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IN *VITRO* EVALUATION OF PROBIOTIC STRAINS FOR ANTI-DIABETIC ACTIVITY THROUGH EXPLORING ISOLATION AND CHARACTERIZATION

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ABSTRACT
Recently, bacterial strains isolated from fermented food resources have gained research focus due to their probiotic attributes, as well as their potential to act as antidiabetics and antioxidants that can help manage diabetes milletus. The purpose of this study was to separate twenty-seven bacteria obtained from curd and Yakult samples using selective MRS agar medium to assess the possibility of their inhibitory effects on antidiabetics as well as antioxidant creation utilizing models of in vitro assays. After initial screening process using three methods included colony morphology and microscopy and safety-related biochemical tests which tested Gram reaction and catalase and motility and hemolysis to identify non-pathogenic lactic acid bacteria-like isolates that would continue to be studied. Among the lactic acid bacteria (LAB) isolated, L8 showed the highest antioxidant (DPPH 61.9%, ABTS 65.8%) and antidiabetic activity (α-amylase 58.4%, α-glucosidase 63.5%), followed by L5 and L9, while L2 and L4 exhibited moderate effects, confirming strong strain-specific antioxidant and antidiabetic potential of selected LAB.

KEYWORDS: Probiotics Starins, Lead acid bacteria, Antidiabetic activity, Antioxidant activity, Gastrointestinal survivability.

1. INTRODUCTION

1.1 Diabetes Mellitus and Its Clinical Significance

Diabetes was defined as a chronic metabolic condition characterized by elevated blood sugar levels caused by reduced insulin synthesis, action, or both. While autoimmune β-cell loss in the pancreas caused absolute insulin insufficiency in people with Type 1 diabetes, patients with Type 2 diabetes mellitus have significant issues with insulin resistance and the related relative insulin deficit [1].Hyperglycemia and metabolic imbalance were the outcomes of the metabolic issues brought on by disrupted insulin activity, which impacted the metabolism of proteins, fats, and carbohydrates [2].

Among the signs of diabetes were polyuria, polydipsia, polyphagia, weight loss, and visual impairment. The elevated blood glucose levels resulted in diabetes mellitus, which in turn produced long-term microvascular problems including nephropathy, neuropathy, and retinopathy as well as macrovascular problems like coronary artery disease and cerebrovascular disease. According to reports, one of the main causes of mortality for people with diabetes is cardiovascular disease [3]. Type 1 diabetes mellitus is characterized by insufficient insulin levels, but Type 2 diabetes mellitus is characterized by insulin resistance, which results in a relative shortage of insulin.

1.2 Limitations of Conventional Therapy and Emerging Alternatives

Although pharmacological agents were used for glycemic control, they were accompanied by side effects and medication dependency. Moreover, these agents did not sufficiently counteract oxidative stress, which is implicated in the progression of complications in diabetes. Therefore, agents with antihyperglycemic and antioxidant activities are being investigated.

In this context, probiotics, especially lactic acid bacteria, gained considerable attention due to their metabolic diversity and safety. Lactic acid bacteria from traditional fermented foods showed considerable enzymatic activities, such as proteolytic, amylolytic, and lipolytic activities, and tolerance to acidic and bile environments, which confirmed their probiotic potential [5].

1.3 Metabolic and Functional Attributes of *Lactic Acid Bacteria*

LAB-mediated fermentation of plant substrates resulted in enhanced production of organic acids, flavonoids, peptides, and antioxidant compounds, thereby increasing the nutritional and functional quality of the substrates [6]. The co-fermentation of dairy substrates with specific strains of LAB resulted in enhanced degradation of proteins and bioactive peptides due to synergistic interactions between the microbes [7].

Metabolomic profiling of various strains of Lactiplantibacillus plantarum revealed specific probiotic, immunomodulatory, and antimicrobial attributes of different strains [8]. LAB-mediated fermentation of fruit substrates such as lychee pulp resulted in enhanced physicochemical attributes, bioactive compounds, and antioxidant activity [9]. Similar results were obtained for tomato juice fermented with LAB strains isolated from traditional food products [10].

LAB-mediated fermentation resulted in enhanced physicochemical stability, antioxidant activity, and shelf life attributes for fruit juice substrates such as Dangshan pear juice [11]. The use of probiotic consortia resulted in enhanced metabolic diversity and functional attributes for fermented beverages [12]. Specific strains of LAB, such as Lactococcus lactis, have been found to produce bioactive metabolites and probiotic attributes following metabolomic evaluation [13].

Metagenomic and metabolomic analysis of the fermented soybean products showed the production of organic acids and bioactive compounds in the process of LAB succession [14]. LAB strains from traditional drinks showed good technological properties and metabolic diversity in industrial applications [15]. Moreover, paraprobiotics and postbiotics of probiotics showed immunomodulatory activity due to the production of bioactive metabolites [16].

Fermented grape juice using Lactiplantibacillus plantarum showed improvement in antioxidant activity and metabolic characteristics [17]. Probiotics from artisanal fish products showed anti-inflammatory and functional properties [18]. LAB strains from human-derived sources showed antioxidant peptides and organic acids with probiotic potential [19]. Fermented camel milk using selected bacterial agents showed improvement in metabolites and probiotic potential [20].

