https://doi.org/10.48047/AFJBS.4.4.2022.294-325

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Isolation and genetic profiling of lactic acid bacteria from traditional fermented foods of assam, northeast india

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Volume 4, Issue 4,2022

Received: 10 Nov 2022

Accepted: 01 Dec 2022

Published: 22 Dec 2022

doi:10.48047/AFJBS.4.4.2022.294-325

Abstract

This research explored various lactic acid bacteria (LAB) strains isolated from traditional fermented foods, including Khorisa, Napham, Hentak, and Soibum. Four LAB strains were chosen for their significant potential as starter cultures and probiotics: Khorisa (28), Berma (B4), Hentak (HTK-5), and Napham (NAP-2). The isolates underwent gram staining, biochemical tests, and physiological evaluations at different pH levels and temperatures using MRS media. The probiotic capabilities of these LAB strains were further examined. All four strains were gram-positive, and most tested positive for proteolytic, lipolytic and carbohydrate fermentation assays, while being negative for hemolytic, catalase and oxidase tests. The strains Khorisa (28), HTK-5, B4, and NAP-2 showed growth at alkaline pH levels (8-11) after 12 hours of incubation at 37°C. They all exhibited acid tolerance (pH 2-3) upto 5 hours but only HTK-5 maintained this tolerance for 12 hours. The LAB strains were tested against 12 antibiotics, showing inhibition zones between 2-4 cm. All the strains were checked for their adherence under in vitro conditions. Due to their strong probiotic properties and fermentation potential, these strains were further investigated and used in developing probiotic food products like curd. Thus, this study confirms the LAB strains as safe and effective probiotic candidates.

Keywords

Lactic Acid Bacteria, Fermented foods, Probiotic potential, Starter culture, Health benefits

1. Introduction

The Northeast region is the eastern-most area of India, known for its rich diversity of traditions. The seven sister states of Northeast—Arunachal Pradesh, Meghalaya, Manipur, Assam, Mizoram, Tripura and Nagaland—are renowned for its diverse populations and ethnic backgrounds. These ethnic communities have traditionally included fermented foods as a fundamental part of their diets (Das et al., 2016). Commonly fermented foods in these communities include soybeans, bamboo shoots, vegetables, fish, and meat. Fermentation

process is considered as the most economical and ancient methods for preparation of food globally, where growth and metabolic activities of microbes assist in food preservation (Nuraida, 2015; Wilburn & Ryan, 2017). Fermentation is used worldwide for preserving a wide range of agricultural products such as fruits, vegetables, cereals, roots, tubers, milk, fish and meat. It is widely practiced in rural areas to produce a variety of beveragesand fermented products, also serving as a source of income for them (Cooke, 1987; Das et al., 2016). Fermentation can occur through two processes: spontaneous and non-spontaneous/traditional. Spontaneous fermentation involves microbes naturally present in the air or materials, whereas non-spontaneous fermentation uses isolated strains of bacteria from spontaneously fermented products. Increase in growth and metabolic actions of microorganisms enhance the flavors, texture, nutrition, and aroma of fermented products(Law et al., 2011). Fermented foods are rich in probiotic microbes that improve the health of consumers. The primary probiotic organisms in fermented foods are lactic acid bacteria, which increase the shelf life of food, modify carbohydrate content, synthesize amino acids, and increase the availability of iron, zinc, and calcium while producing bioactive compounds (Blandino et al., 2003; Muro Urista et al., 2011). LAB are non-pathogenic organisms typically involved in spontaneous food fermentation. They are usually gram- positive, non-sporulating, catalase-negative, anaerobic but also aero-tolerant in nature. Different genera of LAB such as Lactobacillus, Bifidobacterium, Pediococcus,Staphylococcus, Lactococcus, Enterococcus, and Weissella are found in fermented foods (Axelsson et al., 2012; Tamang et al., 2016a). The Lactobacillus genus is the largest group present in these foods (Canchaya et al., 2006). LAB are highly probiotic in nature and exist in various habitats such as the oral cavity, gastrointestinal tract, human and animal vaginal tracts, silages, and composts(Endo et al., 2019). Probiotics, live organisms that provide health benefitsto their host, are recognized for their potential to inhibit pathogenic growth, reduce toxin secretion, and enhance nutritional value. These organisms adhere to epithelial cells and secrete antimicrobial compounds. Effective probiotic isolates should survive and colonize under various temperatures, withstand low pH levels, resist bile salts, exhibit antimicrobial activity, and possess antibiotic resistance and anticancer properties (Bartkiene et al., 2018; Palachum etal., 2018).

2. Materials and Methods

2.1 Sample Collection

Samples from various states of Northeast India, were collected in clean sterile vessel. The samples are being tabulated in Table 1.

Table 1. Samples collected along with its location

Serial number	Sample collected	Location
1.	Khorisa	Assam
2.	Berma	Tripura
3.	Hentak	Manipur
4.	Napham	Assam

2.2 Isolation of the strains

Serial dilution of samples was done in aseptic condition as described by (Blodgett, 2005). Spread plates were prepared with the diluted samples and kept overnight at 37 . Colony morphology of the spread plates was done on basis of its size, shape, colour, texture, form, elevation etc. Each colonies showing different colony type were selected and streaked on plate to fine the pure isolates by keeping overnight at 37 C.

2.3 Screening of starter culture strains by performing proteolytic, lipolytic and carbohydrate fermentation test

2.3.1 Proteolytic Test

Proteolytic test is used to determine microbe's ability the breakdown of protein. The steps of the test are as follows: The proteolytic activity of the microbes was accessed qualitatively using 25g skim milk agar as the substrate for 1000ml distilled water. The cultures were streaked on prepared sterile media plate and incubated at 37°C, overnight. A clearance zone forming around the colonies showed that the microbes can integrate casein, and hence has proteolytic activity as described by (Mazhar et al., 2020).

2.3.2 Lipolytic Test

Lipolytic test is used to determine the microbe's ability to degrade lipid compound. The steps of the test are as follows: The lipolytic activity of the microbes were accessed using Tributyrin Agar whose composition include Peptone, Yeast Extract and Agar (23g in 990ml distilled water) to which 10ml of Tributyrin (FD081) was added as described by (García-Cano et al., 2019). Then the inoculating loop was used for spot inoculation on a prepared sterile media

plate and incubated at 37°C for overnight. Formation of any clearing zone were checked further.

2.3.3 Carbohydrate Fermentation Test

Carbohydrate Fermentation test shows ability of microbes to ferment. The steps of the test are as follows: 1% of sugars such as Sucrose, Lactose, Glucose were mixed to 5ml pre sterilized peptone water containing phenol red indicator. The culture of the isolates was added to the media as described by (Tamang et al., 2016b). It was incubated for 48 hr and production of acids was observed.

2.4 Morphological Characterization of the isolated strains

2.4.1 Simple staining

Clean glass slides were taken. Few drops of sterile distilled water were poured on centre of the slide. Single colony of isolates was picked from the culture plate using sterile loop and thin smear of culture was made on slide. It was air dried and heat fixed. After, crystal violet solution was added, left for 5 min and rinsed with distilled water. The slide was air dried and observed under microscope.

2.4.2 Gram staining

Cleaned grease free glass slides were taken. Few drops of water were poured on the clean slide. A loop of single isolated colony was taken and thin smear of culture was made. It was air dried and heat fixed. After, crystal violet solution was added on the smear and left for 1 min. Crystal violet was discarded using slow running distilled water. Then Gram's iodine was added and left for 1 min. Rinsed with decolourizer i.e., is 95% of ethanol. Counter stain Safranin was added to the smear and kept for 1min and washed off using distilled water. It was left to dry and observed under microscope.

2.5 Biochemical Characterization

2.5.1 Indole Test

Indole test is used to check the capability of organism to degrade amino acid tryptophan as described by (MacWilliams, 2009). The steps of the test are as follows: LAB isolates were inoculated in sterile peptone water and kept for incubation at 37ºC for 24 hours. After the incubation, 0.5 ml of Kovac`s reagent was mixed and the color change in media was observed.

2.5.2 Methyl Red (MR) Test

Methyl Red (MR) test is used to detect the production of acid forming colouration of red, orange, yellow. The steps of the test are as follows: 5ml of glucose phosphate peptone water was filled to sterile test tube. It was inoculated with LAB culture under sterile condition and incubated at 37ºC for 48 - 72 hr as described by (MacWilliams, 2009). after incubation period, few drops of methyl red solution were added and colour change in the media was observed.

2.5.3 Voges Proskauer (VP) Test

Voges Proskauer (VP) test is used to detect if an organism produces acetylmethylcarbinol from glucose fermentation as described by (MacWilliams, 2009). The steps of the test are as follows: LAB culture was inoculated on prepared MR-VP broth media and incubated at 37°C overnight. After 0.6ml of 5% of α-naphthol and 0.2 ml of 40 % KOH was added to it respectively. Tubes were shaken gently and left undisturbed for 10-15 min. The colour change was observed in case of positive test.

2.5.4 Citrate utilisation Test

Citrate utilisation test is to determine the ability to degrade citrate. The steps of the test are as follows: Simmons citrate agar slant was prepared. The next day slant was inoculated with a culture using loop without stabbing the slant. Cotton plugged the tube with culture. Afterwards, it was incubated aerobically at 35°C-37°C overnight as described by (MacWilliams, 2009). Thereafter, color change of the slant was observed.

2.5.5 Catalase Test

Catalase test is used to identify if the organism produces enzyme catalase that detoxify the hydrogen peroxide (Oyeleke, S. B., and Manga, S. B. (2008)). The steps of the test are as follows: Initially, clean grease free slide was taken. Few drops of 3% hydrogen peroxide (H_2O_2) were taken on it. The culture was added into the drop of H_2O_2 and observed for bubble and froth formation.

