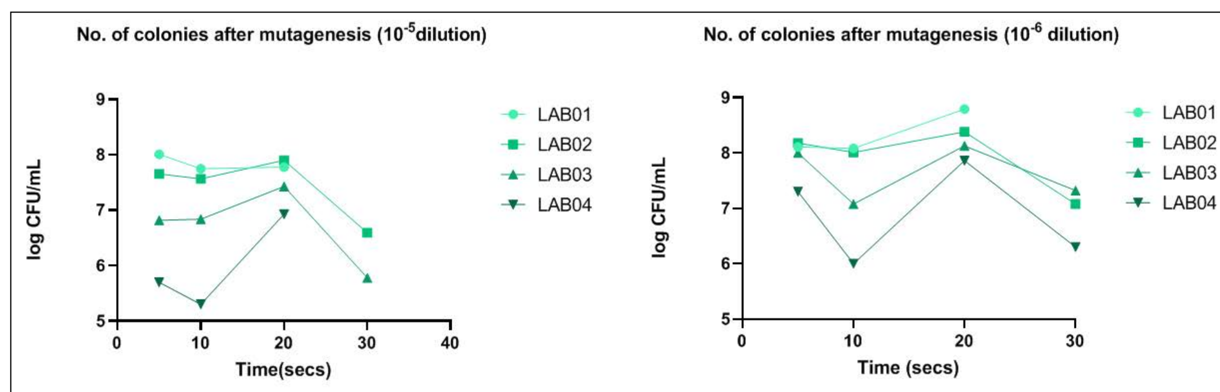

Figure 2: Survival of Isolated Strains After Treatment With UV Endurance to NaCl

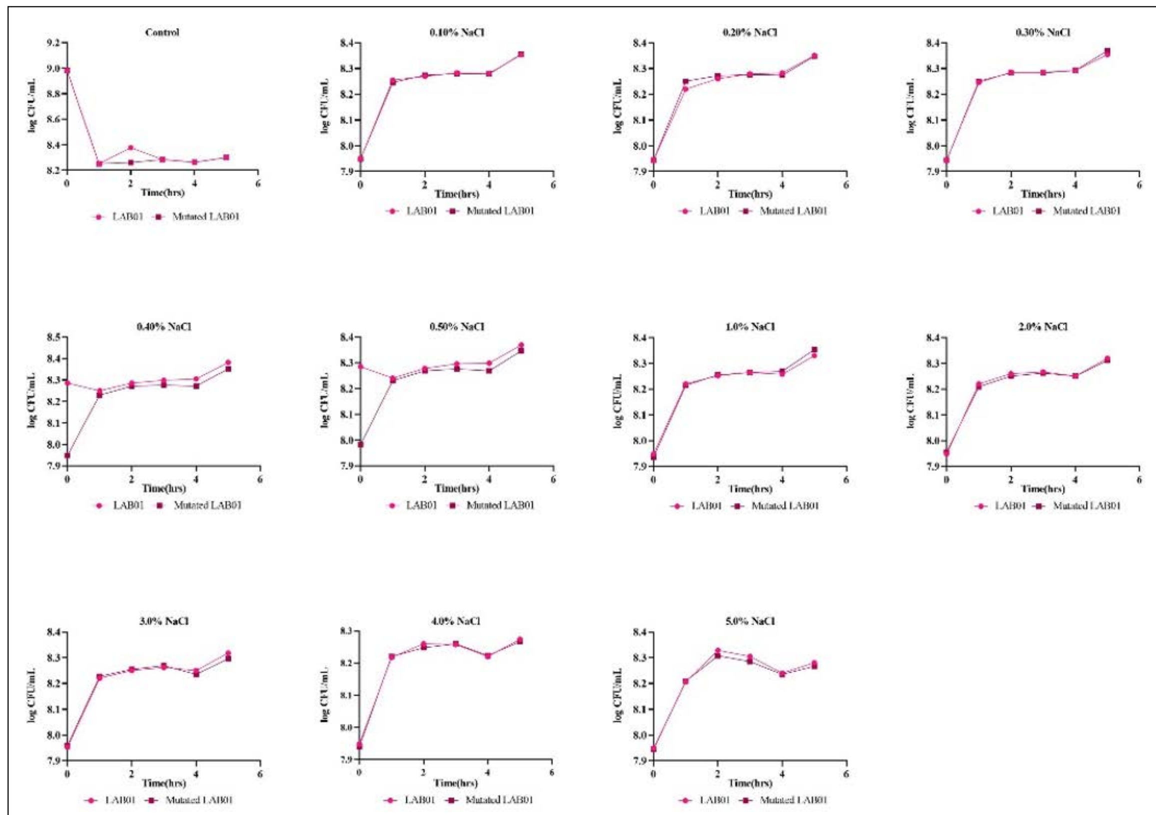

Figure 3: Probiotic Potential Determination of Wild and Mutated Lab01 Strain after Treatment with Varying NaCl Concentrations Ranging from 0.1% to 5%