2. METHODOLOGY

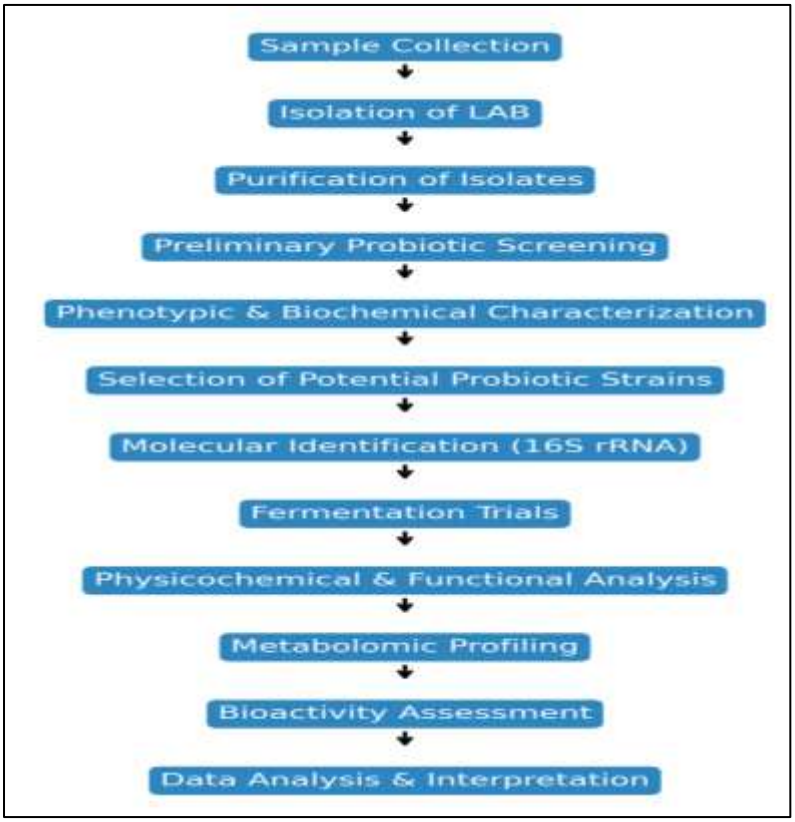


Figure 1: Research Methodology

The fermented milk products, such as Yakult and curd, were removed aseptically from the sterile container and brought to the lab in a refrigerator at 4°C. As soon as the sample was acquired, the analysis was done. The material was serially diluted using a saline solution to extract lactic acid bacteria. The de Man, Rogosa, and Sharpe (MRS) medium was covered with the sample.

After that, the medium was incubated for 24 to 48 hours at 37°C. The morphological traits of the isolated lactic acid bacteria were used to identify them. The procedure of streaking over the MRS medium was repeated in order to purify the isolated lactic acid bacteria. The capacity of the isolated lactic acid bacteria to endure in bile salts (0.3% w/v) and

acidic pH values (2.0 and 3.0) was used as a preliminary screening method. For additional research, the bacteria with the best survival rates were selected.

The selected isolates were phenotypically and biochemically characterized using Gram staining, catalase production, carbohydrate fermentation, and enzymatic activity profiles. The probiotic candidates were then identified using molecular techniques such as 16S rRNA gene sequencing. The selected probiotic candidates were then tested using in vitro fermentation trials, where parameters such as pH, acidity, sugar metabolism, and growth of microorganisms were measured. The selected probiotics were then analyzed using post-fermentation methods such as physicochemical properties, antioxidant and antimicrobial activities, and production of bioactive compounds. Metabolomic analysis of the selected probiotics was also carried out to determine the metabolites of the probiotics. The data generated in this study were analyzed statistically to determine the differences and relationships between the variables.

3. RESULTS

The current research examined six different aspects of probiotic bacterial strains which were obtained from fermented food products. The researchers first collected twenty-seven bacterial isolates from curd and Yakult samples. The researchers selected five isolates which showed the best probiotic properties for advanced testing which included molecular analysis and metabolic assessment and functional testing. The results are presented in a systematic manner which uses quantitative tables and graphical representations to demonstrate the specific probiotic and antidiabetic and antimicrobial and antioxidant capabilities of each strain.

3.1 Isolation and Preliminary Screening of Probiotic Bacteria

Isolation of Probiotic Bacteria

A total of 27 bacterial isolates were obtained from different samples of fermented food products such as curd, Yakult probiotic drink, fermented mango pickle, fermented carrot pickle, and fermented idli batter using selective MRS agar medium. The reason behind the selection of the MRS agar medium is that it is selective and supports the growth of lactic acid bacteria that are naturally found in fermented food products. The isolated bacteria were incubated and observed based on their morphology.

Based on the morphology of the bacteria that is characteristic of lactic acid bacteria, 18 isolates were shortlisted and designated as L1, L2, L3, and so on till L18. The bacteria that were shortlisted were those that exhibited characteristics such as white and creamy, circular, smooth, and convex on the agar medium.

Source-wise Distribution of Isolates

Table 1: Source-wise Distribution of Isolates

Source	Number of Samples	Total Isolates	Morphologically Selected
Curd	2	6	4
Yakult probiotic drink	2	5	3
Fermented mango pickle	2	6	4
Fermented carrot pickle	2	5	3
Fermented idli batter	2	5	4
Total	**10**	**27**	**18**