2.5.6 Oxidase Test

Oxidase test is used to check if the organism possesses cytochrome oxidase enzyme that perform phosphorylation in mitochondria. The steps of the test are as follows: On a clean glass slide an oxidase disc was placed. Few drops of growth culture of the isolates were added to it and observed for colour change (Oyeleke, S. B., and Manga, S. B. (2008)).

2.6 Preparation of curd and its sensory evaluation

Curd preparation began with boiling milk, which was then transferred to culture tubes under aseptic conditions to maintain sterility. Selected lactic acid bacteria strains were inoculated into the milk, starting the fermentation process essential for curd formation (Borthakur et al., 2024). The inoculated culture tubes were incubated at 37°C overnight, allowing the bacteria to proliferate and ferment the milk. This fermentation converts lactose into lactic acid, resulting in the characteristic texture and flavor of curd. Maintaining aseptic conditions during milk transfer and inoculation, along with a controlled incubation period, ensures a consistent curd preparation process. The use of specific lactic acid bacteria strains enhances the final product's quality, ensuring desirable characteristics in the curd. This careful approach ensures a reliable and high-quality curd preparation.

2.7 Screening of probiotic potential of the isolated strains

2.7.1 Effect of Temperature

LAB culture was inoculated on prepared MRS (De Man-Ragosa-Sharpe) media and incubated at different temperatures (4°C, 28°C, 32°C, 37°C & 45°C) in a shaker operating at 150 rpm. The OD of acid production was recorded after 12 hr using spectrophotometer at 600 nm. The result was estimated by comparing the OD of the culture at 0hr & 12hr (Helen Shiphrah et al., 2013).

2.7.2 Effect of pH

For optimizing the effect of pH, MRS (De Man-Ragosa-Sharpe) media were adjusted at different pH (2, 5, 7, 9, 11). It was inoculated with culture and incubated in 37°C shaker operating at 150 revolutions per minute. The Lactic acid production was checked for each pH after 12 hr(Helen Shiphrah et al., 2013). The result was estimated by comparing the OD of the culture at 0hr & 12hr.

$$\text{Survival (\%)} = [OD_{600}(12hr)/OD_{600}(0hr)] \times 100\%$$

where $OD_{600}(12hr)$ describes the optical density at 600 nm at 12 hours and $OD_{600}(0hr)$ describes the optical density at 600 nm at 0 hours.

2.7.3 NaCl tolerance

The survival % of LAB in high percent of NaCl was assessed as described by (Abdel Tawab et al., 2023). Firstly, MRS broth containing 8% NaCl was prepared. Overnight LAB culture was inoculated on the broth and incubated at 37˚C for 6 hr. Optical density (OD) of the

inoculated broth was measured before and after 6hr of incubation using spectrophotometer at 600 i.e. OD of 0hr and 6hr to check the viability of the LAB (Fig. 1).

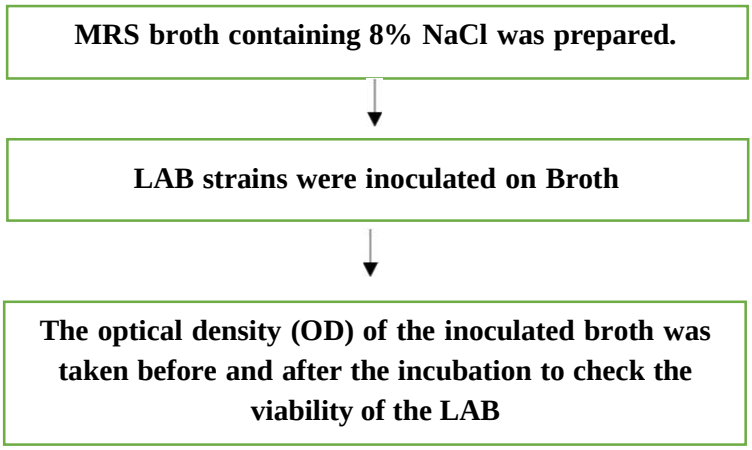


Figure 1. Process of NaCl tolerance

2.7.4 Phenol tolerance

The phenol tolerance capability of the isolates was determined as described by (Abdel Tawab et al., 2023). Initially, MRS broth with 0.2% of phenol was prepared and inoculated with the overnight LAB culture. It was incubated at 37˚C for 24 hr (Fig. 2). The OD of the inoculated broth at 0hr and after 24hr were taken using spectrophotometer at 600nm.

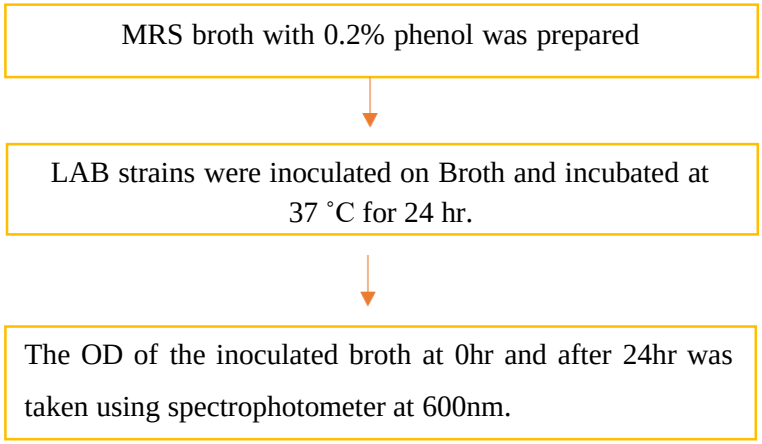


Figure 2. Process of Phenol tolerance

2.7.5 Antibiotic susceptibility test

Antibiotic susceptibility of the isolated LAB was checked using Disc diffusion method (Abdel Tawab et al., 2023). Firstly, MRS agar plate was prepared. The adequate amount of samples

were poured on the prepared media plate under the sterile condition of Laminar. The plates were swirled well with the media and incubated for half an hour. After the incubation the excess sample was pipetted out. Thereafter, the selected antibiotic discs were drop carefully on the surface of the media plate using sterile forecep. These plates were incubated invertedly at 37°C overnight. Next day, diameter of clearing zone around disc was assessed. Afterwards, the results found were interpreted according to the provided guidelines. Antibiotic applied in this study are: Gentamicin (50mcg), Moxifloxacin (5mcg), Ciprofloxacin (5mcg), Amikacin (30mcg), Linezolid (30mcg), Chloramphenicol (50mcg), Ampicillin (2mcg), Imipenem (10mcg), Azithromycin (30mcg), Neomycin (30mcg), Levofloxacin (5mcg).

2.7.6 Auto-Aggregation

The auto-aggregation property of the LAB isolates were assessed as described by (Abdel Tawab et al., 2023; Bindu & Lakshmidevi, 2021). Each isolated LAB strain was harvested by centrifugation of the overnight culture at 8000 rpm for 10 min at 4°C. The pallet formed was washed with PBS buffer twice. PBS buffer was resuspended to it and incubated anaerobically at 37°C for a while to get the upper suspension. The upper suspension was collected to check its absorbance at 600nm.The absorbance of the sample was taken at different interval of 5hr.

$$\text{Auto aggregation (in \%)} = [(A_0 - A_{\text{time}})/A_0] \times 100$$

where A_{time} is the OD at specific time interval and A_0 is the OD at 0 time.

2.7.7 In vitro adhesion to chicken crop epithelial cells

The adhering abilities of the LAB isolates to the epithelial cells was checked as described by (Somashekaraiah et al., 2019). The chicken crop was collected from local market in the sterile falcon. It was transferred in a sterile petri plate, washed 2-3 times using distilled water and store in PBS at 4°C for 30 min. After the crop was washed 3 times with potassium phosphate buffer (pH-7.4) to remove surface mucus of epithelial cells. After washing it was transferred to another sterile petri plate and scrap with sterile cover slip to get the epithelial cell. It was then resuspended with PBS. 400 µl of epithelial cells were pipette out in the sterile microcentrifuge and 100 µl of LAB sample were added to it. Microcentrifuge tube was incubated at water bath for 30 min at 37°C, centrifuged at 3000 rpm at room temperature for 10 minutes and washed the pellet with PBS twice. The pellet formed was resuspended in PBS. Thereafter, it was stained with crystal violet and observed under the microscope.

2.8 Safety evaluation of LAB strain

2.8.1 Hemolytic test

The LAB isolates were streaked on the sterile blood agar plate containing 5% of sheep blood and incubated at 37°C for 48 hr and observed the clearing zone around the colonies denoting blood lysis as described by (Ryan and Ray (2004)).