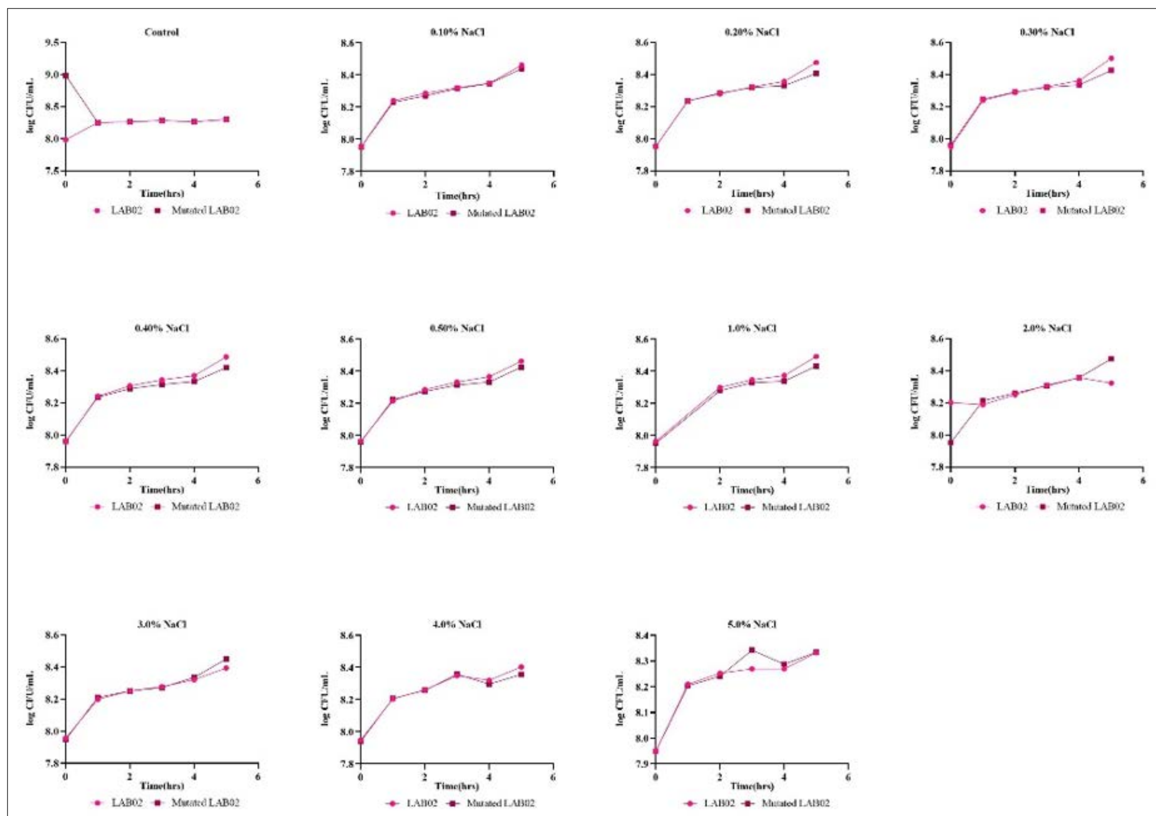

Figure 4: Probiotic Potential Determination of Wild and Mutated LAB02 Strain after Treatment with Varying NaCl Concentrations Ranging From 0.1% to 5%

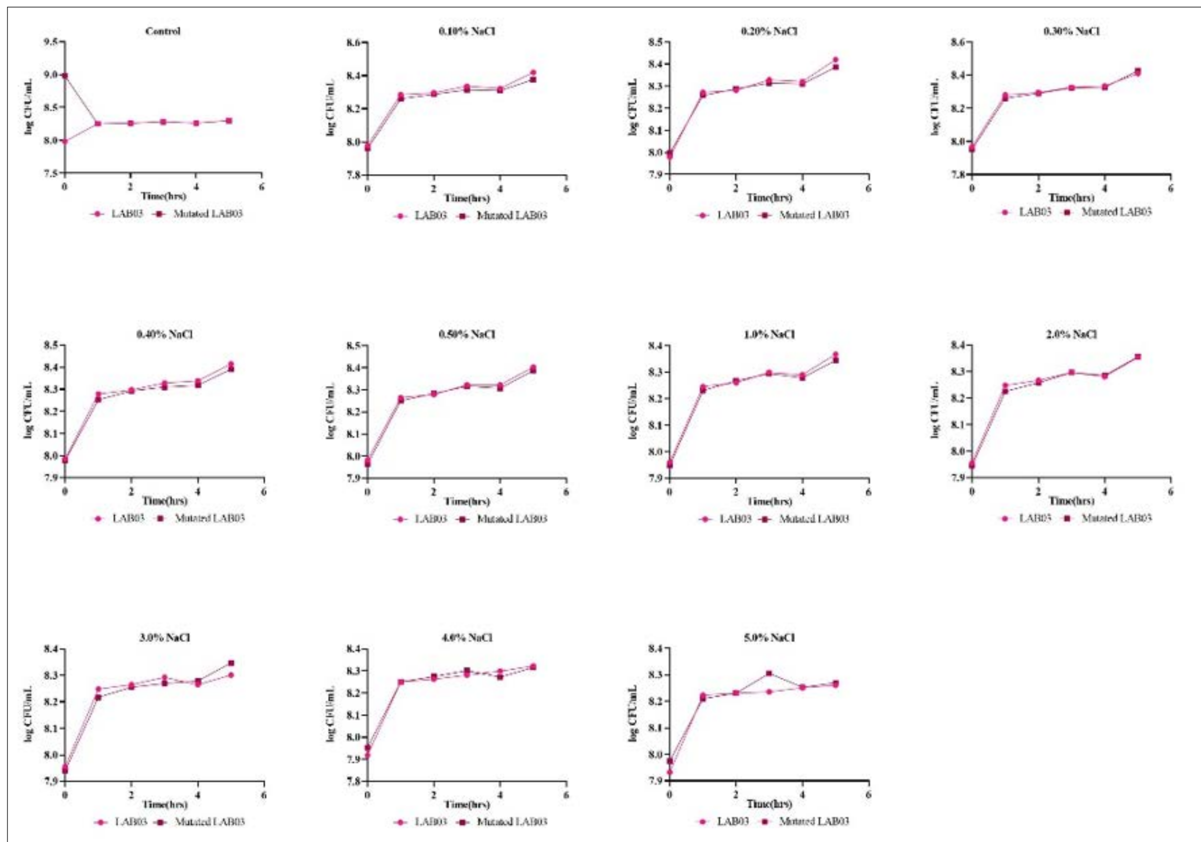

Figure 5: Probiotic Potential Determination of Wild and Mutated LAB03 Strain after Treatment with Varying Nacl Concentrations Ranging from 0.1% to 5%