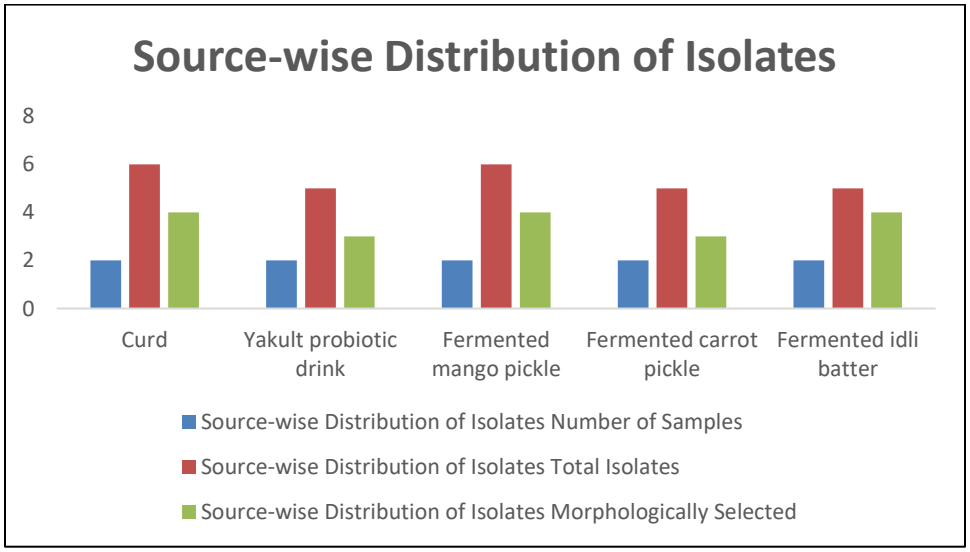
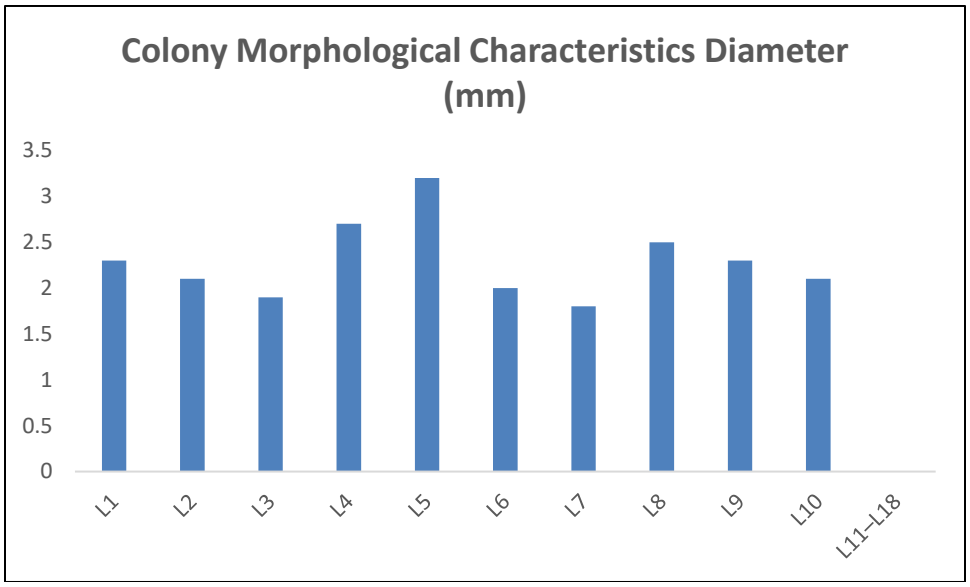


Figure 1: Source-wise Distribution of Isolates

A total of 27 isolates of bacteria were obtained from the fermented food samples used in this study. The results obtained from this study reveal that fermented food is a rich source of probiotic bacteria. Out of the total bacteria obtained from the fermented food samples, 18 bacteria were shortlisted for the screening and characterization of bacteria based on the morphological characteristics of the bacteria colonies, which resemble lactic acid bacteria. These bacteria were marked from L1 to L18 and were further used for the microscopic study in the subsequent experiments. The reduction in the number of bacteria from 27 to 18 is due to the preliminary screening criteria for selecting lactic acid bacteria colonies.

3.1.2 Colony Morphological Characteristics

After 48 hours of incubation, the isolates formed distinct colonies, and all of them belonged to lactic acid bacteria. The colonies formed by the isolates were predominantly white or creamy, circular in shape, and convex or raised in elevation. They also had smooth margins. The size of the colonies formed by the isolates varied from 1.8 to 3.2 mm. The morphological characteristics of the isolates confirmed their affiliation to lactic acid bacteria.



Values expressed as mean ± SD (n = 3)

Figure 2: Colony Morphological Characteristics Diameter (mm)

3.1.3 Microscopic and Biochemical Characterization

The microscopic test confirmed that all isolates were Gram-positive rod-shaped bacteria that occurred singly or in short chains (Table 3; Figure 3). The isolates were not able to form spores and movement capabilities, which are important characteristics in identifying probiotic bacteria. The results obtained in this study were used to enable other researchers to carry out safety-related biochemical studies.

Table 2: Microscopic Characteristics

Parameter	**Observation**
Gram reaction	Gram-positive
Cell shape	Rod-shaped
Arrangement	Single or short chains