2.9 Molecular characterization

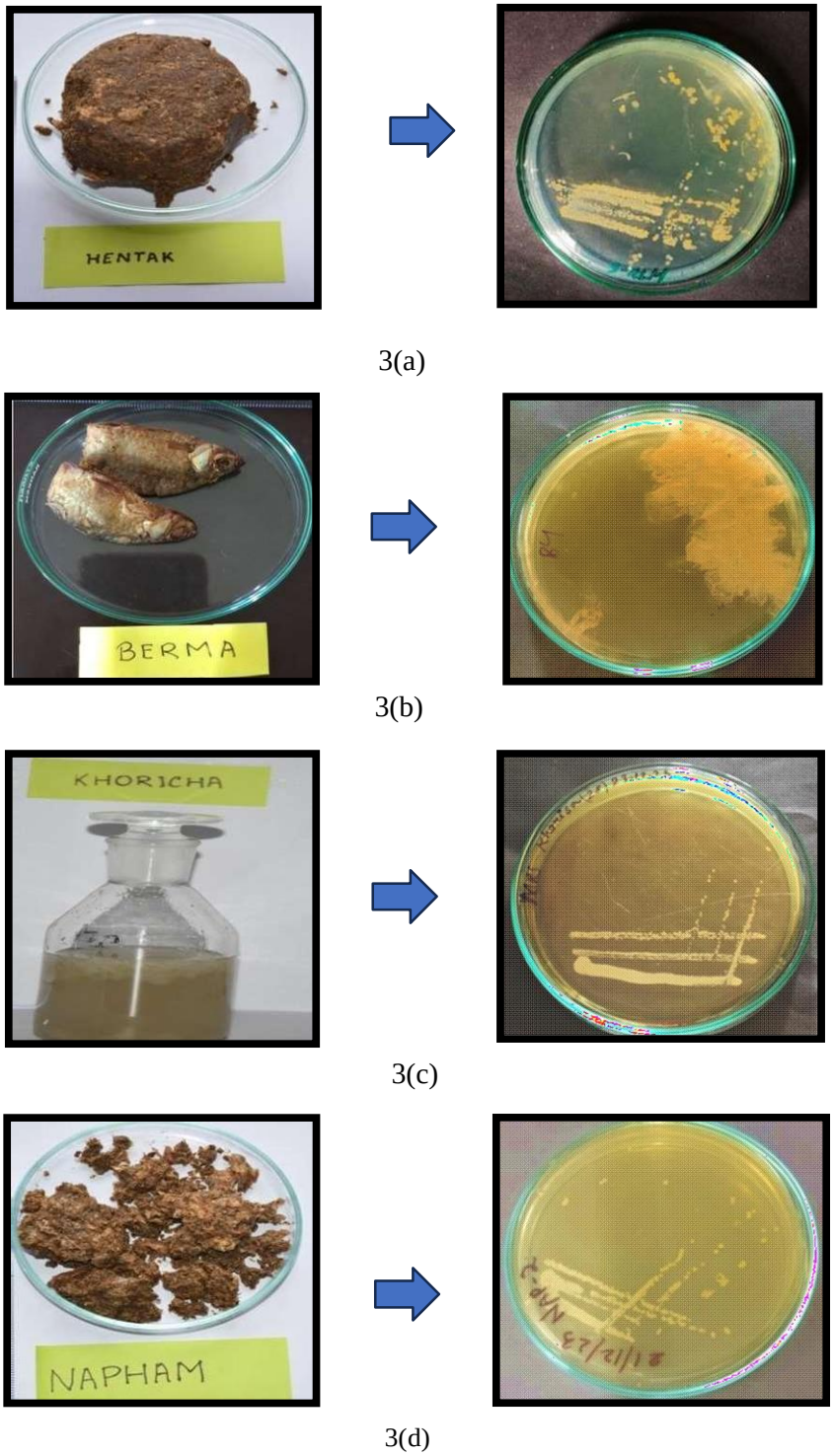
The molecular level identification of the selected strains was assessed on basis 16S rRNA sequencing. Firstly, the isolates were sent for DNA extraction. Thereafter, PCR amplification of 16S rRNA was performed using the primer 16F- 5 GGATGAGCCCGCGGCCTA and 16R-5CGGTGTGTACAAGGCCCGG. The reaction was performed in a TAQ Master Mix using DNA (1 µl), 16S Forward Primer and 16S Reverse Primer (2 µl), Taq DNA Polymerase Enzyme (3U/ ml) (1 µl), 10X Taq DNA polymerase Assay Buffer (10 µl), dNTPs (2.5mM each) (4 µl), Water (30 µl). The optimum condition required for performing PCR are Initial denaturation at 94°C for 3 minutes, denaturation at 94°C for 1 minutes, annealing at 50°C for 1 minutes, extension at 72°C for 2 minutes, final Extension at 72°C for 7 minutes. The products were examined through agarose gel electrophoresis for confirmation. The products were subjected for sanger sequencing. The products obtained were sequenced and compared using BLAST (Basic Local Alignment Search Tool) (2). Thereafter aligned sequence data was searched on GenBank sequence database for accession number and further analysis.

3 Results

3.1 Isolation and Characterization

Isolation of bacterial strains was conducted using MRS media, which is specifically designed for the isolation of LAB strains. As shown in Figure 3, four distinct LAB strains were successfully isolated on MRS media. The characterized features of the isolated LAB were visually observed on the MRS plate; the color of the isolates varies from white to creamy; size varies from pinpoint to large, the margin of colonies formed were curl, entire; elevation were raised, umbonate; consistency varies from smooth to dull; optical density of strains were opaque; and have circular, and lobate form. The result of colony morphology is listed in below

Table 1. On the microscopic analysis, all the strains were found to be gram positive as tabulated in Table 2, in nature and are circular and rod shaped, listed below Fig 4.



3(a)

3(b)

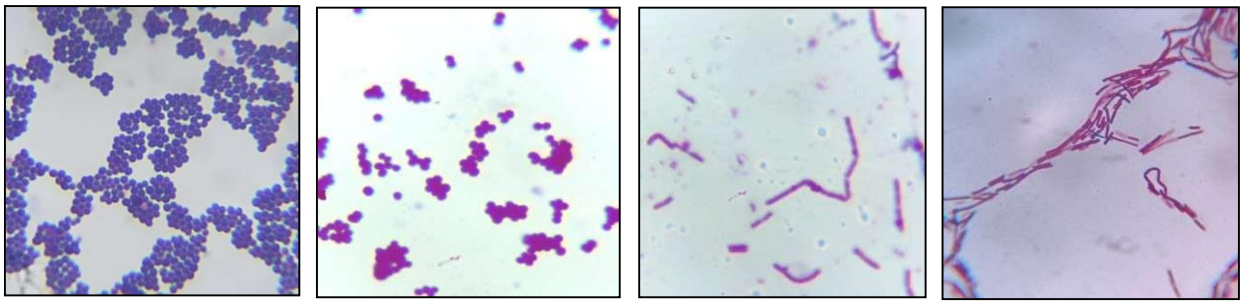
3(c)

3(d)

Figure 3. Isolation of microbes from samples 3(a) Hentak (HTK-5), 3(b) Berma (B4), 3(c) Khorisa (28), 3(d) Napham (NAP-2)

Table 2. Colony morphology of the isolates

Strain	Size	Colour	Margin	Elevation	Consistency	Optical Density	Form
Khorisa (28)	Small	Whitish	Entire	Raised	Smooth	Opaque	Circular
Napham (NAP-2)	Pinpoint /Punctiform	Whitish	Entire	Raised	Smooth	Opaque	Circular
Hentak (HTK-5)	Small	Whitish	Entire	Raised	Smooth	Opaque	Circular
Berma (B4)	Big	Creamish	Curled	Umbonate	Dull	Opaque	Lobate



4(a) 4(b) 4(c) 4(d)

Figure 4: Result of Gram staining: 4(a) Khorisa, 4(b) NAP-2, 4(c) HTK-5, 4(d) B4

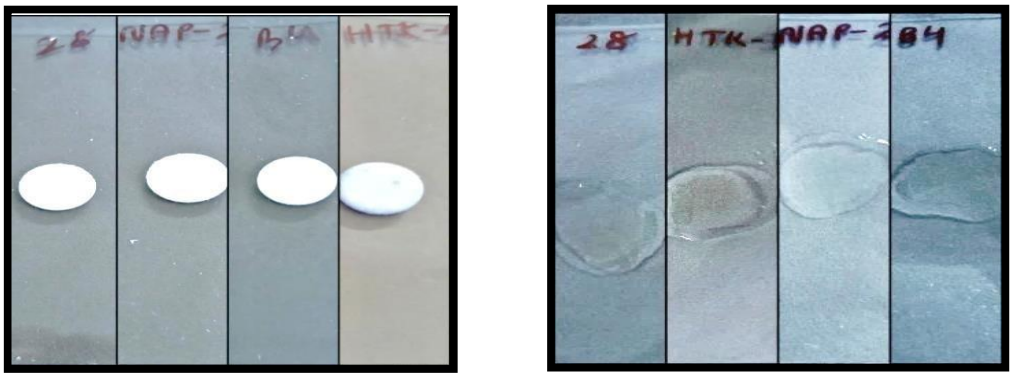
Table 3. Results of Gram staining of the isolates

Strains	**Type**	**Simple staining**	**Gram staining**
Khorisa (28)	Small	Spherical shape, Gram positive	Spherical shape, Gram positive
NAP-2	Pinpoint /Punctiform	Spherical shape, Gram positive	Spherical shape, Gram positive
HTK-5	Small	Rod shape, Gram positive	Rod shape, Gram positive
B4	Big	Rod shape, Gram positive	Rod shape, Gram positive

3.2 Biochemical Characterization

As per the result of biochemical analysis the purified colonies were tested as catalase negative and oxidase negative. All the strains showed positive results in the carbohydrate fermentation test, demonstrating the ability to ferment various sugars, including glucose, sucrose, and lactose. The results have been tabulated in Table 4, with corresponding pictures in Fig. 6. All strains also showed positive results for lipolytic and proteolytic activities (Fig. 7 and Fig. 8), except for the B4 isolate, which tested negative for the proteolytic test, as shown in Table 5. In the IMViC test, most of the strains showed negative results for the Indole, Methyl Red, and

Citrate tests. However, for the Voges-Proskauer test, NAP-2 and Khorisa showed negative results, while the others showed positive results (Fig. 9). The IMViC tests are primarily performed to check for coliform bacteria in isolates. The test results are presented in tabular form in Tables 3 and 6, along with corresponding pictures in Fig. 5.