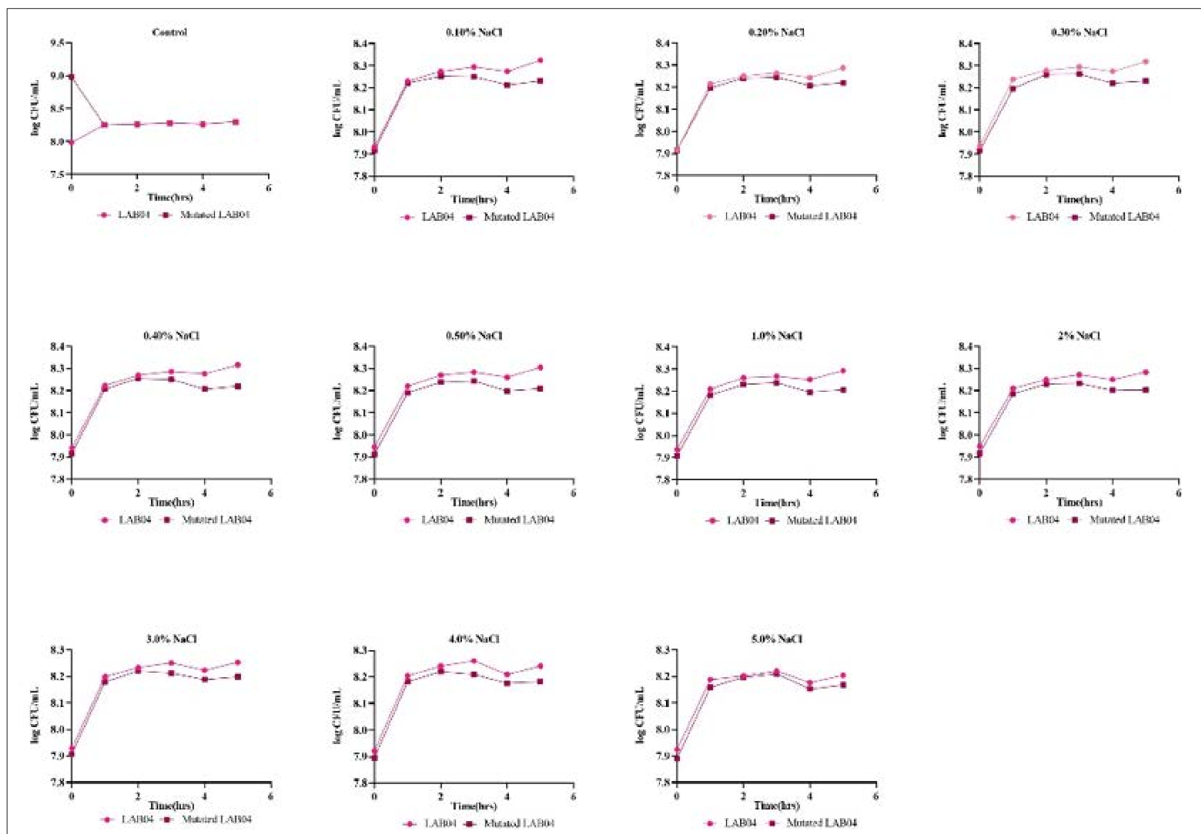

Figure 6: Probiotic Potential Determination of Wild and Mutated LAB04 Strain after Treatment with Varying Nacl Concentrations Ranging From 0.1% to 5%.

The growth of all the mutated LAB01, LAB02, LAB03, and LAB04 strains was observed against that of the originally isolated *Lactobacillus* strain at NaCl concentration range of 0.10% to 5%. As observed from the results in Figure 1-4, up to 0.30% salt concentration very few variations in growth were observed with time wherein, each growth curve of every mutated strain approximately ranged between 1.5*10^8 CFU/mL and 2.5*10^8 CFU/mL. At 0.40% and 0.50% NaCl concentrations, comparatively less growth was observed in the case of all mutated strains and as the salt concentration increased up to 2%, a similar growth pattern was seen in both original and mutated strains except in the case of mutated LAB04 strain wherein decrease in growth was observed till 2% NaCl concentration. At 3-5% NaCl concentrations similar growth pattern was observed for both the control and mutated *Lactobacillus* strains except in the case of the mutated LAB04 strain for which a decrease in growth was observed nearly around 1.5*10^8 CFU/mL at all times intervals. The decrease in growth of the mutated *Lactobacillus* strains and the further increase in growth with time indicates the ability of mutated strains to survive and gain resistance against higher salt concentrations with increasing time.

Phenol Tolerance

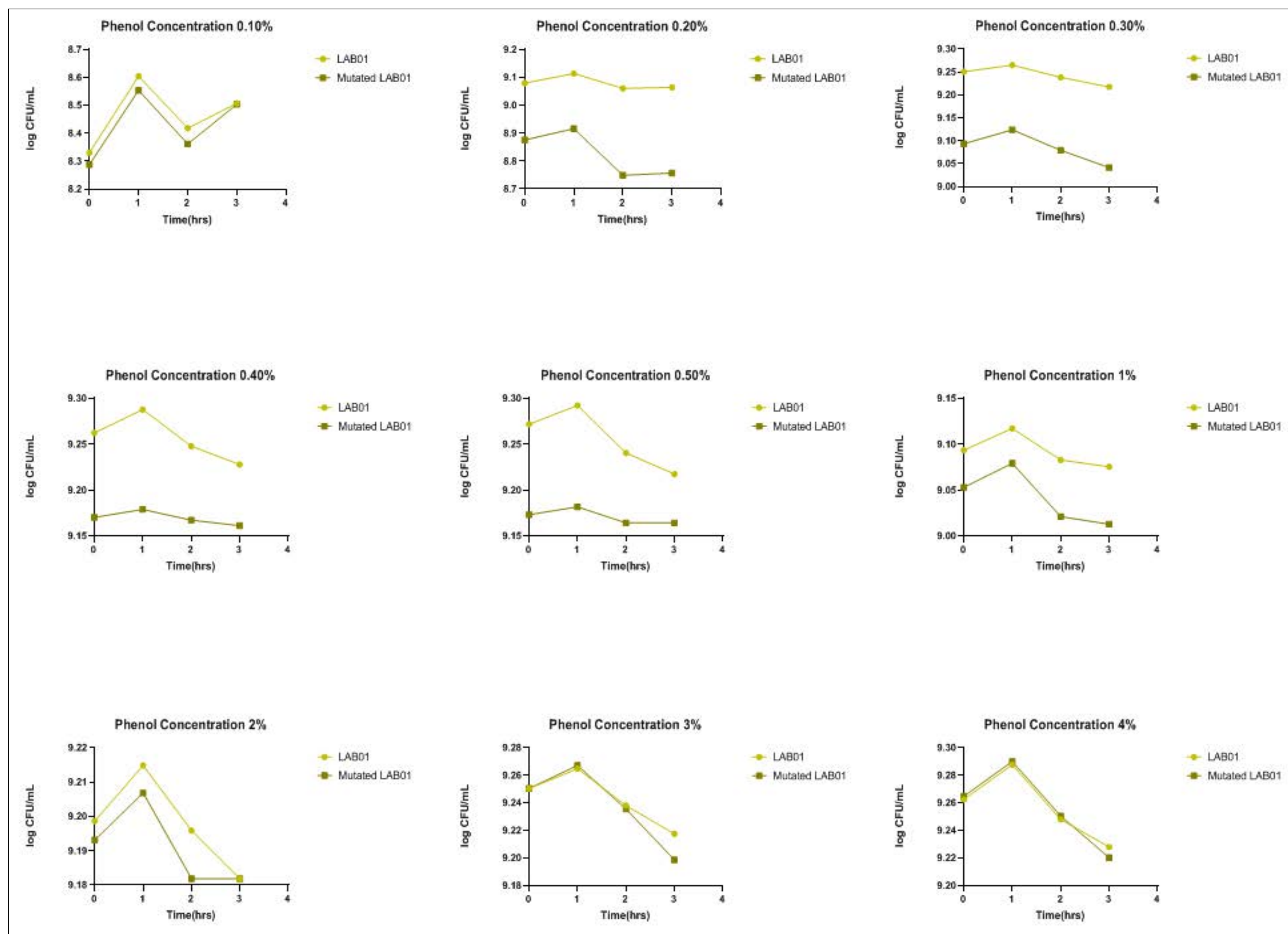

Figure 7: Probiotic Potential Determination of Wild and Mutated LAB01 Strain after Treatment with Varying Phenol Concentrations Ranging From 0.10% to 4%

