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Probiotic Properties of *Lactobacillus Spp*. from Indigenous Curd: Acid, Bile and Antimicrobial Effectiveness

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Abstract:- Probiotic bacteria have assembled an increasing attention for their potential to promote human health through modulation of the gut microbiota. The selection of probiotic strains with suitable properties is crucial for their survival in the gastrointestinal tract and conferring of health benefits. The present study employed a combination of biochemical and molecular techniques to characterize the metabolic traits and identify the species of various probiotic strains. The IMViC test and molecular approaches provided valuable insights into the differentiation of the strains. The acid resistance and bile tolerance of the *Lactobacillus Spp were assessed*, revealing varying degrees of survival potential in simulated gastric and intestinal conditions. The antimicrobial activity of the strains was recorded by measuring the width of clear zone which is commonly known as Zone of Inhibition (ZOI) against distinct pathogens was also evaluated, showing selective inhibition of certain pathogens. The findings provide important information for the selection of probiotic strains for use in dietary supplements or functional foods. Further research is necessary to clarify the probiotic properties of the strains. Such research will contribute to the development of efficacious probiotic formulations with potential to promote human health.

Keywords:- *Human Health, Probiotic Bacteria, Lactobacilli, Acid Resistance, Bile Tolerance, Antimicrobial Activity.*

I. INTRODUCTION

The *Lactobacillus* genus comprises of a genetically and physiologically diverse group of rod-shaped, Gram-positive, non-spore-forming, non-pigmented bacteria (**Soni et al., 2021**). Catalase-negative, microaerophilic to strictly anaerobic lactic acid bacteria (LAB) are widely used in the manufacturing of fermented foods and have been classified as usually considered as safe (GRAS) species, appropriate for medical and veterinary purposes (**Pot et al., 2009**). Lactic acid bacteria (LAB) are widely used as starter cultures in the food industry and are acknowledged as constituents of human microbiota (**Sales et al., 2019**). The gut microbiota is formed shortly after birth, has a reasonably stable composition throughout life, and is essential for sustaining human homeostasis (**Bodke et al., 2022**). Lactobacilli are either naturally present or intentionally added to raw milk and dairy products, such as cheeses, yoghurts, curds, and fermented milks, for technical applications or to provide health benefits to consumers. Curd is among the most recognised probiotic foods. From a health perspective, the consumption of live cells from specific species and strains of lactobacilli, when ingested in sufficient quantities, is believed to provide various advantageous physiological effects on the host, including the maintenance of a healthy and balanced intestinal microbiota and a reduction in the incidence of intestinal infections (**Fuller et al., 2012**). of gastrointestinal infection. The criteria for the in vitro selection of lactobacilli intended for use as health-promoting probiotic components in food and pharmaceutical formulations encompass antibiotic tolerance and lactic acid production, which suppresses the proliferation of other microorganisms, facilitating their establishment in the intestinal tract. Bile tolerance and gastric juice resistance are significant traits of probiotic lactic acid bacteria used as adjuncts, since they facilitate survival, growth, and the execution of beneficial functions inside the gastrointestinal tract. While the specific amount of tolerance necessary for optimal development in the gastrointestinal tract remains unidentified, it is reasonable to favour the selection of species that exhibit the highest resistance to bile and acid.

The objective of this study was to evaluate the probiotic potential of isolated lactobacilli from curd samples by examining their tolerance to acidic and bile environments, as well as their antibacterial efficacy against prevalent pathogenic bacteria. The research aims to find strains that exhibit resistance in gastrointestinal environments and possess antimicrobial characteristics suitable for probiotic use.

II. MATERIALS AND METHODS

10 different curd samples were obtained from a local source in Hyderabad, Telangana. MRS agar (HiMedia) was used for the isolation of *Lactobacillus* cultures. Biochemical methods were used to determine the strains of *Lactobacillus* cultures. Gramm staining, biochemical assays, and molecular procedures were conducted while meticulously observing development at certain temperatures for various research.

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➢ *Isolation of Lactic Acid Bacteria*

Lactic acid bacteria derived from curd produced from many dairy product sources. The samples were examined under sterile conditions. The curd samples were immersed and dissolved in sterile saline, then spread out onto selected medium (MRS) and incubated anaerobically for 24 hours at 37 °C. The Streak plate technique was used to purify the selected colonies. The purified bacterial strains were preserved at -80°C with 15% glycerol.

➢ *Gram Staining and Microscopic Observation*

The bacteria that were isolated were examined using a compound microscope at magnifications of 10, 45, and 100x, with the use of a gramme staining kit.

➢ *Physiological and Biochemical Characterization*

- *Growth at 37°C*

In sterile test tubes, pure colonies were cultured on MRS agar, then transferred to tubes with MRS broth and incubated at 37°C for 12-24 hours. After 12-24 hours, observations were considered positive if turbidity was discovered.

- *Biochemical Characterization (IMViC)*

✓ **Indole Test:** A modified amount of tryptophan broth was prepared and inoculated with the overnight grown culture of the isolated sample and is incubated at 37°C. The turbidity in the broth detects the positivity of the growth in the tryptophan broth.

✓ **Methyl Red & Vogues Proskauer Test:** Certain amount of MRVP broth is used and is inoculated with overnight grown culture and is incubated for 37°C for 12-24 hrs. The turbidity within the broth proves the clear growth of the bacteria in the broth.

✓ **Citrate Utilization:** A modified amount of Simmon's citrate agar slants were used in this test. A purely grown culture is streaked in this agar slant.

➢ *Genomic DNA Isolation*

In this study, total genomic DNA were extracted using the phenol-chloroform method from the cultures of lactobacilli or fermented dairy products samples (**Jafar et al., 2016**). The genomic DNA was extracted from a pure culture that had been grown for 24 hours and centrifuged at 6000 revolutions per minute for fifteen minutes. A total of 500 microlitres of lysozyme was added to the extraction process, and the resulting product was then incubated at a temperature of 37 degrees Celsius for a duration of 45 minutes. Following the addition of the 10% SDS solution, which was 50 µl in volume, and 200 µl of NaCl, the combination was allowed to undergo incubation at room temperature. A combination of phenol, chloroform, and isoamyl alcohol was used in order to extract the DNA from the bacterial cell and get a lysate. A litre of isopropanol that was ice-cold was added, and then the mixture was centrifuged. Following the addition of 70% ethanol, the DNA that had been precipitated was reconstituted with TE buffer for about 50-60 µl's worth of volume. A Gel-Electrophoresis test was performed on the DNA sample that had been extracted in order to verify the samples' level of purity.