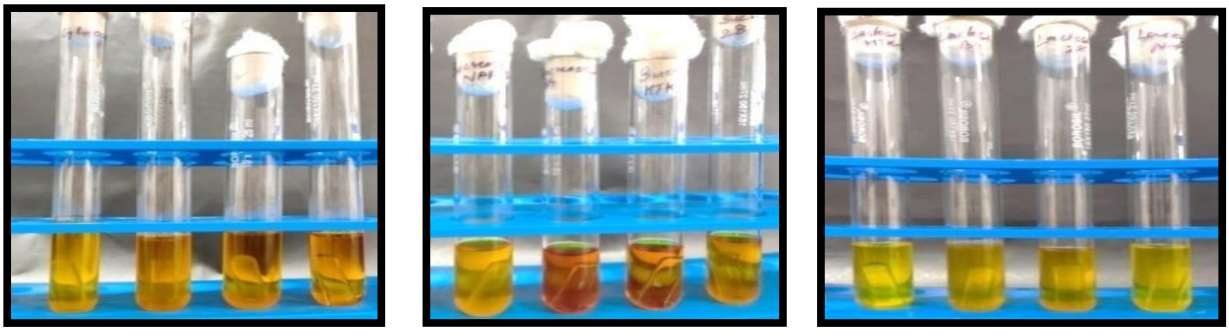


5(a) 5(b)

Figure 5. Result of 5(a) Oxidase test, 5(b) Catalase test

Strains	Catalase test	Oxidase test
Khorisa (28)	Negative	Negative
HTK-5	Negative	Negative
NAP-2	Negative	Negative
B4	Negative	Negative

Table 4. Results of Oxidase and Catalase test of the isolates



6(a) 6(b) 6(c)

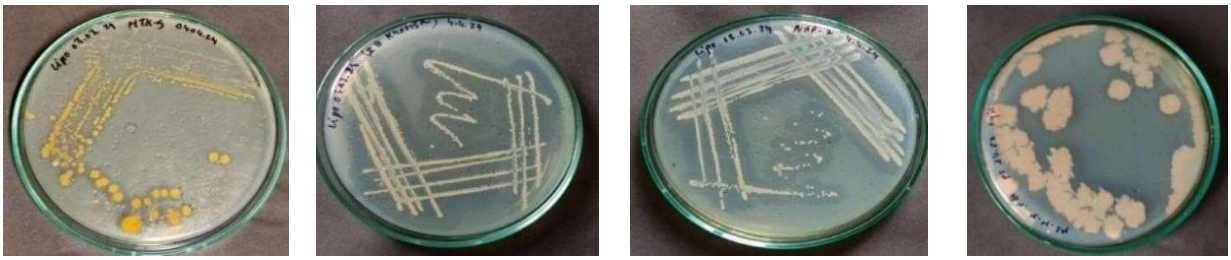
Figure 6: Result of CFT test: 6(a) Glucose test, 6(b) Sucrose test, 6(c) Lactose test

Table 5. Results of Carbohydrate fermentation test of the isolates.

Strains	**Carbohydrate**		
	Glucose	**Sucrose**	**Lactose**
NAP 2	Positive	Positive	Positive
KHORISA (28)	Positive	Positive	Positive
B4	Positive	Intermediate	Positive
HTK 5	Positive	Intermediate	Positive

7(a) 7(b) 7(c) 7(d)

Figure 7: Result of Proteolytic test: 7(a) NAP-2, 7(b) khorisa, 7(c) HTK-5, 7(d) B4

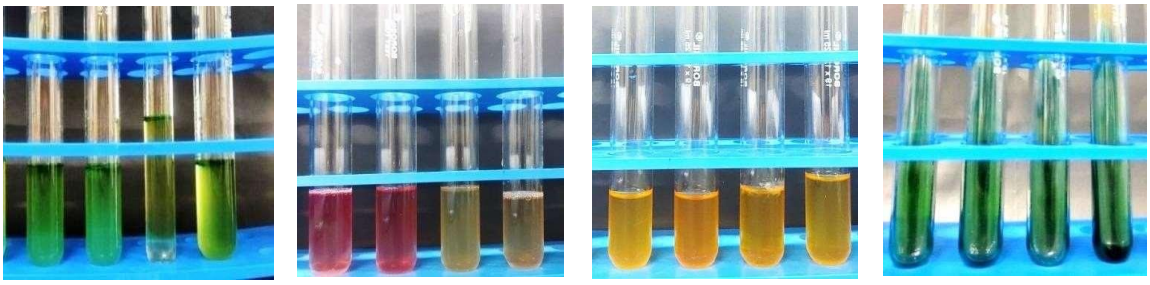


8(a) 8(b) 8(c) 8(d)

Figure 6. Result of Lipolytic test: 8(a) HTK-5, 8(b) Khorisa, 8(c) NAP-2, 8(d) B4

Strains	Proteolytic test	Lipolytic test
HTK 5	Positive	Positive
KHORISA (28)	Positive	Positive
NAP 2	Positive	Positive
B4	Negative	Positive

Table 6. Results of Proteolytic and Lipolytic test of the isolates.



9(a) 9(b) 9(c) 9(d)

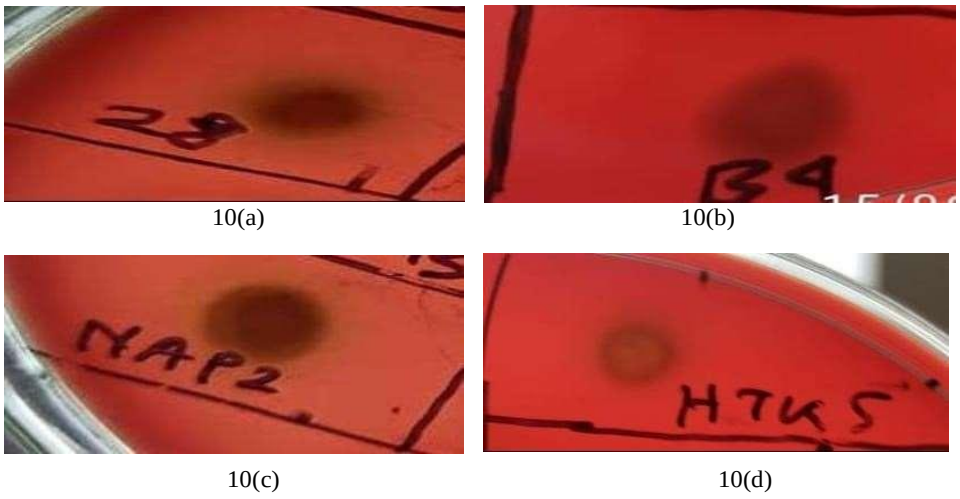
Figure 9: Result of IMViC test 9(a) Indole test, 9(b) Methyl Red Test, 9(c) Voges-Proskauer test, 9(d) Citrate test.

Table 7. Results of IMViC test of the isolates

Strains	**IMViC test**			
	Indole test	**Methyl Red test**	**Voges -Proskauer test**	**Citrate test**
NAP 2	Negative	Negative	Negative	Negative
KHORISA (28)	Negative	Negative	Negative	Negative
B4	Negative	Negative	Positive	Negative
HTK 5	Negative	Negative	Positive	Negative

3.3 Safety evaluation of lab strain

For the safety evaluation of the strains, the isolates were tested using the Hemolytictest. These tests were performed to determine if the isolates possess exoenzymes capable of lysing or blood. According to the results, all strains showed negative results for both the hemolytic tests, except for HTK-5, which showed a positive result for the DNase test. The results are shown in Fig. 10 and have been tabulated in Table 7.



10(a) 10(b)

10(c) 10(d)

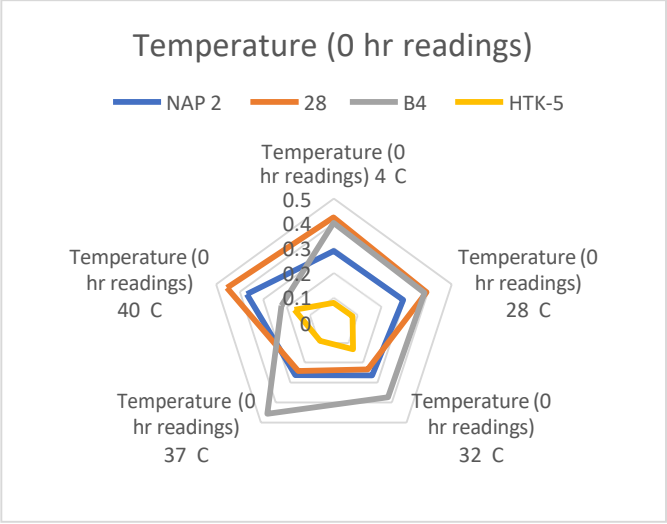
Figure 10. Hemolytic test:10(a) 28, 10(b) B4, 10(c)NAP-2, 10(d) HTK-5

Strains	Hemolytic test
KHORISA (28)	Negative
B4	Negative
NAP 2	Negative
HTK- 5	Negative

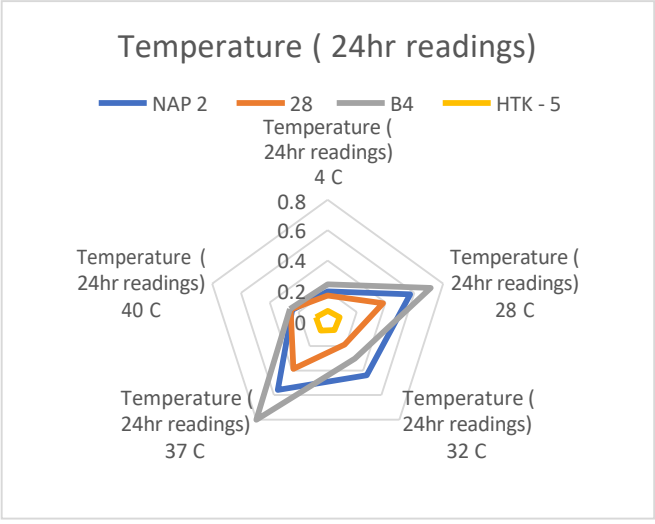
Table 8. Results of Hemolytic test of the isolates

3.4 Physiological test

Criteria for selecting probiotic microbes include their ability to survive in the gastrointestinal tract with low pH and their capability to withstand temperature changes in the body. To survive in the gastrointestinal tract, microbes must be resistant to the acidic pH caused by gastric juices in the stomach. The survival ability of the four isolated LAB strains was tested at different temperatures (4°C, 30°C, 32°C, 37°C, and 40°C). Based on the optical density (OD) recorded at 0 hours and 24 hours, the strains showed higher growth at temperatures between 30°C and 37°C and were able to survive all selected temperatures. The strain HTK-5 showed stable growth across all temperatures, with minimal fluctuation. The OD records for 0 hours and 24 hours are represented in Fig. 11, and the results have been tabulated in Table 9. The four isolated LAB strains were also tested at different pH levels, both acidic and basic, to assess their viability and ability to withstand the gut environment. The results showed that thestrains are tolerant to acidic conditions. The survival percentage of the LAB strains between 0 hours and 12 hours ranged from 107% to 127%. The OD records for 0 hours and 12 hours are represented in Figure 12.