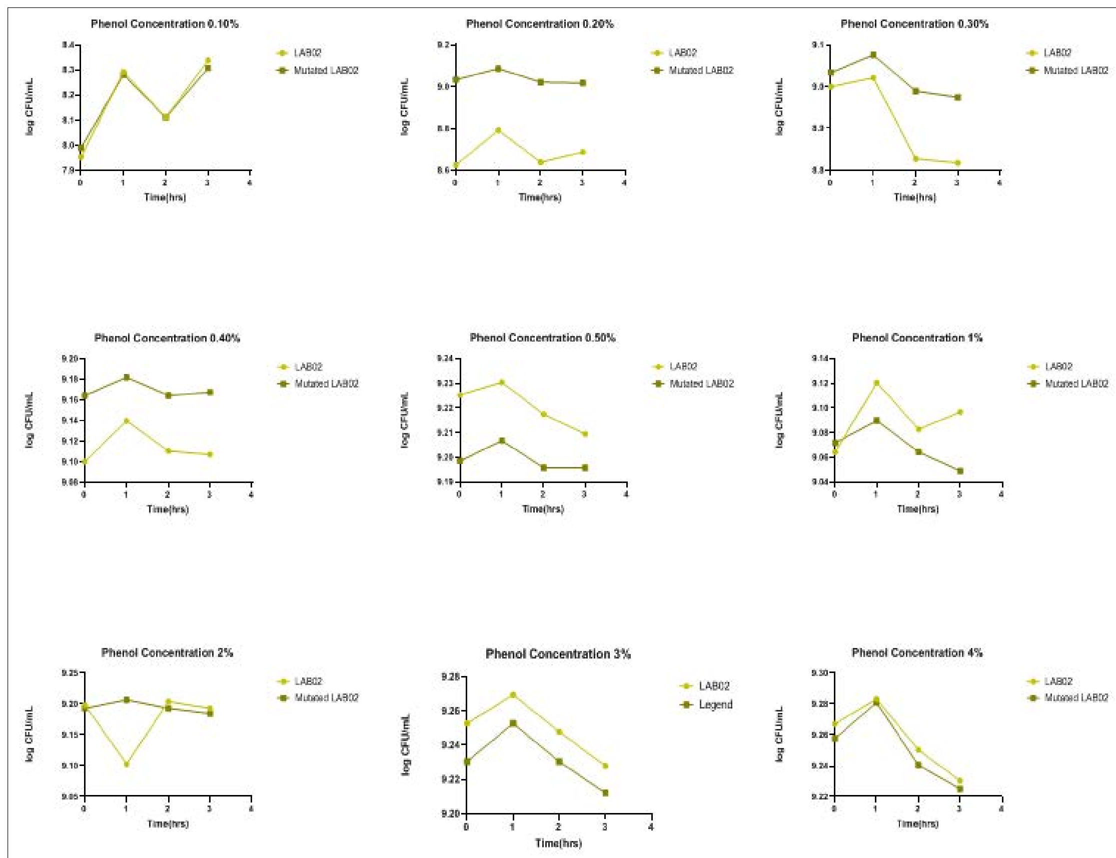

Figure 8: Probiotic Potential Determination of Wild and Mutated LAB02 Strain after Treatment with Varying Phenol Concentrations Ranging From 0.10% to 4%

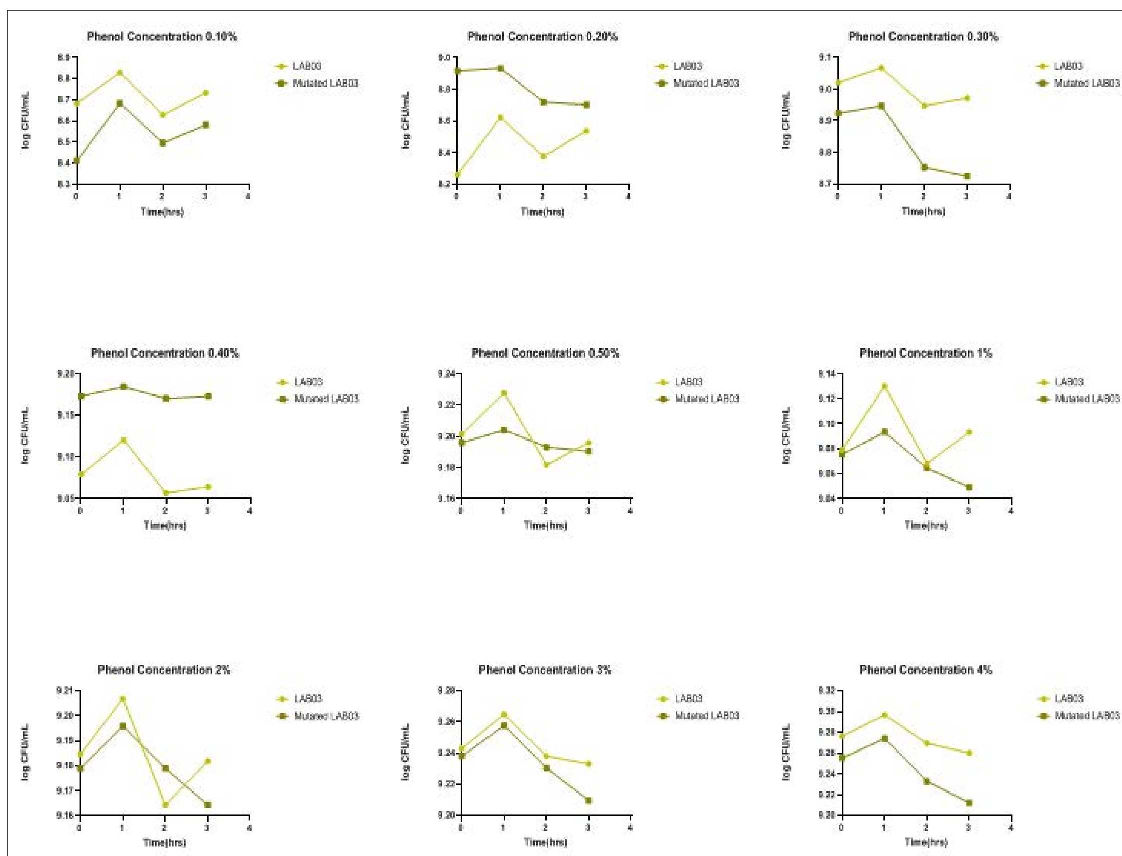

Figure 9: Probiotic Potential Determination of Wild and Mutated LAB03 Strain after Treatment with Varying Phenol Concentrations Ranging From 0.10% to 4%