Spore formation	Absent

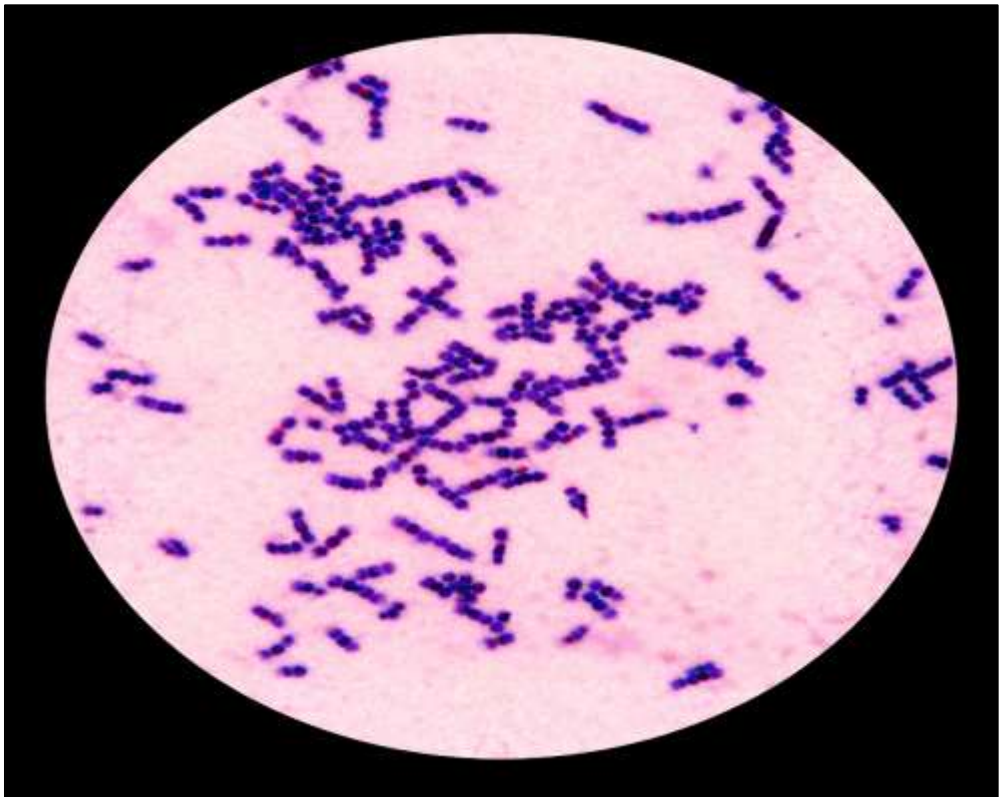


Figure 3: Gram-stained microscopic view (1000×) of selected isolates showing Gram-positive, rod-shaped bacteria arranged singly and in short chains.

3.1.4 Safety-related Biochemical Screening

The researchers conducted tests on all isolates to determine their catalase activity, motility, hemolysis, indole production, and nitrate reduction capabilities (Table 4).

Table 3: Biochemical Test Results	
Test	**Observation**
Catalase activity	Negative
Motility	Non-motile
Hemolysis on blood agar	γ-hemolytic (non-hemolytic)
Indole production	Negative
Nitrate reduction	Negative

The five isolates which included L2, L4, L5, L8 and L9 showed non-catalase activity while they remained non-motile and non-hemolytic (γ-hemolysis), thus satisfying crucial safety standards for probiotic use (Table 5). The researchers chose these isolates to conduct subsequent molecular and functional investigations.

Table 4: Preliminary Safety and Phenotypic Screening of All Isolated Probiotic Strains (n = 18)					
Isolate Code	**Gram Reaction**	**Catalase Activity**	**Motility**	**Haemolytic Activity**	**Suitability for Further Screening**
L1	Positive	Negative	Non-motile	γ (Non-haemolytic)	No
L2	Positive	Negative	Non-motile	γ (Non-haemolytic)	**Yes**
L3	Positive	Negative	Non-motile	β (Haemolytic)	No

L4	Positive	Negative	Non-motile	γ (Non-haemolytic)	**Yes**
L5	Positive	Negative	Non-motile	γ (Non haemolytic)	**Yes**
L6	Positive	Positive	Non-motile	γ (Non-haemolytic)	No
L7	Positive	Negative	Motile	γ (Non-haemolytic)	No
L8	Positive	Negative	Non-motile	γ (Non-haemolytic)	**Yes**
L9	Positive	Negative	Non-motile	γ (Non-haemolytic)	**Yes**
L10	Positive	Negative	Motile	γ (Non-haemolytic)	No
L11	Positive	Positive	Non-motile	γ (Non-haemolytic)	No
L12	Positive	Negative	Motile	γ (Non-haemolytic)	No
L13	Positive	Negative	Non-motile	α (Haemolytic)	No
L14	Positive	Negative	Non-motile	β (Haemolytic)	No
L15	Positive	Positive	Motile	γ (Non-haemolytic)	No
L16	Positive	Negative	Motile	γ (Non-haemolytic)	No
L17	Positive	Positive	Non-motile	γ (Non-haemolytic)	No
L18	Positive	Negative	Non-motile	α (Haemolytic)	No

3.2 Molecular Characterization of Selected Probiotic Isolates

Molecular identification of the selected probiotic isolates was performed using 16S rRNA gene sequencing. The analysis confirmed that the isolates belonged to lactic acid bacteria, predominantly within the genus *Lactobacillus*. The identified strains corresponding to isolates L2, L4, L5, L8, and L9 were classified as probiotic lactic acid bacteria based on sequence similarity with reference strains