➢ *PCR (Polymerase Chain Reaction)*

The PCR amplification was carried out in a thermal cycler which has a capacity of 32 wells (BR-PCR-32T 800x480). The universal primers (100kB base pair) were used in this and was employed as an efficient method for identifying the *Lactobacillus* species and strains. The reaction mixture had a total volume of 20µl, comprising 10µl of Norgen 2X Master Mix, 1µl of 10 µM of primer, 1 µl of DNA template and 7 µl of nuclease free water. The amplification process began with an initial denaturation at 94°c for 5 minutes followed by 30 cycles of denaturation 94°c, annealing at 55°c and extension at 72°c each lasting 1 minute. A final extension was carried out for 8 minutes at 72°c. After amplification, PCR products (amplicons) were analysed via gel electrophoresis and visualized under ultraviolet light using a gel documentation system.

➢ *Resistance to pH*

The acid resistance of *Lactobacillus Spp.* was assessed using the method of **Tambekar et al., 2010**. The strains were grown overnight in MRS broth, 100 µl of the culture was inoculated into the broths with pre-adjusted pH levels of 2, 3 & 4, alongside a control. After centrifugation, the bacterial pellet was resuspended in PBS. This suspension (100 µl) was added to the prepared pH broths, and the growth was monitored every 4 hours by measuring the optical density at 620nm.

➢ *Bile Tolerance*

The tolerance of bile was determined using a 0.3 percent (w/v) intestinal bile concentration and a 4-hour food stay period in small intestine. MRS broth with a bile concentration of 0.3 percent (w/v) was inoculated with lactic acid bacteria culture overnight and the growth was monitored every hour for 3 intervals by measuring the optical density at 620nm.

➢ *Antimicrobial Activity*

The disc diffusion process was, as defined by **Hassanzadazar et al 2012** was used to evaluate the antimicrobial activity of the lactic acid bacteria isolates. Nutrient agar (20ml) was poured onto the Petri plates. On the surface of nutrient agar, pathogenic strains (*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*) were spread and wells were created using pipette tips wells and 50µl of the culture was introduced into the wells and kept for incubation for overnight. The antimicrobial activity of the *Lactobacillus* isolates was recorded by measuring the width of clear zone. The resultant zone of inhibition (ZOI) formation confirms the activity of the isolates.

III. RESULTS

➢ *Isolation of Lactic Acid Bacteria (LAB)*

The 10 extracted curd samples yielded a total of 3 lactic acid bacteria. The curd samples were taken from different locations from the Hyderabad local. LAB isolates were labelled as A3, A6, B3, B6, A, B(A), S(B), C, D, E based on physiological and biochemical characteristics (Figure,1).

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➢ *Gram Staining and Microscopic Observation of Isolates*

Under an electronic microscope with magnifications of 10x, 45x and 100x, the gram staining technique was performed and observed for the presence of different isolates. Many of the isolates were gram-positive.

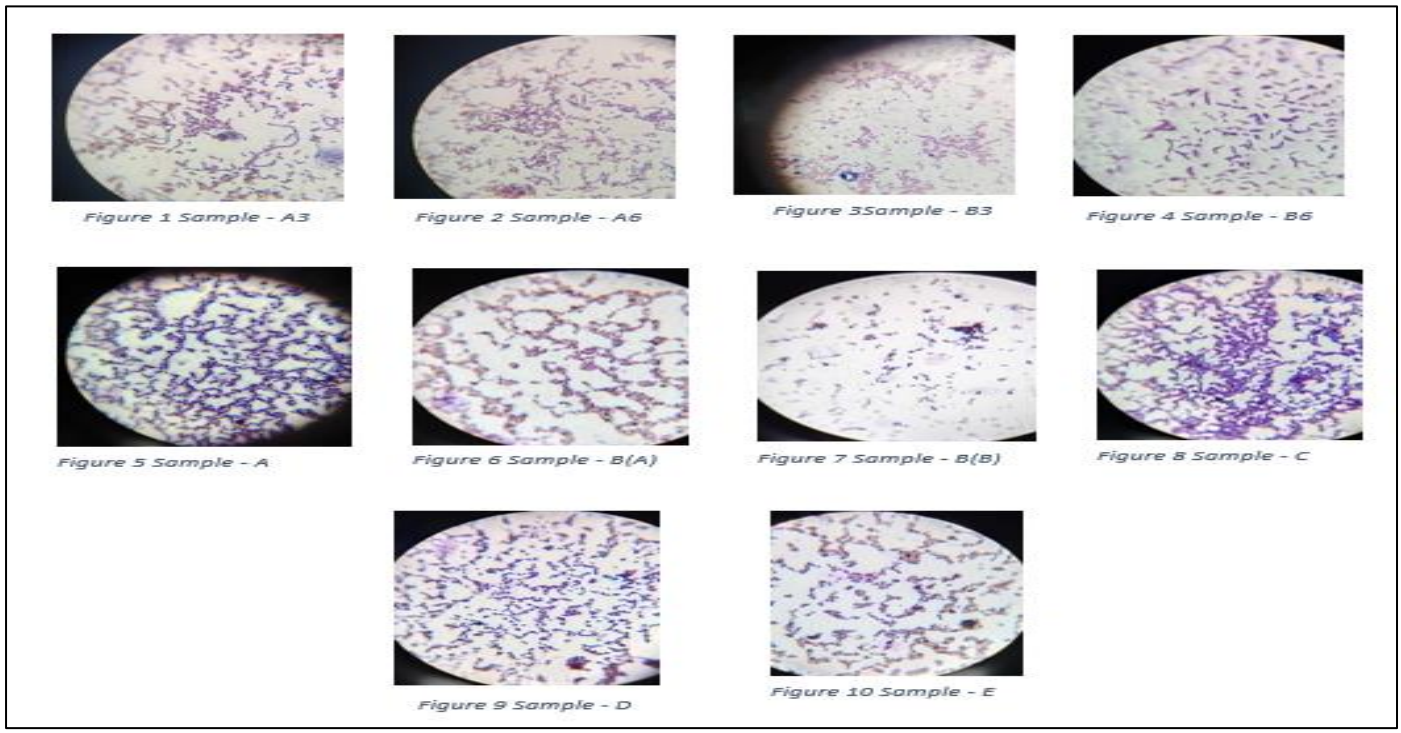

Fig 1 Gram Staining and Microscopic Observation of Probiotic Isolates

➢ *Physiological and Biochemical Characterization*

All studied isolates (Sample A3, A6, B3, B6, A, B(A), B(B), C, D, E) were grown at 37°C according to the biochemical characterization. The findings of IMViC experiments were used to identify *Lactobacillus* strains. Indole production in tryptophan broth was not observed and the results shown as negative, Methyl red was tested positive for all the samples with the indication of red colour and Vogues Proskauer tested Positive for sample B3 and negative for the remaining samples. The Citrate utilization test has shown positive for sample A3, A6, B3, D & E and negative for samples B6, A, B(A), B(B) & C (Figure-2, Table-1).