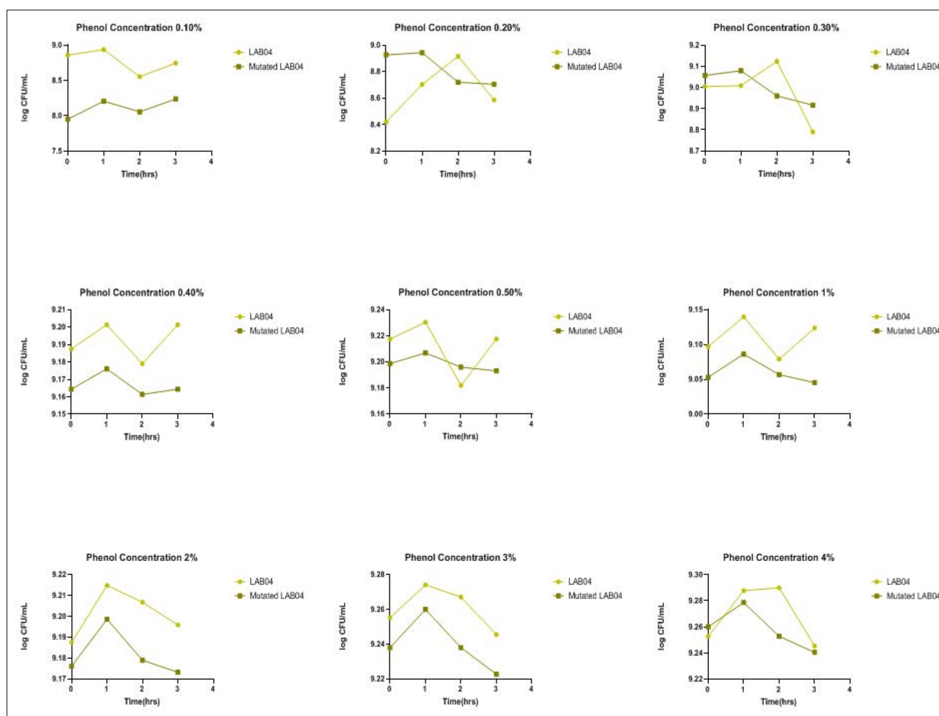

Figure 10: Probiotic Potential Determination of Wild and Mutated LAB04 Strain after Treatment with Varying Phenol Concentrations Ranging From 0.10% to 4%

Phenol tolerance or a probiotic organism is essential so as to survive the gastrointestinal conditions caused by the various reactions involved in the digestion process. Phenols are known to be bactericidal and are derived after the breaking down of dietary proteins [please cite]; surviving in gastrointestinal phenol concentrations is a plus point for probiotic bacteria. The wild and mutated strains were both tested against various phenol concentrations from 0.10% to 4%. The strains survived well in high concentrations (2% to 4%) with a satisfactory amount of CFU/mL. All strains showed maximum survival in the first hour and then gradually decreased over time. Both strains of LAB01 had near 1.9×10^9 CFU/mL in the mentioned high concentrations. LAB02 wild strain showed better activity in 2% phenol concentration with 1.6×10^9 CFU/mL at 1 hour while the mutated strain had the same result at 2 hours. For LAB03 the wild strains showed a slightly higher number of CFU/mL with the average being 1.8×10^9 CFU/mL, than the mutated strains which showed about 1.75×10^9 CFU/mL. The wild-type strain of LAB04 had better survival at high concentrations than the mutated type. It showed 1.65×10^9 CFU/mL, 1.9×10^9 CFU/mL, and 19.5×10^9 CFU/mL in increasing concentrations. The mutated type however had only 1.77×10^9 CFU/mL on average. The wild-type strain of LAB04 could survive at 4% phenol concentration for two hours. According to these results, it is seen that the selected strains gave similar results.

Resilience to Simulated Gastric fluid

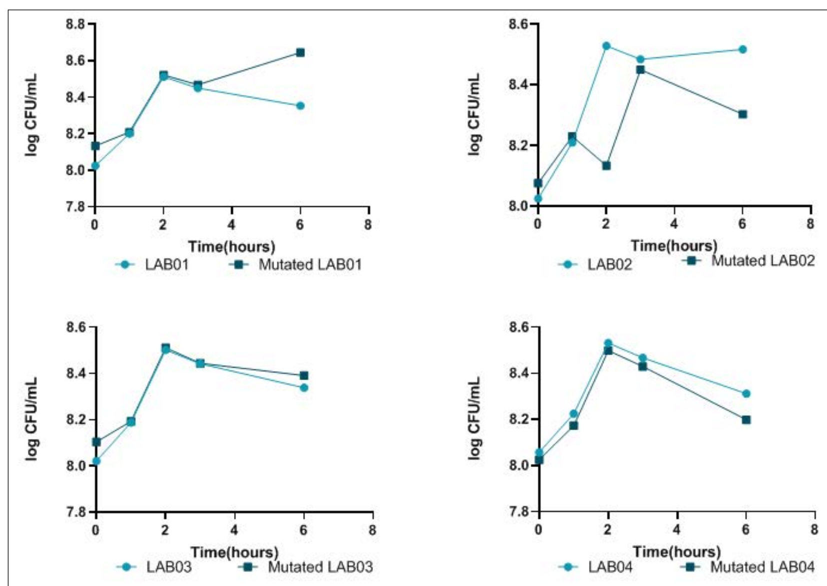

Figure 11: Probiotic Potential Determination of Wild and Mutated Strain after Treatment with Simulated Gastric Fluid

Probiotic bacteria are frequently supplied through food products by passing through the digestive tract towards the stomach via the mouth. As a result, they should be resistant to oral cavity enzymes (e.g., lysozyme) along with the process of digestion in the stomach and lower intestinal tract [25]. The probiotic characterization is therefore also dependable on the degree of resilience towards acid and bile component content in the upper and lower gastrointestinal tract.[26]. Comparative survival analysis between the originally isolated lactobacillus and mutated strains was carried out under the acidic conditions presented by the simulated gastric juice (dissolving 2.0g sodium chloride, 3.2g pepsin, and 7.0mL HCl in 1L of distilled water), pH of 1.2 for over 360 minutes (6 hours) [27]. As shown in figure (x), initial exposure of isolated and mutated LAB01 to the simulated gastric juice stabilized the cell value at 8.2 log CFU/mL followed by a steady increase up to 8.6 log CFU/mL. However, with an increase in the exposure rate, the LAB01 isolate showed a rather decrease in the cell value whilst the mutated LAB01 strain presented an increase up to 8.6 log CFU/mL. Both LAB03 and LAB04 isolates showed similar growth patterns to the LAB01 isolate. Although a cell value of 8.5 log CFU/mL was showcased during 2nd-hour exposure to the simulated gastric juice, a sudden decline was observed for the LAB isolates, whereas the mutated strains showed opposite results during the extended exposure [28]. Conversely, LAB02 isolate on exposure to the simulated gastric juice showed a better survival pattern when compared to the mutated LAB02. Whilst other LAB isolates showed a decreased survival rate in the population following a 2nd-hour exposure to the simulated gastric juices, the LAB02 isolate presented a slow but steady surge of the growth rate to 8.5 log CFU/mL. Parallelly, the mutated LAB02 strain showed a disproportionate growth pattern with an increased exposure time of the simulated gastric juice. The results were very similar to those reported by commenting on the low pH tolerance of *L. acidophilus* [29]. The pH of the human stomach tends to vary from 1.0 during fasting to 4.5 after a meal, and all of the strains tested were resistant to synthetically produced gastric juice, pH of 1.2 after 3 to 4 hours. Overall, the data revealed that Lactobacillus species exhibit distinct survival rates, which are also evident at the strain level.