11(a)



11(b)

Figure 11. 11(a) Viability of strains at 0hr on different temperature. 11(b) Viability of the

strains at 24hr on different temperature.

Table 9. 9(a) Results of viability of strains at 0hr on different pH. 9(b) Results of viability of the strains at 12hr on different pH.

Strains	Temperature (OD readings at 0hr)				
	4 ℃	28 ℃	32 ℃	37 ℃	40 ℃
NAP 2	0.289	0.295	0.265	0.263	0.369
28	0.424	0.395	0.235	0.243	0.454
B4	0.403	0.385	0.375	0.457	0.222
HTK-5	0.079	0.079	0.134	0.093	0.167

9(a)

Strains	Temperature (OD readings at24hr)				
	4 ℃	28 ℃	32 ℃	37 ℃	40 ℃
NAP 2	0.196	0.569	0.438	0.559	0.247
28	0.169	0.382	0.188	0.385	0.257
B4	0.243	0.712	0.303	0.8	0.263
HTK - 5	0.068	0.081	0.073	0.078	0.082

9(b)

3.5 Preparation of curd and its sensory evaluation

The LAB isolates were tested for their ability to prepare curd in the laboratory. The results indicated that these strains exhibited excellent fermentative properties, as evidenced by the appearance, aroma, flavor, and texture of the curd. Sensory evaluation conducted with 10-12 people showed that Khorisa (28) received the highest ratings for curd formation compared to the other strains. Therefore, Khorisa (28) is recommended for application in functional food formulations.

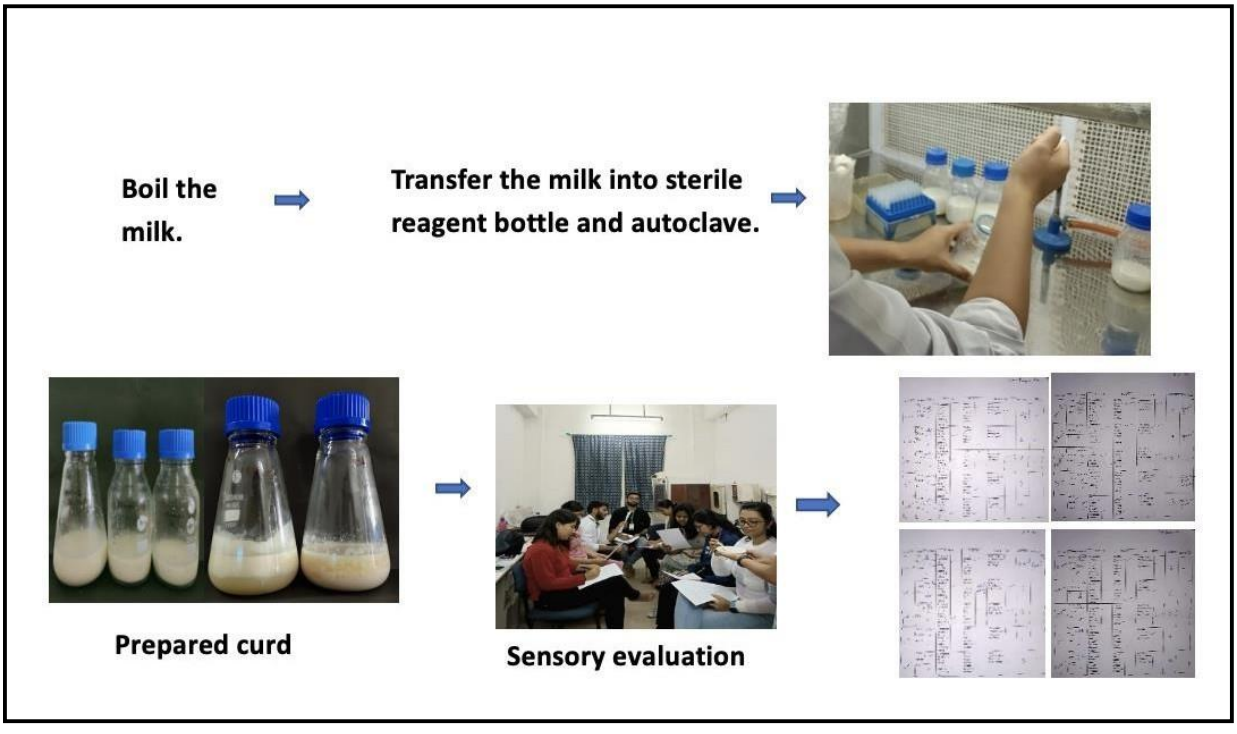


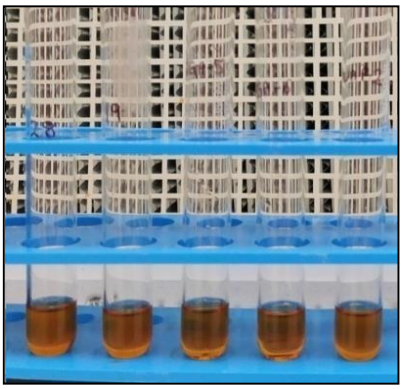
Figure 19: Process of sensory evaluation of curd

3.6 Screening of probiotic potential of the isolated strains

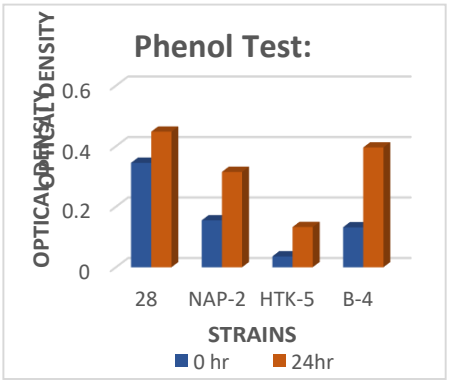
3.6.1 Phenol test and NaCl test

Probiotic microbes are known for their tolerance to phenol and high levels of NaCl. In the human body, phenol serves as an antioxidant, protecting tissues from oxidative stress, while NaCl aids in nutrient absorption, transportation, and maintaining blood fluidity. All four isolates were tested for their tolerance to high concentrations of phenol and NaCl. The isolates NAP-2 and B4 showed good tolerance to phenol at an OD value of 0.2 compared to the other isolates under a 0-24 hour incubation period. The NAP-2 and Khorisa isolates exhibited good

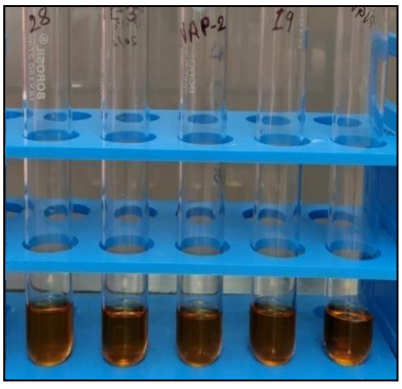
tolerance to high NaCl levels after a 6-hour incubation period. Conversely, the growth of HTK-5 and B4 was reduced at high NaCl concentrations. The test results have been tabulated in Table 10, and the pictures are displayed in Fig. 13.



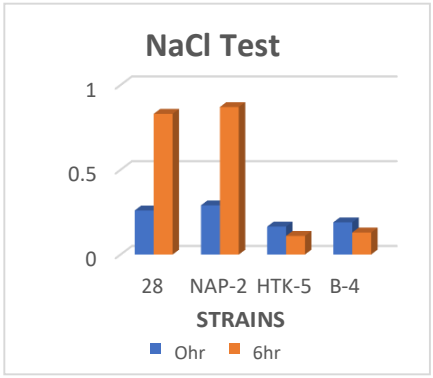
13(a)



13(b)



13(c)



13(d)

Figure 13: 13(a) Phenol test, 13(b) Representation of Optical Density of the strains at 0hr & 24hr.13(c) NaCl test, 13(d) Representation of Optical Density of the strains at 0hr & 6hr.

Strains	0 hr	24hr
Khorisa (28)	0.345	0.448
Napham (NAP-2)	0.155	0.315
Hentak (HTK-5)	0.036	0.133
Berma (B4)	0.132	0.396

10(a)

Strains	0 hr	6hr
Khorisa (28)	0.26	0.83
Napham (NAP-2)	0.29	0.87
Hentak (HTK-5)	0.165	0.11
Berma (B4)	0.19	0.13

10(b)

Table 10. 10(a) Results of Phenol test of the strains at 0hr & 24hr, 10(b) Results of NaCl test of the strains at 0hr & 6hr.

3.6.2 Tolerance to Acidic pH

Tolerance to acidic pH is another criterion that potential probiotics must fulfill. The isolates demonstrated viability at an acidic pH of 2, as shown in Fig. 14. This ability helps them survive the acidic conditions of the stomach or the host's gastrointestinal tract. The results have been tabulated in Table 11.

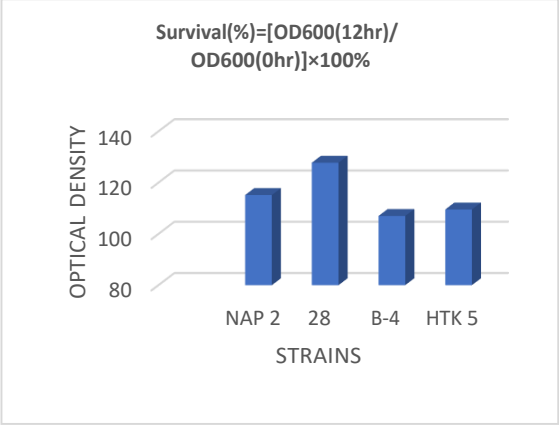


Figure 14. Survival % of the strains at acidic pH (2).