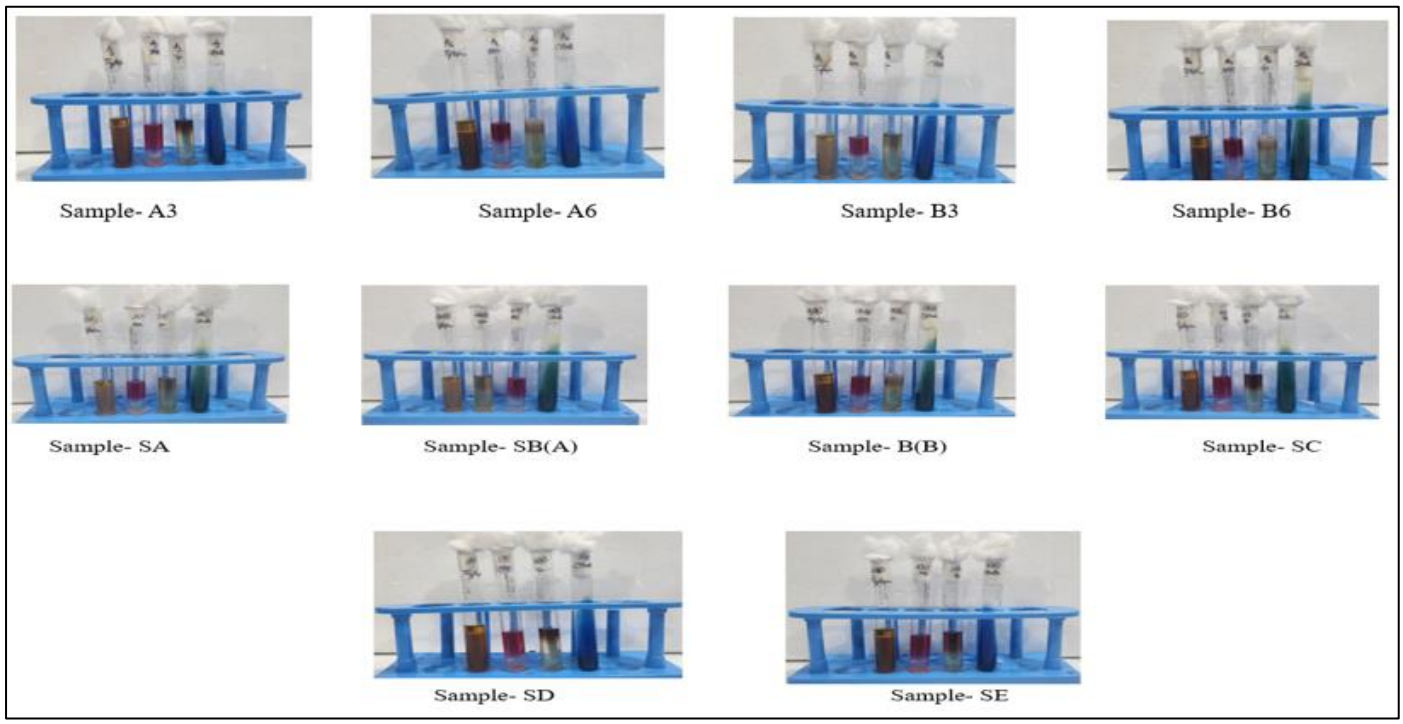

Fig 2 Biochemical Test of Probiotic Isolated Samples

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Table 1 IMViC Test Results of the Probiotic Samples

Sample Name	Indole Test	Methyl Red	Voges Proskauer	Simmon Citrate
Sample - A3	(-ve)	(+ve)	(-ve)	(+ve)
Sample - A6	(-ve)	(+ve)	(-ve)	(+ve)
Sample - B3	(-ve)	(+ve)	(+ve)	(+ve)
Sample - B6	(-ve)	(+ve)	(-ve)	(-ve)
Sample - A	(-ve)	(+ve)	(-ve)	(-ve)
Sample- B(A)	(-ve)	(+ve)	(-ve)	(-ve)
Sample- B(B)	(-ve)	(+ve)	(-ve)	(-ve)
Sample-C	(-ve)	(+ve)	(-ve)	(-ve)
Sample- D	(-ve)	(+ve)	(-ve)	(+ve)
Sample - E	(-ve)	(+ve)	(-ve)	(+ve)

➢ *Genomic DNA Extraction*

The that genomic DNA was effectively isolated from all probiotic samples. All 10 samples (A3, A6, B3, B6, A, B(A), B(B), C, D, and E) yielded equal amounts of genomic, as shown by the strength of the bands in the gel electrophoresis (Figure-3).