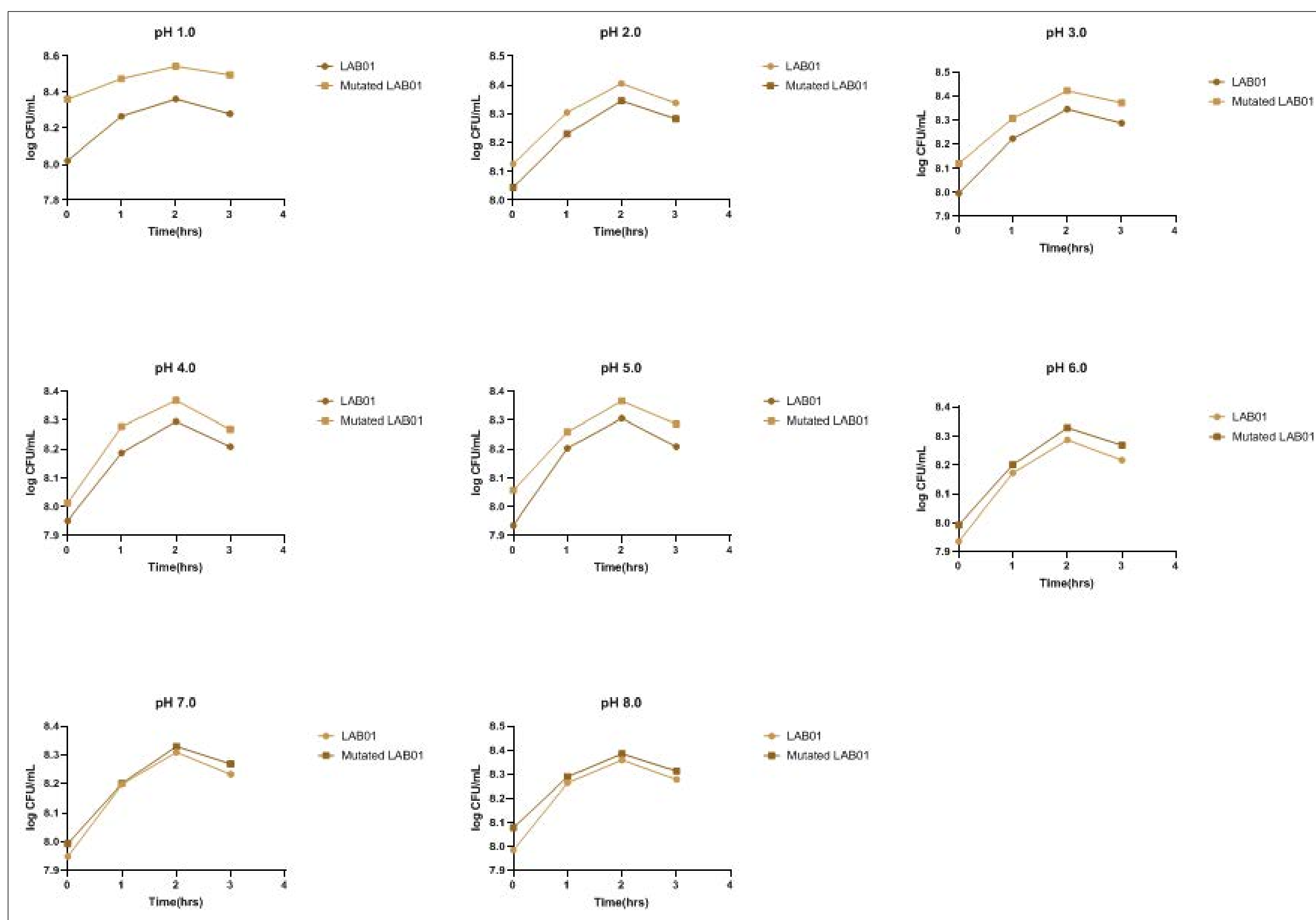

Figure 12: Probiotic Potential Determination of Wild and Mutated LAB01 Strain after Treatment with Varying Ph Concentrations Ranging From 1 to 8

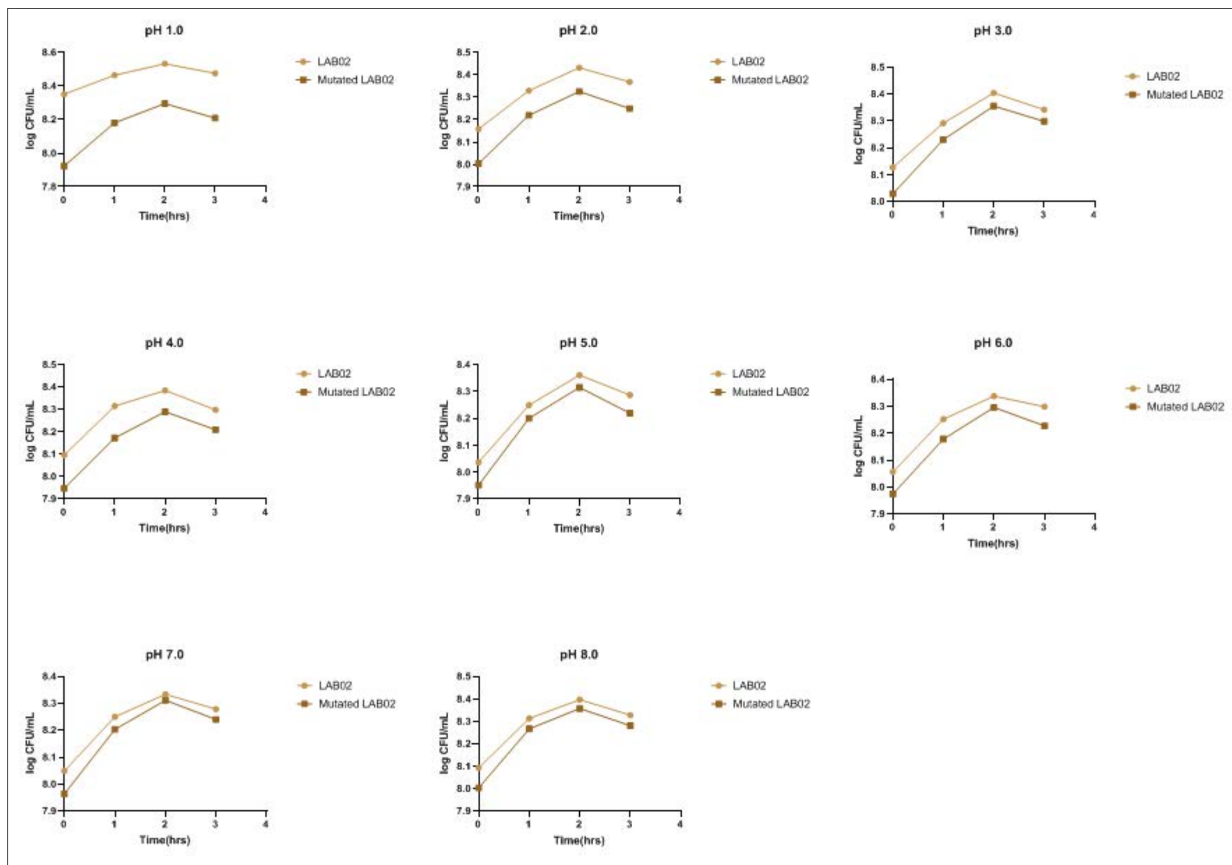

Figure 13: Probiotic Potential Determination of Wild and Mutated LAB02 Strain after Treatment with varying pH Concentrations Ranging from 1 to 8