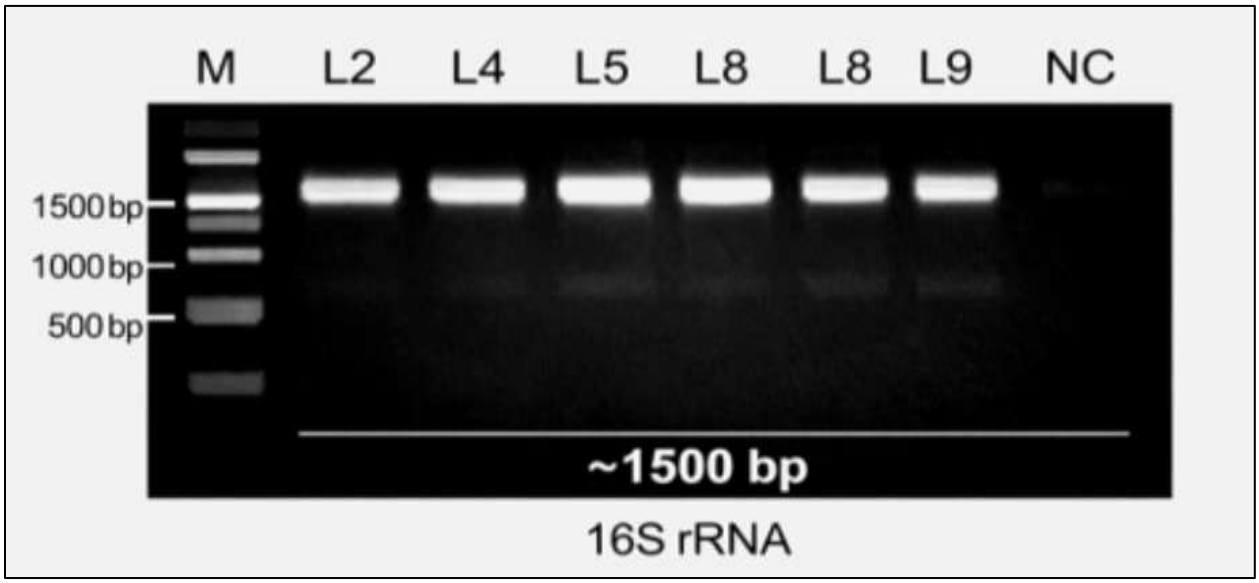


Figure 4: PCR amplification of the 16S rRNA gene (~1500 bp) in selected probiotic isolates. Lane M: DNA marker; lanes L2, L4, L5, L8, and L9 represent probiotic isolates; NC: negative control.

The functional gene analysis identified essential glucose metabolism genes which included glk and pfk and ldh in every isolate according to Tables 6 and Figure 5. The results established both the taxonomic identity and metabolic capacity of the selected probiotic strains.

Table 6: Detection of Glucose Metabolism–Related Functional Genes

Isolate Code	glk (320 bp)	pfk (410 bp)	ldh (290 bp)
L2	+	+	+
L4	+	+	+
L5	+	+	+
L8	+	+	+
L9	+	+	+
NC	-	-	-

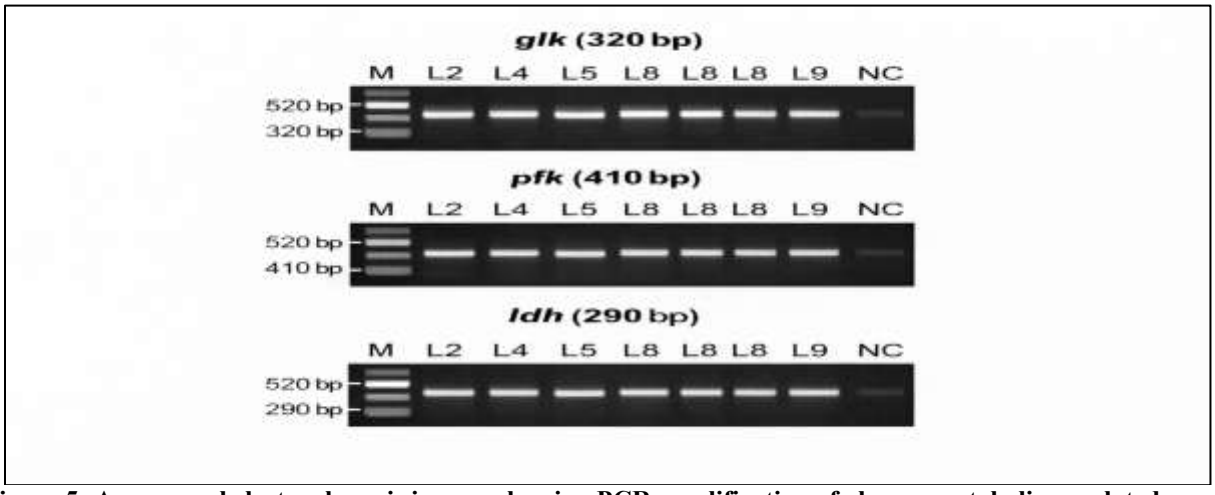


Figure 5: Agarose gel electrophoresis images showing PCR amplification of glucose metabolism–related genes (*glk* ~320 bp, *pfk* ~410 bp, and *ldh* ~290 bp) in selected probiotic isolates. Lane M: DNA marker; lanes L2, L4, L5, L8, and L9 represent probiotic isolates; NC: negative control.

3.3 Metabolic Profiling of Selected Probiotic Isolates

3.3.1 Glucose Utilization and Lactic Acid Production

The selected isolates achieved a high glucose utilization rate which ranged from 76.0 to 84.5 percent while they retained only minimal residual glucose according to Table 7 and Figure 6.

Table 7: Glucose Utilization Ability of Selected Isolates

Isolate Code	Initial Glucose (%)	Residual Glucose (%)	Glucose Utilization (%)
L2	2.0	0.48 ± 0.03	76.0 ± 1.5
L4	2.0	0.42 ± 0.02	79.0 ± 1.2
L5	2.0	0.36 ± 0.02	82.0 ± 1.4