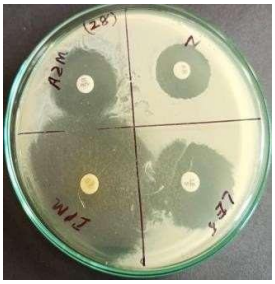
Strains	Survival(%)=[OD_{600}(12hr)/ OD_{600}(0hr)]×100%
NAP 2	115.15
KHORISA (28)	127.77
B4	107
HTK 5	109.52

Table 11: Results of tolerance of the strains at acidic pH(2)

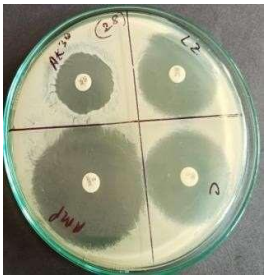
3.6.3 Susceptibility of LAB to antibiotics

The antibiotic susceptibility of the LAB isolates was assessed using the disc diffusion method. With growing concerns about antibiotic resistance, it is important for LAB strains to show susceptibility to antibiotics, indicating their potential positive impact in the future. Most isolates demonstrated sensitivity to a range of antibiotics, including Gentamicin (50 µg), Moxifloxacin (5 µg), Ciprofloxacin (5 µg), Amikacin (30 µg), Linezolid (30 µg), Chloramphenicol (50 µg), Ampicillin (2 µg), Imipenem (10 µg), Azithromycin (30 µg), Neomycin (30 µg), and Levofloxacin (5 µg). While the majority of LAB strains were sensitive to the tested antibiotics, a few strains showed inhibition by certain antibiotics. Notably, most strains were resistant to Ciprofloxacin (CIP) and Ampicillin (AMP), which are inhibitors of DNA and protein synthesis, respectively. The details of antibiotic sensitivity and resistance are listed in Table 12.

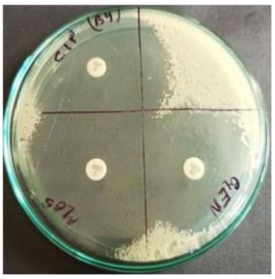
15(a)



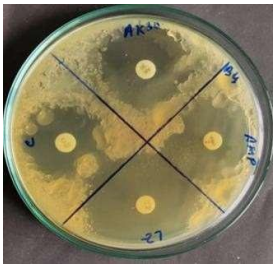
15(b)



15(c)

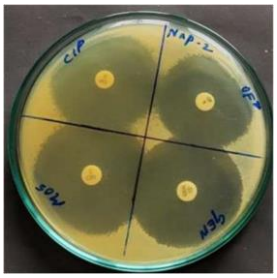


15(d)

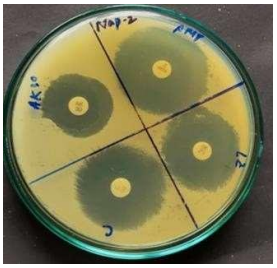


15(e)

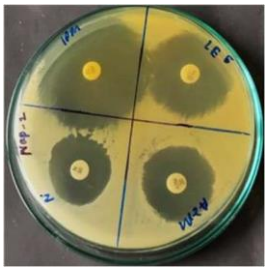
15(f)



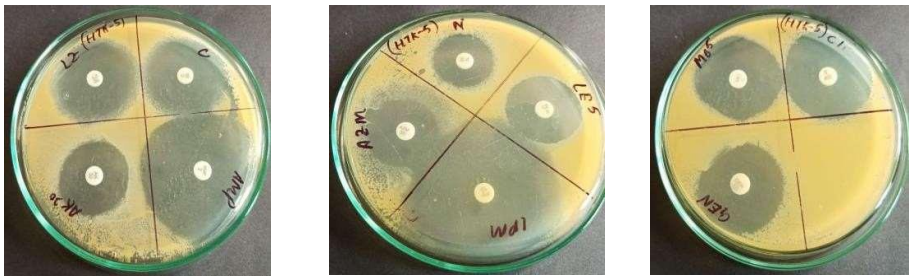
15(g)



15(h)



15(i)



15(j) **15(k)** **15(l)**

Figure 15: Antibiotic susceptibility test. 15(a),15(b),15(c) : Khorisa(28); 15(d),15(e),15(f) : Berma(B4); 15(g),15(h),15(i) : Napham(NAP-2); 15(j),15(k),15(l) : Hentak(HTK-5)

Table 12. Results of Antibiotic susceptibility test of the strains

Antibiotics	**Hentak(HTK-5)**	**Berma (B4)**	**Napham(NAP-2)**	**Khorisa (28)**
Gentamicin (GEN)	S	S	S	S
Moxifloxacin ($M0^{5}$)	S	S	S	S
Ciprofloxacin (CIP)	R	S	S	R
Ofloxacxin ($0F^{2}$)	-	S	S	-
Amikacin (AK^{30})	S	S	S	S
Linezolid (Lz)	S	S	S	S
Chloramphenicol (C)	S	R	S	S
Ampicillin (AMP)	S	S	S	S
Imipenem (IPM)	S	S	S	S
Azythromycin (AZM)	S	S	S	S
Neomycin (N)	S	S	S	S
Levofloxacin (LE^{5})	R	S	S	S

3.6.4 In vitro adhesion to chicken crop epithelial cell

Probiotic microbes have the ability to adhere to the surface of epithelial cells, which is crucial for their retention in the gastrointestinal tract. To assess this adherence capability, the isolates were tested for their ability to adhere to gastrointestinal epithelial cells. A result is considered positive if around 10-15 microbes are attached to the epithelial cells, while fewer than 10 is considered negative. Staining results are shown in Figure 16. All strains demonstrated adherence to chicken crop epithelial cells, with HTK-5 and NAP-2 exhibiting the highest levels

of adherence. The results have been tabulated in Table 13 and are displayed in Fig. 17.

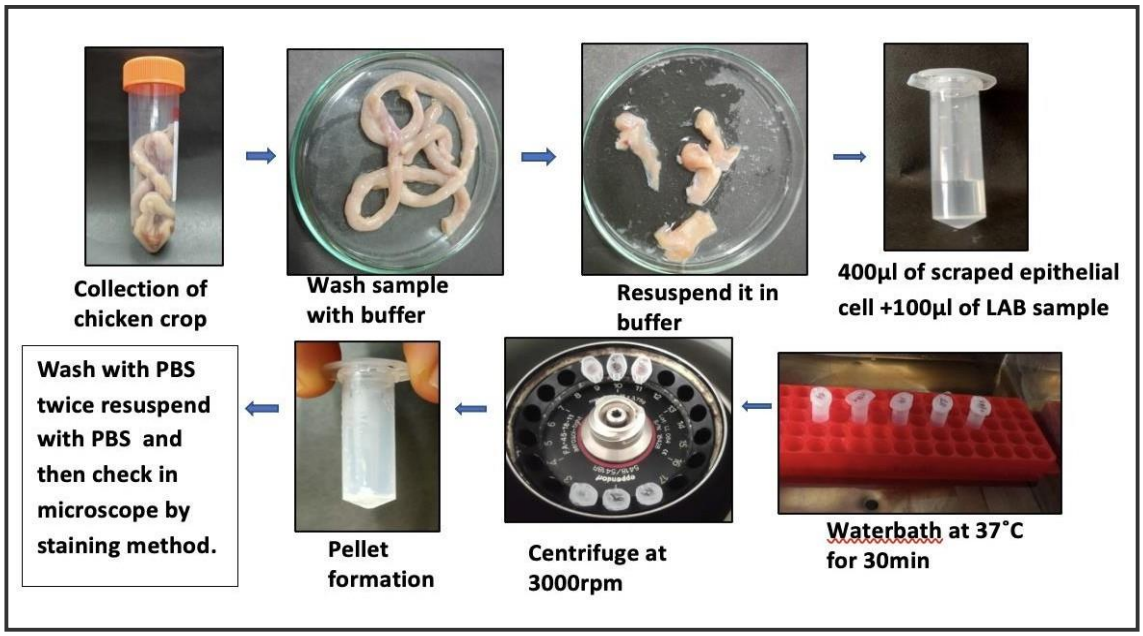
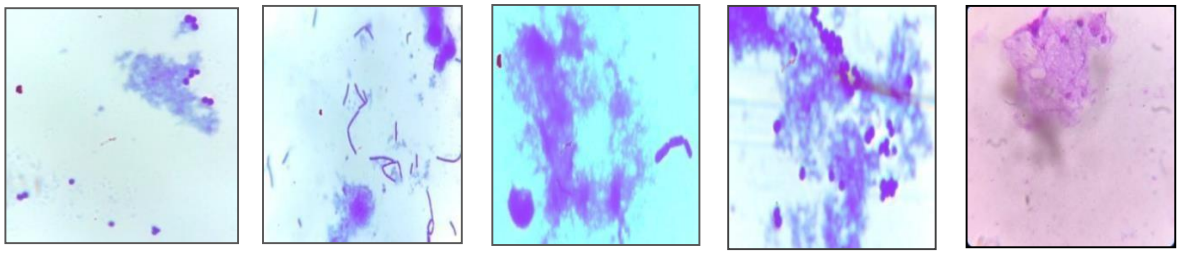


Figure 16: Process of In vitro adhesion to chicken crop epithelial cell

Table 13. Table for adherence to chicken crop epithelial cells

Sl. No.	Strains	Adhesion to Chicken Crop Epithelial cells
1.	Khorisa (28)	+
2.	B4	+
3.	HTK-5	+++
4.	NAP-2	+++
5.	Control	++



17(a) **17(b)** **17(c)** **17(d)** **17(e)**

Figure 17: Result of staining 17(a) Khorisa, 17(b) B4, 17(c) HTK-5, 17(d) NAP-2, 17(e) Control

3.6.5 Auto aggregation

Autoaggregation refers to the ability of microbial cells of the same strain to aggregate with each other in a non-specific manner. This property is crucial for probiotics as it indicates their ability to adhere to the mucosal surfaces of the small intestine. Autoaggregation helps in

forming biofilms, which can protect against pathogenic microbes. According to the results, the NAP-2 and B4 strains exhibited high autoaggregation abilities, with percentages ranging from 67.74% to 82.85% after a 5-hour incubation period, as shown in Fig. 18.