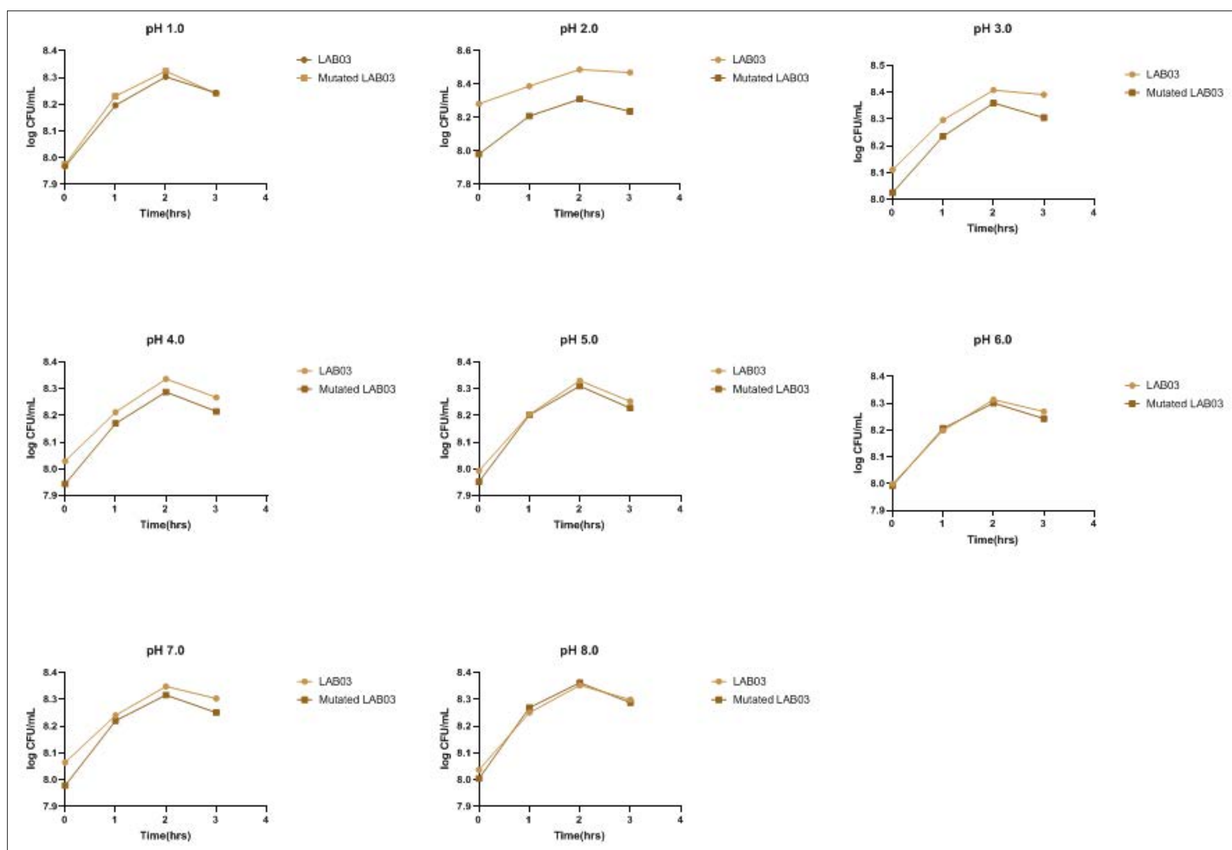

Figure 14: Probiotic Potential Determination of Wild and Mutated LAB03 Strain after Treatment with varying pH Concentrations Ranging from 1 to 8