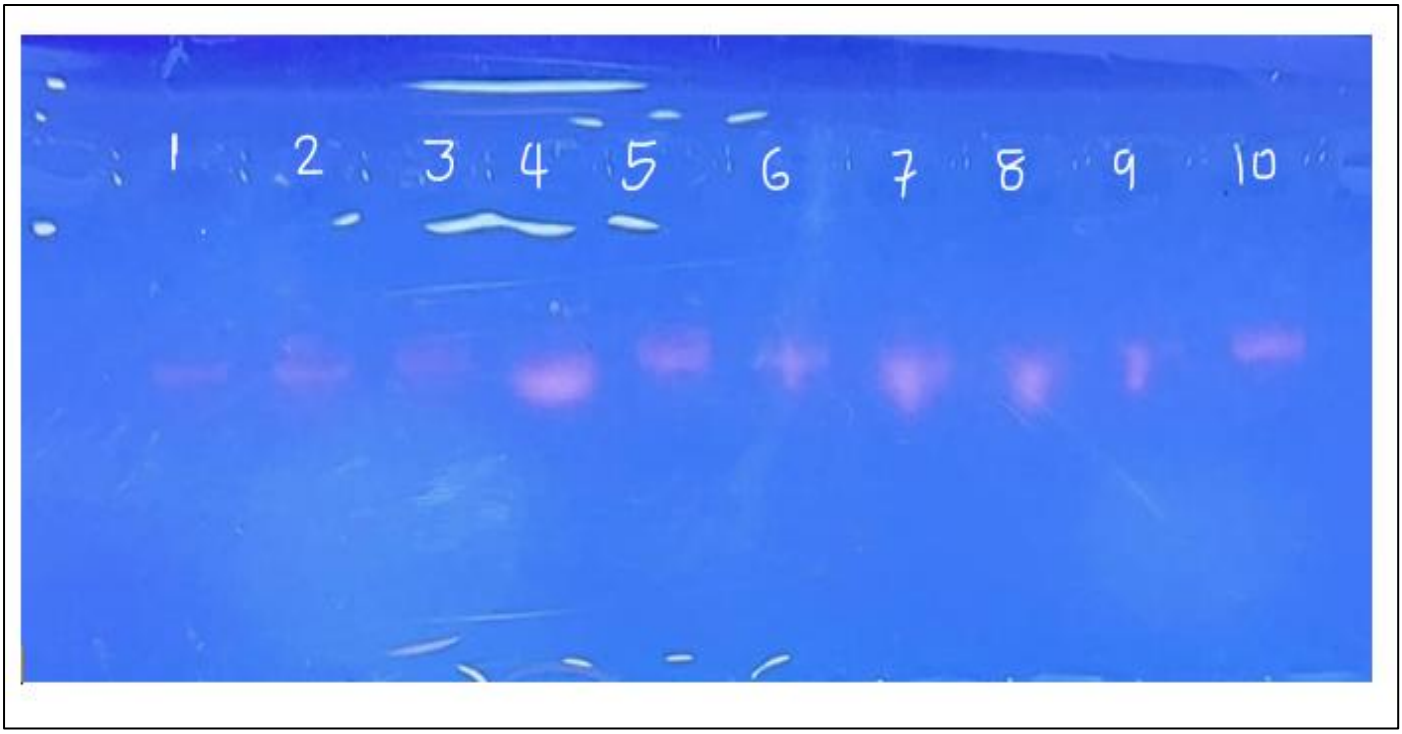

Fig 3 Genomic DNA Extraction and Gel Electrophoresis Visualization

➢ *PCR*

The clear bands observed in the gel electrophoresis, which indicates that the PCR amplification was successful for the probiotic samples, which include A6, B3, B6, A, B(A), B(B), C, D & E. The presence of the distinct bands at the expected positions confirms that the primers used were specific to the target genomic regions, likely related to the genus or species of the probiotic bacteria (Figure-4).

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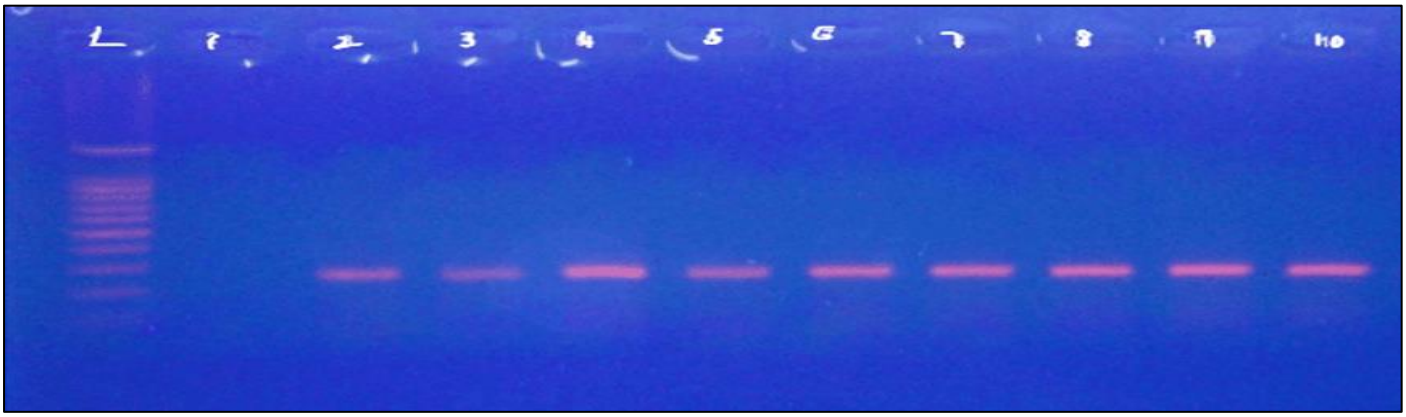

Fig 4 PCR Amplification of Probiotic samples DNA Extracts with Gel Electrophoresis Visualization

➢ *Acid Resistance of Probiotic Isolates*

The highest acid resistance was observed in Sample B(B) under control (neutral pH) conditions. The resistance increased from 0.14 in the first period to 0.48 in the fourth interval, showing a substantial development in resistance. This indicates that sample (B) exhibits a strong capacity to resist stress in neutral pH environments, highlighting its resilience in less acidic conditions. The lowest acid resistance was noted in Sample B(A) at pH 4 (mildly acidic) conditions, where resistance started at 0.02 in the first interval and gradually rose to 0.12 by the 4th hour. This slow increase indicates that sample B(A) demonstrates limited ability to withstand mildly acidic environments, showing the susceptibility to acid stress at pH 4 (Table-2, Figure-5).