L8	2.0	0.31 ± 0.01	84.5 ± 1.3
L9	2.0	0.39 ± 0.02	80.5 ± 1.1

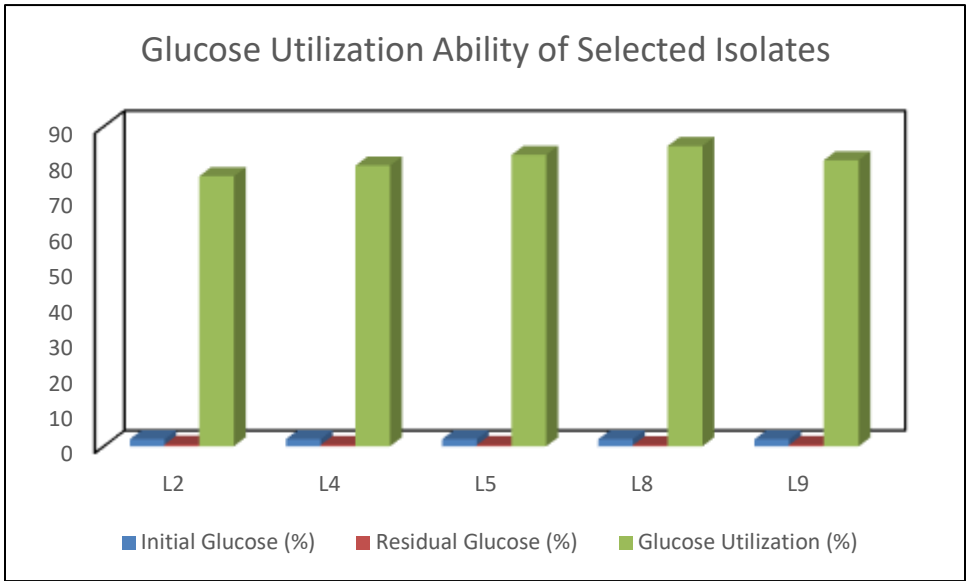


Figure 6: Glucose Utilization Ability of Selected Isolates

The tests showed that the isolates produced lactic acid at a rate between 7.8 and 9.3 grams per liter while the pH level showed a major decrease according to Table 8 and Figure 7. The L8 isolate demonstrated superior metabolic efficiency because its glycolytic genes were confirmed through molecular testing.

Table 8: Lactic Acid Production by Selected Probiotic Isolates

Isolate Code	Lactic Acid Produced (g/L)	Final pH
L2	7.8 ± 0.3	4.3 ± 0.1
L4	8.4 ± 0.2	4.1 ± 0.1
L5	8.9 ± 0.3	3.9 ± 0.1
L8	9.3 ± 0.4	3.8 ± 0.1
L9	8.6 ± 0.2	4.0 ± 0.1

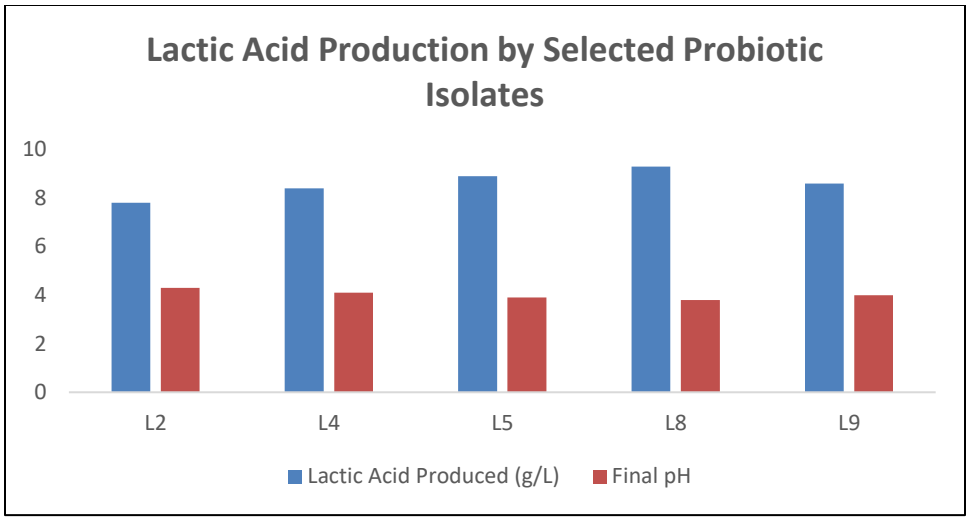


Figure 7: Lactic Acid Production by Selected Probiotic Isolates

3.3.2 Effect of Metformin on Glucose Metabolism

The presence of metformin reduced glucose consumption by all isolates; however, the isolates maintained most of their metabolic functions according to Table 9 and Figure 8. The L8 isolate showed the best glucose consumption ability which demonstrated its ability to withstand antidiabetic treatments.

Table 9: Effect of Metformin on Glucose Metabolism of Selected Probiotic Isolates

Isolate Code	Glucose Utilization Without Metformin (%)	Glucose Utilization With Metformin (%)
L2	76.0 ± 1.5	72.3 ± 1.4
L4	79.0 ± 1.2	75.5 ± 1.3
L5	82.0 ± 1.4	78.1 ± 1.2
L8	84.5 ± 1.3	80.8 ± 1.1
L9	80.5 ± 1.1	77.0 ± 1.0

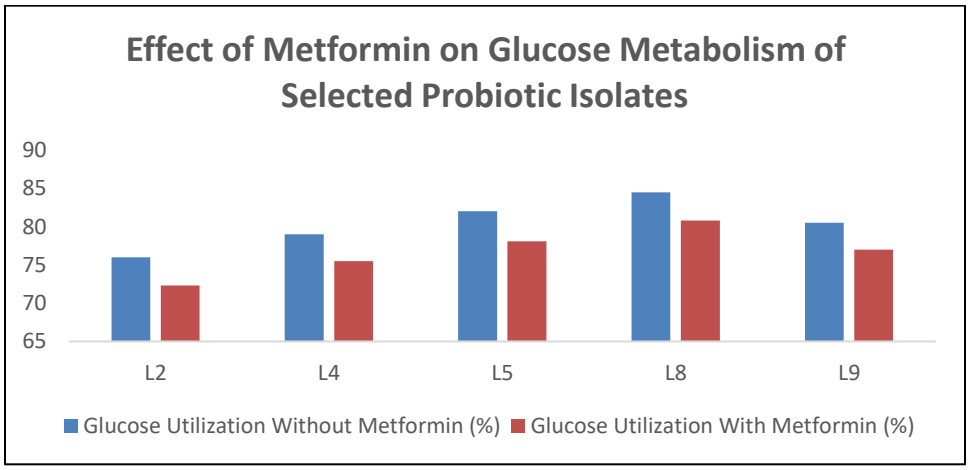


Figure 8: Effect of Metformin on Glucose Metabolism of Selected Probiotic Isolates

3.4 Gastrointestinal Survivability of Selected Isolates

All isolates exhibited high survival rates under simulated gastric (pH 2.0 and 3.0) and intestinal (0.3% bile) conditions (Tables 10 and 11; Figures 9 and 10). L5 and L8 showed exceptional survival ability through all tests while L2 L4 and L9 maintained their survival ability at high levels. The overall gastrointestinal survival assessment showed that all isolates qualified as effective probiotic strains (Table 12).