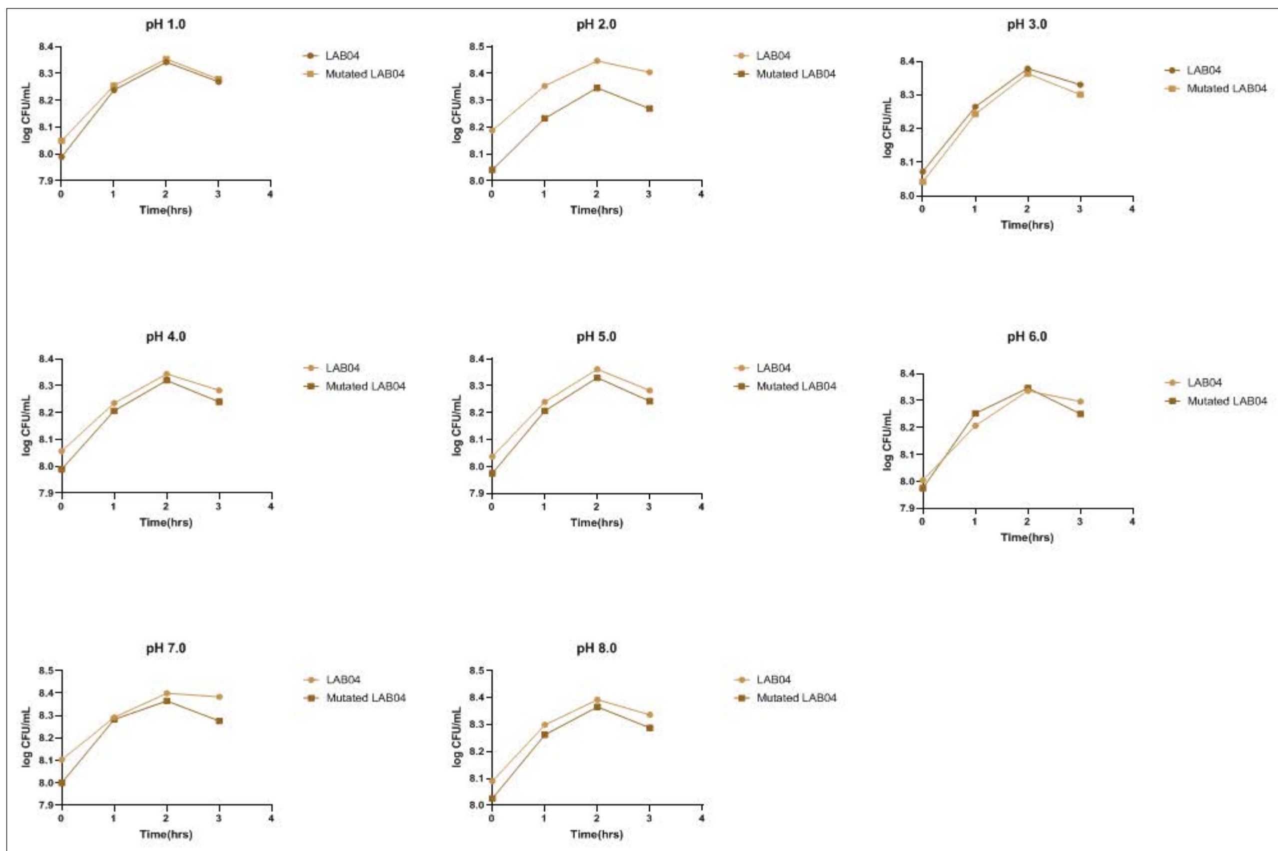

Figure 15: Probiotic Potential Determination of Wild and Mutated LAB04 Strain after Treatment with varying pH Concentrations Ranging from 1 to 8

Probiotic bacteria are considered to play a vital role to promote and maintain gut health in the host. However, to confer the benefits, they have to pass through the unfavourable physiological conditions of the gastrointestinal tract and then persist in the gut [30]. Therefore, to be utilized as potential probiotics, microbial strains need to be screened for their capacity to tolerate variable pH conditions in the gastrointestinal tract. In the current study, to assess pH endurance, a comparative analysis of wild-type, as well as randomly mutated Lactobacillus strains, was carried out under controlled conditions. According to the Figures this displays tolerance at an extreme pH range of 1 to 3. Apart from mutated LAB01, which showed better endurance at pH 1 to 3 than its wild type, all other mutated strains of LAB demonstrated slightly lower tolerance to the given pH range, when compared to their original forms. Furthermore, considering the pH range between 4 to 6, which is represented in the Figures above illustrates that the same pattern was followed by mutated LAB01strain, which exhibited high growth, showing its resistance to the mentioned pH range, whereas, mutated LAB04 demonstrated better tolerance compared to its non-mutated strain and mutated LAB03, revealed resistance that is equivalent to its wild type strain. In addition to the capability of the strains to resist acidic conditions, the given bacterial variants were also tested at neutral (pH-7) and alkaline conditions (pH-8). In the neutral and alkaline environment again mutated LAB01 showed better growth results in terms of log CFU/mL compared to its counterparts, except LAB03, which demonstrate almost similar endurance at pH 8 to its mutated form. Previous studies have provided enough evidence that consumption of probiotics not only promotes intestinal health but also has a beneficial influence on dental caries, diabetes (type-2), and Osteoporosis, enhances the immune response and has also been reported to play a positive role in cancer prevention [31].

Resistance to Antibiotics

Table 4: Antibiotic Susceptibility and Resistance Profile for LAB Isolates

ANTIBIOTICS			LAB ISOLATES Zone of inhibition in mm							
Sr.no.	**Name**	**Conc. in µg**	**LAB01**	**Mutated LAB01**	**LAB02**	**Mutated LAB02**	**LAB04**	**Mutated LAB04**	**LAB03**	**Mutated LAB03**
1.	Ceftriaxone	10	2.8	2.4	2.3	**R**	2.8	**R**	2.5	2.8
2.	Cefoperazone	50	2.6	1.9	2.0	**R**	2.6	**R**	2.6	2.4
3.	Ampicillin	25	2.9	2.5	2.7	**R**	2.8	**R**	3.0	2.2
4.	Tetracycline	10	1.5	1.5	1.6	**R**	1.8	**R**	1.6	1.7
5.	Trimethoprim	30	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
6.	Ciprofloxacin	30	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
7.	Ofloxacin	2	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
8.	Nalidixic acid	30	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
9.	Vancomycin	30	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
10.	Gentamicin	10	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
11.	Amikacin	10	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
12.	Chloramphenicol	30	2.9	2.6	2.8	**R**	3.0	**R**	2.6	2.8
13.	Levofloxacin	5	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
14.	Erythromycin	10	2.7	2.4	**R**	**R**	2.6	**R**	2.9	2.4

A multitude of antibiotics were used to test the antibiotic susceptibility of the four identified LAB isolates. Trimethoprim, Ciprofloxacin, Ofloxacin, Nalidixic acid, Vancomycin, Gentamicin, Amikacin, and Levofloxacin resistance was found in all isolates. LAB01 and Mutated LAB01 were reported to be vulnerable to Ceftriaxone, Cefoperazone, Ampicillin, Tetracycline, Chloramphenicol, and Erythromycin. In the case of LAB02, a zone of inhibition was seen for Ceftriaxone, Cefoperazone, Ampicillin, Tetracycline, and Chloramphenicol (2.3, 2.0, 2.7, 1.6mm respectively), but mutant LAB02 is impervious to all antibiotics. Trimethoprim, Ciprofloxacin, Ofloxacin, Nalidixic acid, Vancomycin, Gentamicin, Amikacin, and Levofloxacin resistance was observed in LAB03 and mutated LAB03. Mutant LAB04 is resistant to all antibiotics whereas LAB04 is sensitive to Ceftriaxone, Cefoperazone, Ampicillin, Tetracycline, and Chloramphenicol. A zone of inhibition of 2.6 and 2.8mm was observed in LAB04 and mutated LAB04 respectively for Chloramphenicol. From the above table, it can be concluded that LAB02, LAB03, LAB04, and mutated LAB02 and LAB04 showed antibiotic resistance, whilst mutated LAB01 and LAB03 demonstrated antibiotic sensitivity.

Conclusion

According to the findings of this study, numerous fermented foods are a source of lactic acid bacteria. Further research of isolates for their probiotic potential on characteristics such as inhibitory substance tolerance, antibiotic resistance, and extracellular antimicrobial production suggests that all isolates are promising probiotics. The altered strains have not proven to be significantly superior to their pure counterparts. As a result, as this research progresses, alternative mutagenesis procedures, such as site-directed mutagenesis, can be used to produce controlled and significant changes, resulting in altered strains that outperform pure strains. Aside from that, more than one modified bacterial colonies can be isolated, cultured, and tested for various tolerances to have a diverse spectrum of higher-functioning mutant strains. Other assays, such as aggregation, co-aggregation, biofilm formation, anti-biofilm formation, tolerance to different temperatures, and the time required for curdling milk, secondary metabolite profiling, FAME profiling, impact on cholesterol reduction, impact on fermentation, and so on, can be performed to gain a better understanding of a bacterial strain's ability to function as a probiotic [32, 33].

Author Contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability Statement

1. All data generated or analyzed during this study are included in this published article.
2. All the DNA sequences were published in NCBI Database

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