Table 2 Acid Resistance of the Probiotic Samples at Different pH and Time Intervals

Sample Name	Sample Type	1 hr	2 hr	3 hr	4 hr
Sample -A3	Control	0.04	0.06	0.07	0.16
	pH 2	0.06	0.08	0.08	0.13
	pH 3	0.06	0.07	0.09	0.14
	pH 4	0.07	0.07	0.07	0.12
Sample-A6	Control	0.06	0.07	0.11	0.21
	pH 2	0.08	0.08	0.09	0.17
	pH 3	0.06	0.08	0.09	0.15
	pH 4	0.05	0.07	0.08	0.16
Sample-B3	Control	0.02	0.03	0.05	0.14
	pH 2	0.04	0.06	0.07	0.09
	pH 3	0.04	0.06	0.08	0.11
	pH 4	0.03	0.05	0.07	0.12
Sample-B6	Control	0.05	0.06	0.08	0.14
	pH 2	0.06	0.08	0.12	0.15
	pH 3	0.09	0.11	0.13	0.15
	pH 4	0.07	0.09	0.11	0.12
Sample-A	Control	0.12	0.21	0.32	0.45
	pH 2	0.12	0.16	0.17	0.19
	pH 3	0.06	0.09	0.11	0.13
	pH 4	0.09	0.11	0.12	0.14
Sample-B(A)	Control	0.09	0.11	0.14	0.21
	pH 2	0.08	0.11	0.12	0.15
	pH 3	0.05	0.07	0.09	0.11
	pH 4	0.02	0.05	0.08	0.12
Sample-B(B)	Control	0.14	0.21	0.37	0.48
	pH 2	0.12	0.14	0.16	0.18
	pH 3	0.03	0.05	0.06	0.09
	pH 4	0.02	0.04	0.05	0.08
Sample-C	Control	0.23	0.28	0.33	0.38
	pH 2	0.21	0.25	0.29	0.32
	pH 3	0.19	0.21	0.25	0.29
	pH 4	0.14	0.17	0.19	0.22
Sample-D	Control	0.13	0.14	0.16	0.18
	pH 2	0.18	0.19	0.21	0.23
	pH 3	0.11	0.14	0.16	0.18
	pH 4	0.12	0.13	0.15	0.16
Sample-E	Control	0.08	0.11	0.15	0.25
	pH 2	0.09	0.11	0.12	0.14
	pH 3	0.07	0.09	0.11	0.13
	pH 4	0.05	0.07	0.09	0.11

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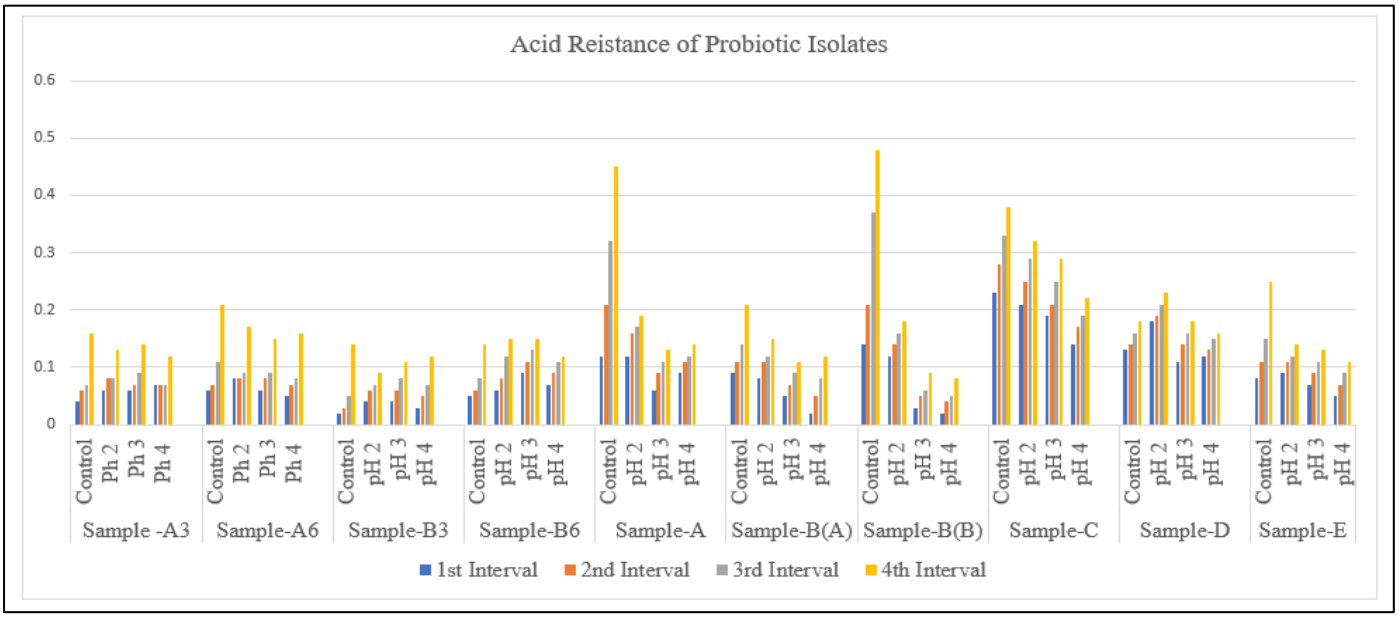

Fig 5 Graphical Representation of Acid Resistance of Probiotic Samples

➢ *Bile Tolerance of Probiotic Isolates*

The highest bile resistance in control of Sample C with no bile is 0.28 and increased significantly by 0.66 by the third interval, showing the most substantial growth in resistance in the absence of the bile. The resistance in the sample which is treated with Bile was much lower but increased slightly from 0.28 to 0.39 b7y the third interval. The lowest bile tolerance in control of sample D is observed from 0.03 to 0.09 increase over three intervals. The bile treated sample followed a similar pattern as the control rising from 0.05 to 0.09 by the third interval, showing almost negligible variation between bile-treated and untreated sample (Table-3, Figure-6)

Table 3 Bile Tolerance of the Probiotic Isolated Samples at Different Interval of Time

Sample Name	Sample Type	1st Interval	2nd Interval	3rd Interval
Sample -A3	Control	0.17	0.29	0.39
	Bile	0.13	0.15	0.17
Sample -A6	Control	0.33	0.57	0.69
	Bile	0.26	0.29	0.3
Sample -B3	Control	0.16	0.28	0.36
	Bile	0.14	0.15	0.16
Sample -B6	Control	0.41	0.56	0.63
	Bile	0.29	0.31	0.35
Sample -A	Control	0.18	0.31	0.41
	Bile	0.14	0.16	0.18
Sample -B(A)	Control	0.17	0.31	0.46
	Bile	0.16	0.18	0.21
Sample -B(B)	Control	0.12	0.18	0.26
	Bile	0.13	0.16	0.19
Sample - C	Control	0.28	0.47	0.66
	Bile	0.28	0.34	0.039
Sample - D	Control	0.03	0.04	0.09
	Bile	0.05	0.07	0.09
Sample - E	Control	0.03	0.05	0.07
	Bile	0.04	0.05	0.06