Table 10: Acid Tolerance of Selected Probiotic Isolates- Survival rate

Isolate Code	pH 2.0 (%)	pH 3.0 (%)
L2	68.5 ± 1.6	85.2 ± 1.4
L4	72.1 ± 1.5	88.6 ± 1.3
L5	70.6 ± 1.4	90.0 ± 1.2
L8	74.3 ± 1.3	91.4 ± 1.1
L9	69.8 ± 1.5	87.6 ± 1.4

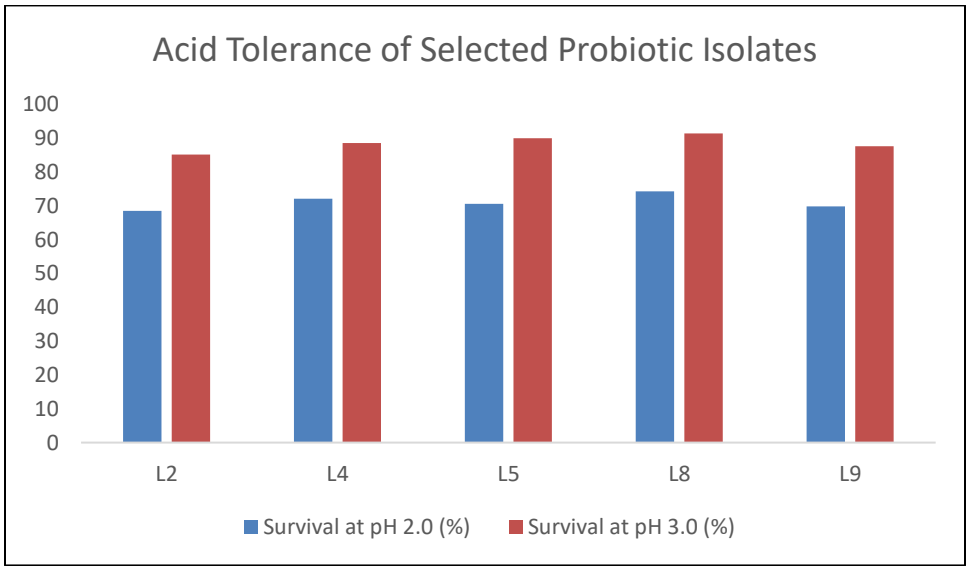


Figure 9: Acid Tolerance of Selected Probiotic Isolates

Table 11: Bile Salt Tolerance of Selected Probiotic Isolates

Isolate Code	Survival in 0.3% Bile (%)
L2	76.0 ± 1.4
L4	81.4 ± 1.5
L5	83.8 ± 1.3
L8	86.1 ± 1.2
L9	79.6 ± 1.4

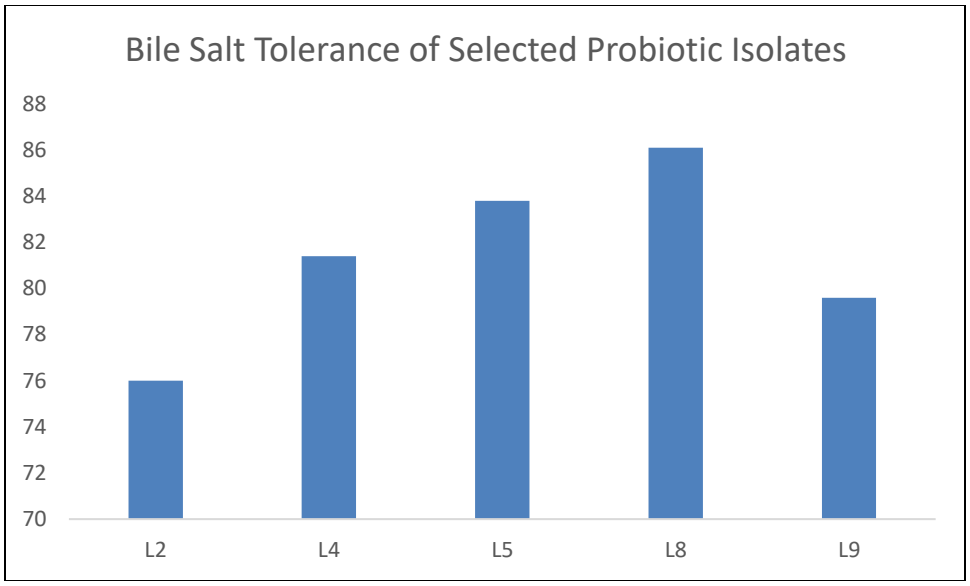


Figure 10: Bile Salt Tolerance of Selected Probiotic Isolates

Table 52: Gastrointestinal Survivability Profile of Selected Isolates

Isolate Code	Acid Tolerance (pH 2.0)	Acid Tolerance (pH 3.0)	Bile Tolerance (0.3%)	GI Survivability Status
L2	High	High	High	Suitable
L4	High	High	High	Suitable
L5	High	Very High	Very High	Highly Suitable
L8	Very High	Very High	Very High	Highly Suitable
L9	High	High	High	Suitable

3.5 Antimicrobial Activity

Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, and E. coli were all susceptible to the broad-spectrum antibacterial activity of the chosen probiotic isolates (Table 13; Figure 11).