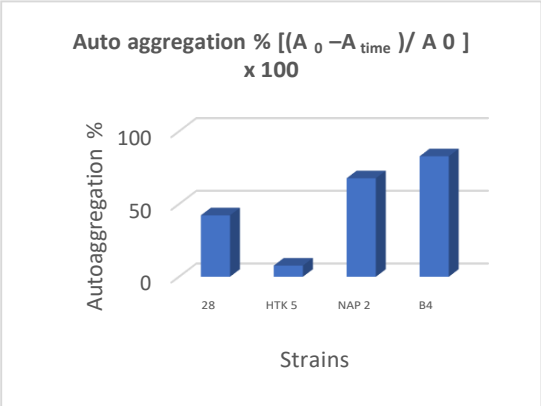


Figure 18. Auto aggregation % of the strains.

Strains	0hr	5 hr	Auto aggregation % $[(A_0 - A_{time})/A_0] \times 100$
KHORISA (28)	0.071	0.041	42.25
HTK 5	0.013	0.012	7.69
NAP 2	0.031	0.010	67.74
B4	0.035	0.006	82.85

Table 14. Results of Autoaggregation of the strains

3.6.6 Molecular characterisation of 16S rRNA

The four LAB isolates with high probiotic potential were identified at the molecular level through 16S rRNA sequencing, which allowed for accurate phylogenetic classification. The sequencing results identified the isolates as *Bacillus cereus* strain RS01 (Khorisa), with accession number PP406892.1; *Staphylococcus carnosus* strain LS370 (NAP-2), with accession number MT409929.1; *Bacillus paralicheniformis* strain PB61 (B4), with accession number MN689683.1; and *Exiguobacterium acetylicum* strain B001 (HTK-5), with accession number MN587993.1. This detailed molecular characterization confirms the identities of these strains and underscores their potential for use in probiotic applications. The identification provides a solid foundation for further research and application in functional food formulations.

3.6.6.1 Khorisa(28)

3.6.6.1.1 Aligned sequence data

GACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGC
GATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGA
GAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGCTCTTTGTACCGTCC
ATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCC
CACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAATGATGG
CAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACA
CGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCT
ATCTCTAGGGTTTTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCG
AATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTC
AGCCTTGCGGCCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTA
AAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAG
GGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACC
AGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTAC
ACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCT
CCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCT
TTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGG
CACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAA
CTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACT
CACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGC
CTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCA
GGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCG
ACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGC
GGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATG
GGCAGGTTACCCACGTGTTACTCA ACCCGTCCCGCCGCTAACTTC

- The Microbe was found to be *Bacillus cereus* strain RS01 16S ribosomal RNA gene
- Sequence ID: PP406892.1
- The next closest homologue was found to be *Bacillus cereus* strain J6 16S ribosomal RNA gene
- Sequence ID: PP414204.1

3.6.6.1.2 Phylogenetic tree

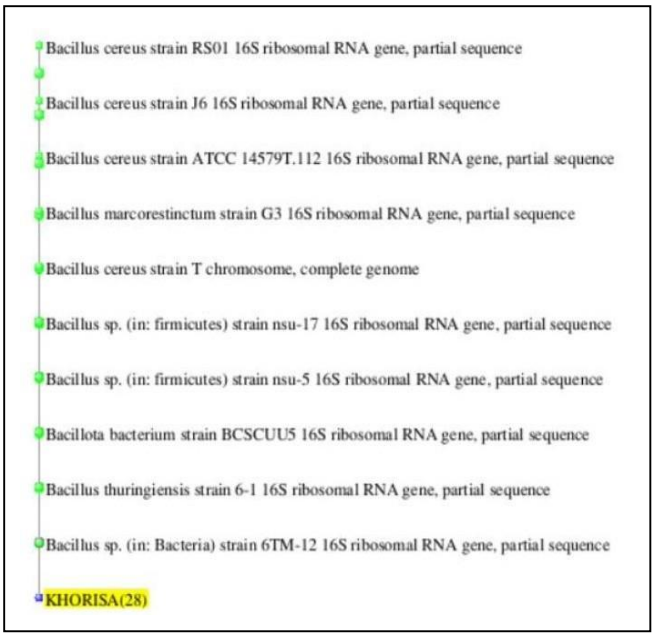


Figure 20: Phylogenetic tree of Khorisa(28)

3.6.6.2 NAP 2

3.6.6.2.1 Aligned Sequence data (1324 bp):

GTTGTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGTAGCATGCTGA
TCTACGATTACTAGCGATTCCAGCTTCATGTAGTCGAGTTGCAGACTACAATCCGA
ACTGAGAACAGCTTTATGGGATTTGCTTGACCTCGCGGTTTCGCTGCCCTTTGTAC
TGTCCATTGTAGCACGTGTGTAGCCCAAATCATAAGGGGCATGATGATTTGACGTC
ATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCAACTTAGAGTGCCCAACTTAAT
GCTGGCAACTAAGCTTAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCA
CGACACGAGCTGACGACAACCATGCACCACCTGTCACTTTGTCCCCCGAAGGGG
AAGACTCTATCTCTAGAGCGGTCAAAGGATGTCAAGATTTGGTAAGGTTCTTCGCG
TTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGTCCCCGTCAATTCCTT
TGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGC
AGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGA
CTACCAGGGTATCTAATCCTGTTTGATCCCCACGCTTTCGCACATCAGCGTCAGTT
GCAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTGCGCATTTCA
CCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTTTTCCAGTTTCCAAT
GACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAAAAACCGCCTACG
CGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCT

GCTGGCACGTAGTTAGCCGTGGCTTTCTGATTAGGTACCGTCAAGGTGCGCATAGT
TACCTACGCACTTGTTCTTCCCTAATAACAGAGTTTTACGATCCGAAGACCTTCATC
ACTCACGCGGCGTTGCTCCGTCAGGCTTTCGCCCATTGCGGAAGATTCCCTACTGC
TGCCTCCCGTAGGAGTCTGGACCGGGTCTCAGTTCCAGTGGGGCCGATCACCCTC
TCAGGTCGGCTACGTATCGTTGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATA
CGGCGCGGGTCCATCTATAAGTGACAGCAAAACCGTCTTTCATTGCGGAACCATG
CGGTTCCGCATATTATCCGGCATTAGCCCCGGTTTCCCGGAGTTATTCCAGTCTTAT
AGGTAGGTTACCCACGTGTT ACTCAACCCGTCCGCCCGC

- The Microbe was found to be *Staphylococcus carnosus* strain LS370 16S ribosomal RNA gene
- Sequence ID: MT409929.1
- The next closest homologue was found to be *Staphylococcus condimenti* SKJ-1 gene for 16S ribosomal RNA
- Sequence ID: LC511700.1