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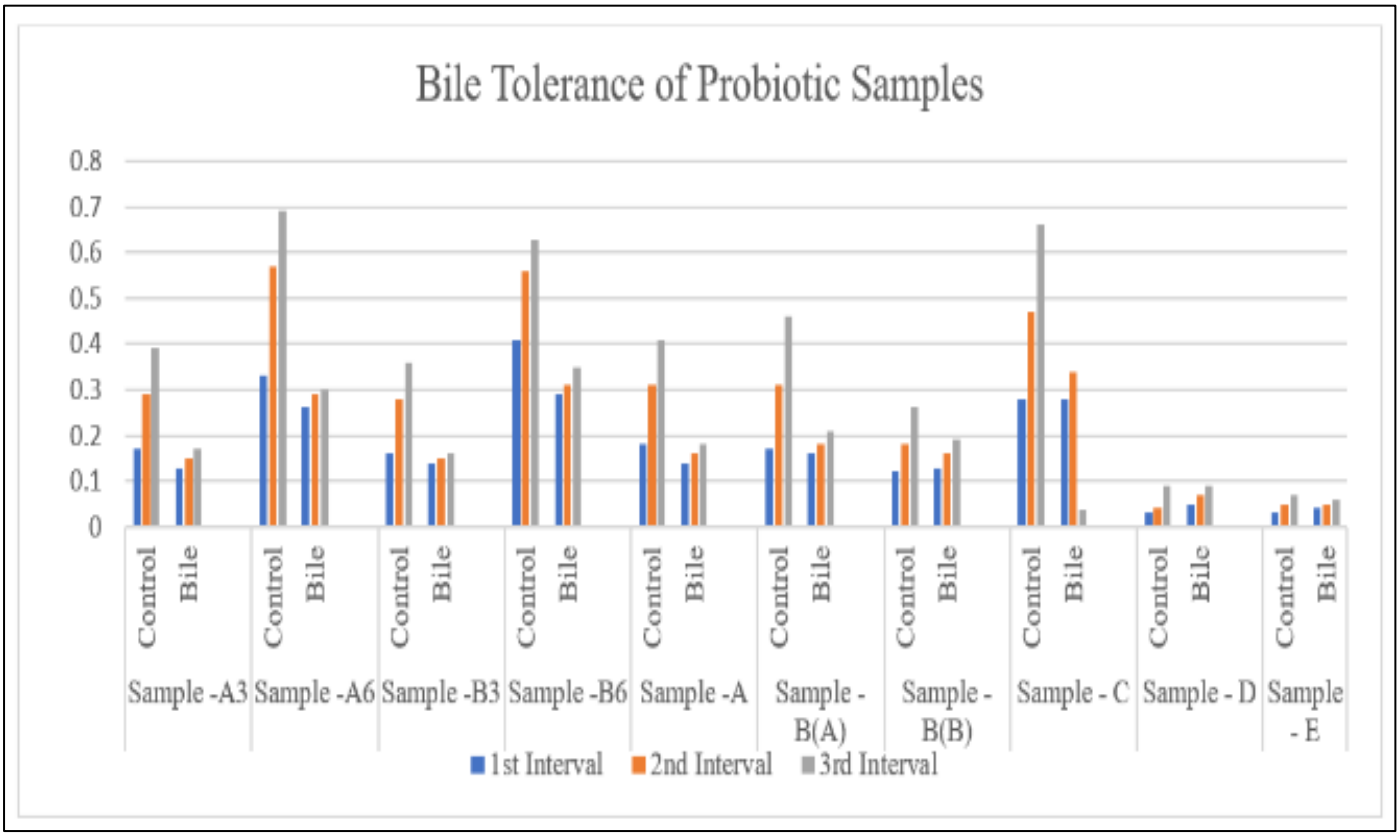

Fig 6 Graphical Representation of Bile Tolerance of Probiotic Isolated Samples

➢ *Antimicrobial Activity*

The antimicrobial activity of different probiotic isolates against three distinct pathogens: *Pseudomonas*, *Staphylococcus*, and *E. coli*. The activity is quantified by the existence and dimensions of the inhibition zone, which reflects the efficacy of each sample in suppressing pathogen development. The results indicate diverse antimicrobial activity of the probiotic isolates against *Pseudomonas*, *Staphylococcus*, and *E. coli*. The most potent action was seen against *Pseudomonas*, exhibiting substantial zones of inhibition in samples A (2.7 mm), B(b) (2.6 mm), and others. Only Sample A (0.6 mm) and B6 (0.2 mm) exhibited minimal inhibition against *Staphylococcus*, whilst the majority of samples shown no impact. Likewise, for E. coli, only Sample A (0.4 mm) and A6 (0.3 mm) exhibited limited activity, while the majority of samples were ineffectual. *Pseudomonas* had the highest susceptibility among pathogens (Figure-7, Table-4, Figure-).