3.6.6.2.2 Phylogenetic tree :

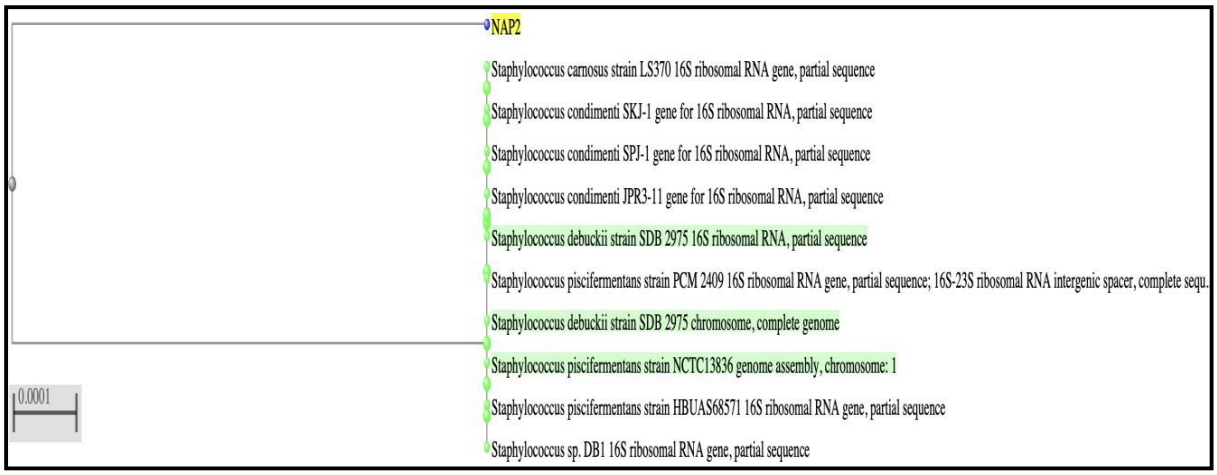


Figure 21. Phylogenetic tree of NAP-2

3.6.6.3 HTK 5

3.6.6.3.1 Aligned sequence data(1384bp):

ACCTCACCGTGCTTCGGGTGTTGCAAACTCTCGTGGTGTGACGGGCGGTGTGTAC
AAGACCCGGGAACGTATTCACCGCAGTATGCTGACCTGCGATTACTAGCGATTCCG

ACTTCATGCAGGCGAGTTGCAGCCTGCAATCCGAACTGGGAACGGCTTTATGGGAT
TGGCTCCACCTCGCGGTCTCGCTGCCCTTTGTACCGTCCATTGTAGCACGTGTGTA
GCCCAACTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGT
CACCGGCAGTCTCCCTAGAGTGCCCAACTGAATGCTGGCAACTAAGGATAGGGGT
TGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCA
TGCACCACCTGTCACCATTGTCCCCGAAGGGAAAACTTGATCTCTCAAGCGGTCA
ATGGGATGTCAAGAGTTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGC
TCCACCGCTTGTGCGGGTCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTA
CTCCCCAGGCGGAGTGCTTAATGCGTTAGCTTCAGCACTGAGGGGCGGAAACCCC
CCAACACCTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTT
GCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAAAGAGTCGCCTTCGC
CACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCACTCT
TCTCTTCTGTACTCAAGCCTTCCAGTTTCCAATGGCCCTCCCCGGTTGAGCCGGGG
GCTTTCACATCAGACTTAAAAGGCCGCCTGCGCGCGCTTTACGCCCAATAATTCCG
GACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGC
TTTCTCGTAAGGTACCGTCAAGGTACGAGCATTTCCTCTCGTACGTGTTCTTCCCTT
ACAACAGAGTTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCATCA
GACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCC
GTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCGTCGCC
TTGGTGGGCCGTTACCCCACCAACTAGCTAATGCACCGCAAGGCCATCTCAAGGTG
ACGCCGAAGCGCCTTTCATCAGCGGACCATGCGGTCCGTTGAACTATCCGGTATTA
GCTCCGATTTCTCGGAGTTATCCCAATCCTTGAGGCAGGTTCCTTACGTGTTACTCA
CCCGTCCGCTCGCTCGATTCCACTGCCTTCCCTCCGAAAGA

- The Microbe was found to be *Exiguobacterium acetylicum* strain B001 16S ribosomal RNA gene
- Sequence ID: MN587993.1
- The next closest homologue was found to be Exiguobacterium sp. CSQXZ2N3.7.7 gene for 16S ribosomal RNA
- Sequence ID: LC484818.1

3.5.6.3.2 Phylogenetic tree :

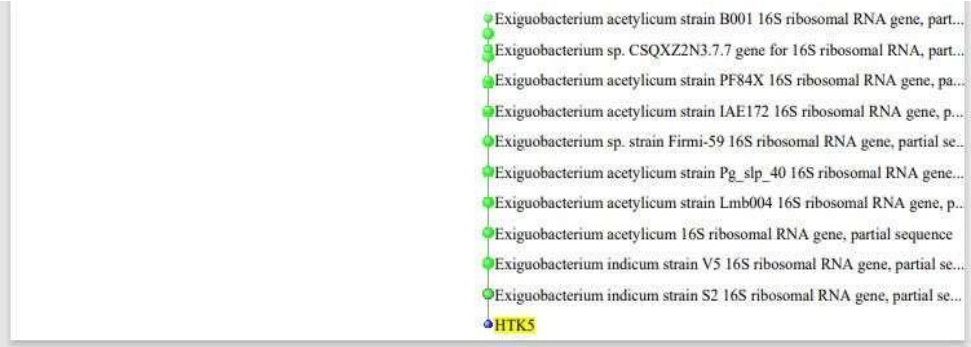


Figure 22. Phylogenetic tree of HTK-5

3.6.6.4 B4

3.6.6.4.1 Aligned sequence data(1402 bp):

AAAGGTTACCTCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGT
GTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCG
ATTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTT
GTGGGATTGGCTTAGCCTCGCGGCTTCGCTGCCCTTTGTTCTGCCCATTGTAGCAC
GTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTC
CGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGA
TCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGAC
GACAACCATGCACCACCTGTCACTCTGCCCCCGAAGGGGAAGCCCTATCTCTAGG
GTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACC
ACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCG
ACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTTGCTGCAGCACTAAAGGGCGGA
AACCCTCTAAACACTTAGCACTCATCGTTTTACGGCGTGGACTACCAGGGTATCTA
ATCCTGTTTCGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAGAGAG
TCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGG
AATTCCACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCCCGG
TTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCGCGCTTTACGC
CCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTA
GTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACCGCCCTATTCGAACGGTA
CTTGTTCTTCCCTAACAACAGAGTTTTACGATCCGAAAACCTTCATCACTCACGCG

GCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCG
TAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGG
CTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGG
GTCCATCTGTAAGTGGTAGCTAAAAGCCACCTTTTATAATTGAACCATGCGGTTCA
ATCAAGCATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAG
GTTACCCACGTGTTACTCACCCGTCCGCCGCTGACATCAGGGAGCAAGCTCCCAT
CT GTCCGCTCGAC

- The Microbe was found to be *Bacillus paralicheniformis* strain PB61 16S ribosomal RNA gene
- Sequence ID: MN689683.1
- The next closest homologue was found to be *Bacillus paralicheniformis* strain A4-3 chromosome
- Sequence ID: CP043501.1

3.6.6.4.2 Phylogenetic tree:

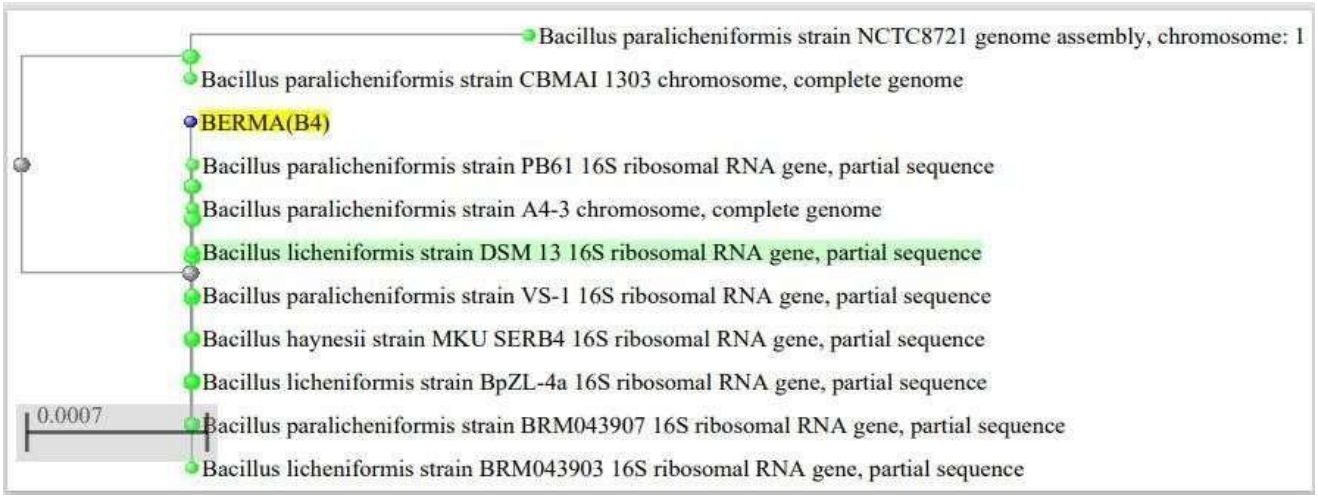


Figure 23. Phylogenetic tree of B4

4 Discussion

In this study, traditional fermented foods from various locations in Assam were collected, and bacteria were isolated from these samples. The isolates were characterized based on colony morphology, including size, shape, color, texture, form, and elevation. Cellular morphology was also examined, and various biochemical tests were conducted, including

IMViC, catalase, oxidase, lipolytic, proteolytic, and carbohydrate fermentation tests. Additionally, physiological tests assessed their viability across different temperatures and pH levels. The results indicated that the isolates were Gram-positive, with rod and spherical shapes, and were negative for catalase and oxidase tests. Most strains exhibited positive results for lipolytic and proteolytic activities and demonstrated resistance to phenol and high NaCl concentrations. They also maintained viability in acidic pH and various temperature ranges. Notably, the Khorisa, B4, and NAP-2 isolates showed good adherence to epithelial cells, with B4 and NAP-2 exhibiting high autoaggregation percentages.

The antibiotic susceptibility of the strains was tested against Gentamicin (50 mcg), Moxifloxacin (5 mcg), Ciprofloxacin (5 mcg), Amikacin (30 mcg), Linezolid (30 mcg), Chloramphenicol (50 mcg), Ampicillin (2 mcg), Imipenem (10 mcg), Azithromycin (30 mcg), Neomycin (30 mcg), and Levofloxacin (5 mcg). All strains showed sensitivity to most antibiotics, distinguishing them from antibiotic-resistant bacteria—a significant finding given the current concerns about antimicrobial resistance. Molecular characterization revealed the strains as *Bacillus cereus* strain RS01, *Staphylococcus carnosus* strain LS370, *Bacillus paralicheniformis* strain PB61, and *Exiguobacterium acetylicum* strain B001. The study highlights the potential of LABs associated with northeastern Indian fermented foods, which remain largely unexplored. This work provides a foundation for future research and underscores the need to explore the antimicrobial properties of these LABs.

5 Conclusion

From this study, we can conclude that the selected isolates demonstrated strong probiotic properties, as evidenced by their high auto-aggregation percentages and resistance to phenol, high concentrations of NaCl, low pH, and various antibiotics. These characteristics suggest that the isolates can effectively survive and thrive in the human gastrointestinal tract. The study highlights that fermented foods are rich in lactic acid bacteria (LAB) with notable probiotic qualities. Most of the selected strains exhibited excellent fermentative properties.

Based on these findings, it is recommended that these probiotic strains be considered for use in formulating various food products across different industries. Their proven benefits suggest potential applications in enhancing gut health and contributing to the production of functional foods. The study underscores the importance of understanding microbial dynamics in fermented foods, paving the way for the development of probiotic products that can provide

significant health benefits. Expanding the use of these beneficial microbes in food formulations could lead to improved gut health and overall well-being for consumers.

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