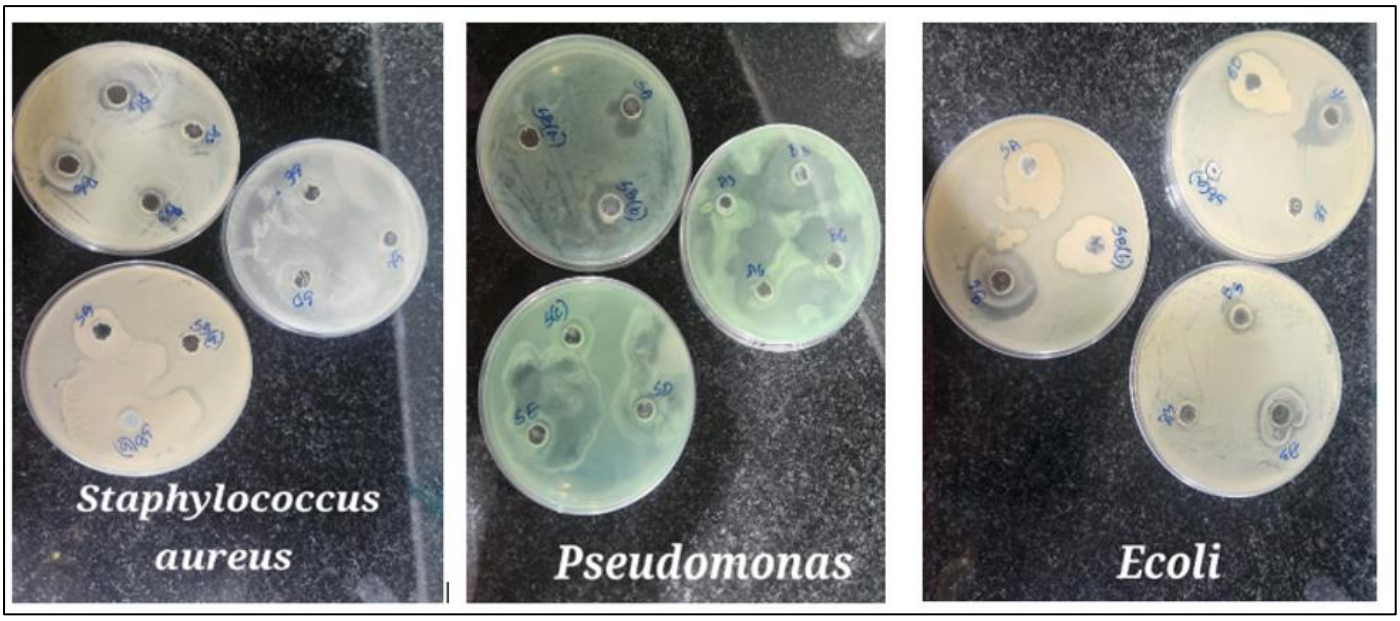

Fig 8 Formation of Zone of Inhibition in Antimicrobial Activity

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Table 4 Antimicrobial Activity of the Probiotic Isolates on Different Strains of Pathogens.

Sample	Pathogen Type	Zone of Inhibition Observed	Measurement of the Zone Observed
Sample -A3	Pseudomonas	Not Observed	-
Sample - A6		Observed	0.3
sample -B3		Not Observed	-
sample - B6		Not Observed	-
sample - A		Observed	2.7
sample - B(a)		Observed	0.9
sample -B(b)		Observed	2.6
sample - C		Observed	0.4
sample - D		Not Observed	-
sample - E		Observed	0.7
Sample -A3	Staphylococcus	Not Observed	-
Sample - A6		Not Observed	-
sample -B3		Not Observed	-
sample - B6		Observed	0.2
sample - A		Observed	0.6
sample - B(a)		Not Observed	-
sample -B(b)		Not Observed	-
sample - C		Not Observed	-
sample - D		Not Observed	-
sample - E		Not Observed	-
Sample -A3	E-coli	Not Observed	-
Sample - A6		Observed	0.3
sample -B3		Not Observed	-
sample - B6		Not Observed	-
sample - A		Observed	0.4
sample - B(a)		Not Observed	-
sample -B(b)		Not Observed	-
sample - C		Not Observed	-
sample - D		Not Observed	-
sample - E		Not Observed	-

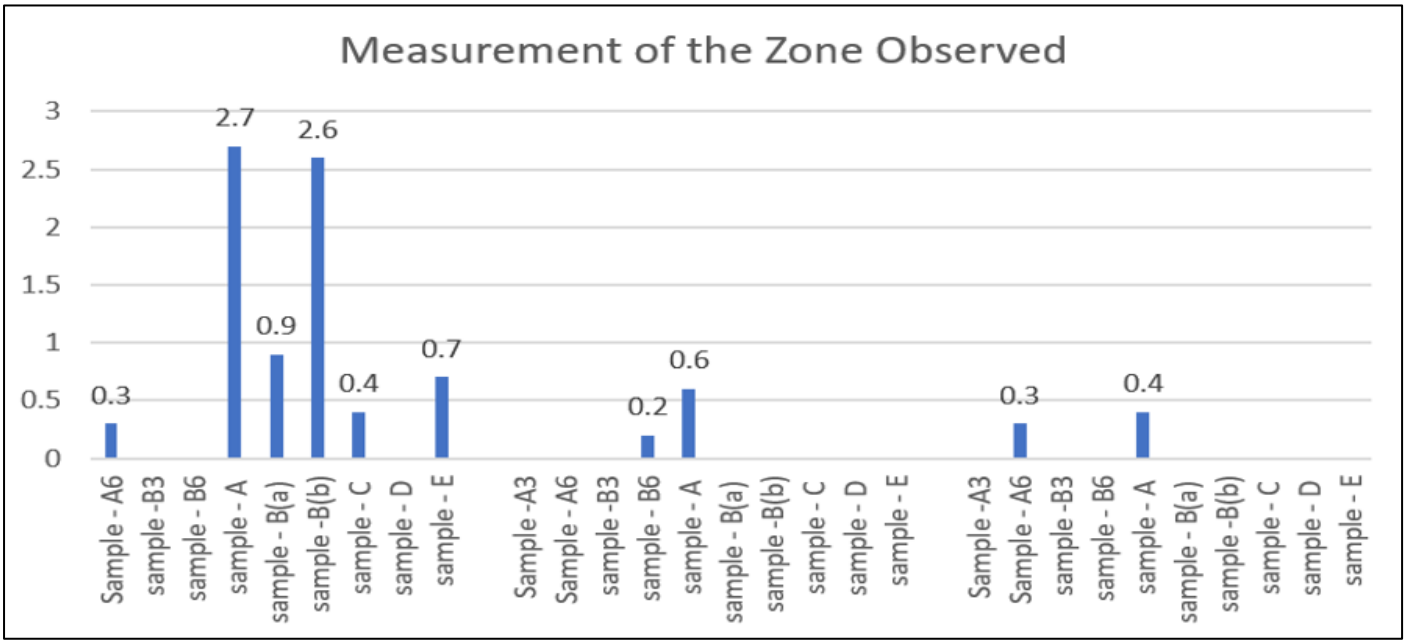

Fig 8 Measurement of Zone of Inhibition

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IV. DISCUSSION

Researchers have been looking for ways to replace hazardous pharmaceuticals with organic alternatives. Various natural substances and technology are now used to prevent or treat infections. One of these methods is the application of probiotics. Lactobacilli and bifidobacteria are natural intestinal flora that perform an important role in the health by avoiding intestinal infection, decreasing cholesterol, stimulating the immune system, and lowering the risk of colon cancer. Probiotic bacteria develop lactic acid and organic acids, lower the pH of the atmosphere, and attempt to keep many bacteria from growing. **Boris et al. (2001)**, found that lactobacilli strains obtained from dairy products suppress the growth of P. aeruginosa, E. coli, and Salmonella typhimurium, exhibiting the most significant inhibitory impact. Different probiotic LAB can be identified using a variety of techniques. The following experiments were used in this investigation:

- *IMViC Tests:*

The IMViC test, comprising the indole, methyl red, Voges-Proskauer, and citrate utilization tests, provided valuable insights into the metabolic profiles of the probiotic samples. The results of these biochemical assays, presented in Table 1, allowed for the differentiation of lactic acid bacteria (LAB), highlighting the utility of the IMViC test in the preliminary identification and characterization of probiotic isolates. Indole production in tryptophan broth was not observed and the results shown as negative, Methyl red was tested positive for all the samples with the indication of red colour and Vogues Proskauer tested Positive for sample B3 and negative for the remaining samples. The Citrate utilization test has shown positive for sample A3, A6, B3, D & E and negative for samples B6, A, B(A), B(B) & C. As per other studies, **Kajal et al., 2017** has Indole test negative, MR-VP test positive, citrate test negative, glucose positive, lactose positive test.

- *DNA Isolation:*

The successful isolation of genomic DNA from all probiotic samples (A3, A6, B3, B6, A, B(A), B(B), C, D, E) using the phenol chloroform method indicates the effectiveness of this technique in extracting high-quality DNA from *Lactobacillus Spp.* The uniform yield across all 10 samples, as evidenced by the equal band intensities in the gel-electrophoresis, suggests that the method was consistent and reliable in isolating genomic DNA from each sample. As per other studies, **Abed et al., 2013** obtained he modified wizard technique yields the greatest DNA purity and yield compared to the other four methods. The DNA yield varied greatly based on the extraction technique utilised. DNA extracted using five procedures and was analysed for degradation using 1% Agarose gel electrophoresis. All DNA extracted using the modified wizard approach showed crisp bands, whereas the other four methods generated smeared bands. Lysozyme doses that resulted in the greatest genomic DNA recovery from all isolates tested were effective.

- *PCR:*

The clear bands were observed, indicates that the PCR amplification was successful for the probiotic samples using (100kb bp) primers, which included A6, B3, B6, A, B(A), B(B), C, D & E. The presence of the distinct bands at the expected positions confirms that the primers used were specific to the target genomic regions, likely related to the genus or species of the probiotic bacteria. As per in the other studies, **Abed et al., 2013** has obtained the PCR results with pure DNA isolated from Lactobacilli and other bacterial isolates is crucial for sensitive and effective biological studies like PCR. The research found that the commercial wizard kit/Promega with modifications was the most effective approach for amplification of *Lactobacillus Spp.* 16S r DNA. PCR of six amplicons (216 bp) for *Lactobacillus spp.* aligns with the target gene size and yields a single band. Observations showed no non-specific amplification products, such as primer dimmers. Amplification of the target gene of *Lactobacillus Spp.* provides a helpful diagnostic tool, indicating particular primers, optimised DNA extraction, and PCR technique.

- *Acid Resistance:*

In Acid resistance, Sample B(B) had the strongest acid resistance when subjected to control circumstances, which would be considered neutral in pH. The resistance showed a significant rise, going from 0.14 in the first period to 0.48 in the fourth interval. This indicates that resistance has been growing significantly. Based on this, it can be concluded that sample (B) has a robust ability to withstand stress in surroundings with a neutral pH, hence showing its resilience in situations with a lower acidity level. The acid resistance of Sample B(A) was found to be the lowest when it was subjected to circumstances with a pH 4 (mildly acidic). The resistance began at 0.02 in the first interval and steadily increased to 0.12 by the fourth hour. This gradual rise provides evidence that sample B(A) has a limited capacity to resist settings that are moderately acidic, indicating that it is susceptible to acid stress at a pH of 4 while in other studies **Shirisha et al., 2021** investigated and demonstrated that five different isolates were able to tolerate a pH of 3. An impressively high pH tolerance has been shown by *Lactobacillus Spp.* The isolates that were able to hold their own at a pH of 3.0 were transferred to the subsequent round of testing.

- *Bile Tolerance:*

The highest bile resistance in control of sample C with no bile is 0.28 and increased significantly by 0.66 by the third interval, showing the most substantial growth in resistance in the absence of the bile. The resistance in the sample which is treated with Bile was much lower but increased slightly from 0.28 to 0.39 b7y the third interval. The lowest bile tolerance in control of sample D is observed from 0.03 to 0.09 increase over three intervals. The bile treated sample followed a similar pattern as the control rising from 0.05 to 0.09 by the third interval, showing almost negligible variation between bile-treated and untreated sample whereas **Shirisha et al., 2021** all of the isolates were resistant to a concentration of 0.3 percent bile salts, including *Lactobacillus delbrueckii*, which

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was much more resistant than the other isolates. The data indicate that all of the isolates were resistant.

➢ *Antimicrobial Activity:*

The antimicrobial activity of different probiotic isolates against three distinct pathogens: *Pseudomonas*, *Staphylococcus*, and *E. coli*. The activity is quantified by the existence and dimensions of the inhibition zone, which reflects the efficacy of each sample in suppressing pathogen development. The results indicate diverse antimicrobial activity of the probiotic isolates against *Pseudomonas*, *Staphylococcus*, and *E. coli*. The most potent action was seen against *Pseudomonas*, exhibiting substantial zones of inhibition in samples A (2.7 mm), B(b) (2.6 mm), and others. Only Sample A (0.6 mm) and B6 (0.2 mm) exhibited minimal inhibition against *Staphylococcus*, whilst the majority of samples shown no impact. Likewise, for E. coli, only Sample A (0.4 mm) and A6 (0.3 mm) exhibited limited activity, while the majority of samples were ineffectual. *Pseudomonas* had the highest susceptibility among pathogens while **Shirisha et al., 2021** had three pathogenic strains were tested and all of the isolates showed antimicrobial activity. The zone of inhibitions' diameters was calculated. The *Lactobacillus* delbrueckii strain has a strong inhibition zone.

V. CONCLUSION

In the present study, biochemical and molecular methods were utilised to characterise the metabolic properties and species of many probiotic strains. The IMViC test and molecular methods revealed strain differentiation information. The investigators further evaluated the cultures' acid and bile tolerance and antibacterial efficacy against numerous pathogens. The strains have varying acid resistance, bile tolerance, and antibiotic activity, suggesting they had diverse gastrointestinal survival potentials and health benefits. The findings may help choose probiotic strains for functional foods and supplements. More research is needed to fully understand the probiotic properties of the strains found in this study. They can live and colonise in vivo, modulate the immune system, and attach to epithelial cells. This research will create effective probiotic compositions that may benefit human health.

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