Table 13: Antimicrobial Activity of Selected Probiotic Isolates against Pathogenic Bacteria

Isolate Code	*E. coli* (mm)	*Staphylococcus aureus* (mm)	*Salmonella typhi* (mm)	*Pseudomonas aeruginosa* (mm)
L2	12.4 ± 0.6	14.2 ± 0.5	11.8 ± 0.4	10.6 ± 0.5
L4	14.1 ± 0.5	16.0 ± 0.6	13.5 ± 0.5	12.3 ± 0.4
L5	15.6 ± 0.6	17.8 ± 0.5	14.9 ± 0.6	13.7 ± 0.5
L8	17.2 ± 0.5	19.1 ± 0.6	16.4 ± 0.5	15.2 ± 0.4
L9	14.8 ± 0.4	16.5 ± 0.5	13.9 ± 0.4	12.8 ± 0.5

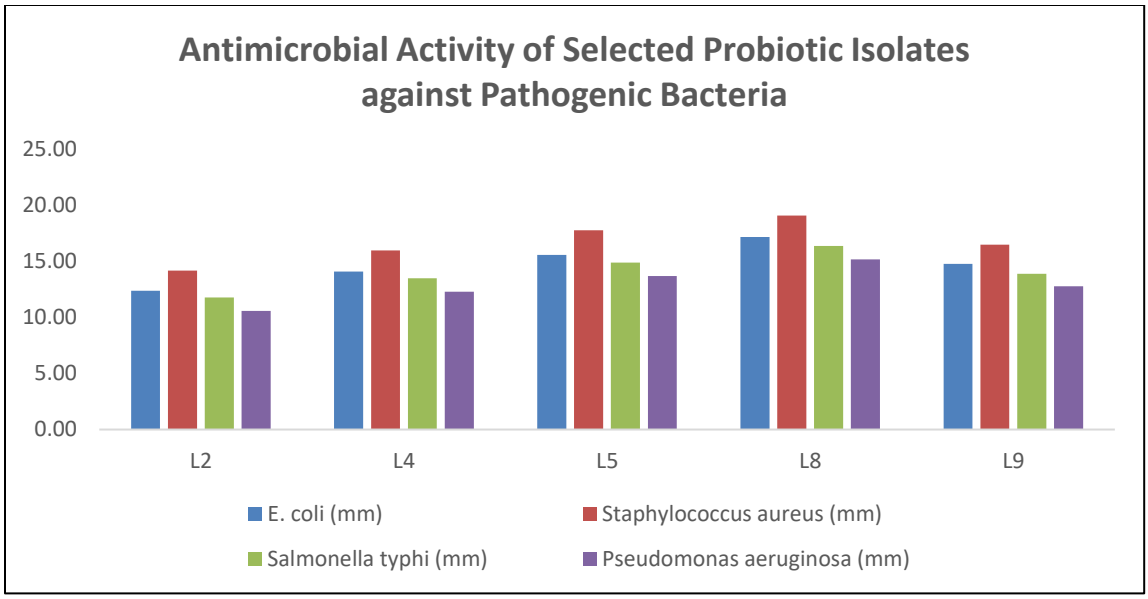


Figure 21: Antimicrobial Activity of Selected Probiotic Isolates against Pathogenic Bacteria

The largest zones of inhibition resulted from isolate L8 which was followed by L5 and L4. Comparative analysis ranked L8 as having very high antimicrobial activity, highlighting its strong pathogen-inhibitory potential (Table 14; Figure 12)

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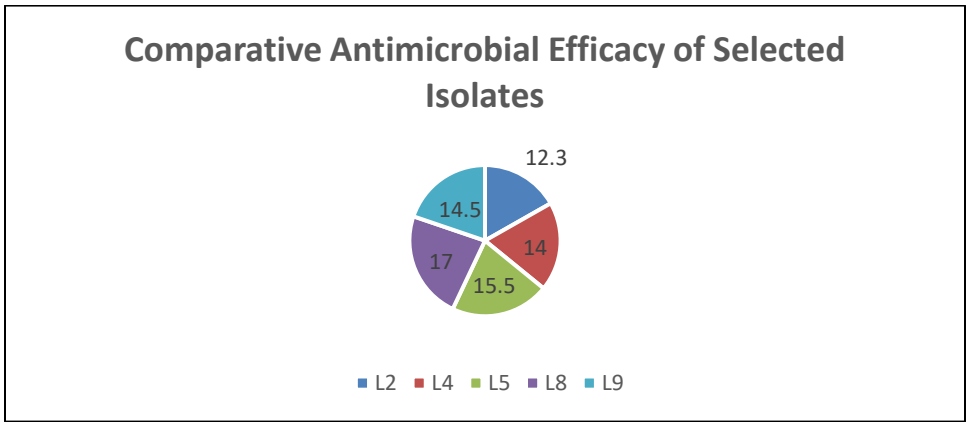
Table 64: Comparative Antimicrobial Efficacy of Selected Isolates

Isolate Code	Mean Zone of Inhibition (mm)	Antimicrobial Activity Level
L2	12.3 ± 0.5	Moderate
L4	14.0 ± 0.5	High
L5	15.5 ± 0.6	High
L8	17.0 ± 0.5	Very High
L9	14.5 ± 0.4	High



Values expressed as mean zone of inhibition (mm) ± SD (n = 3)

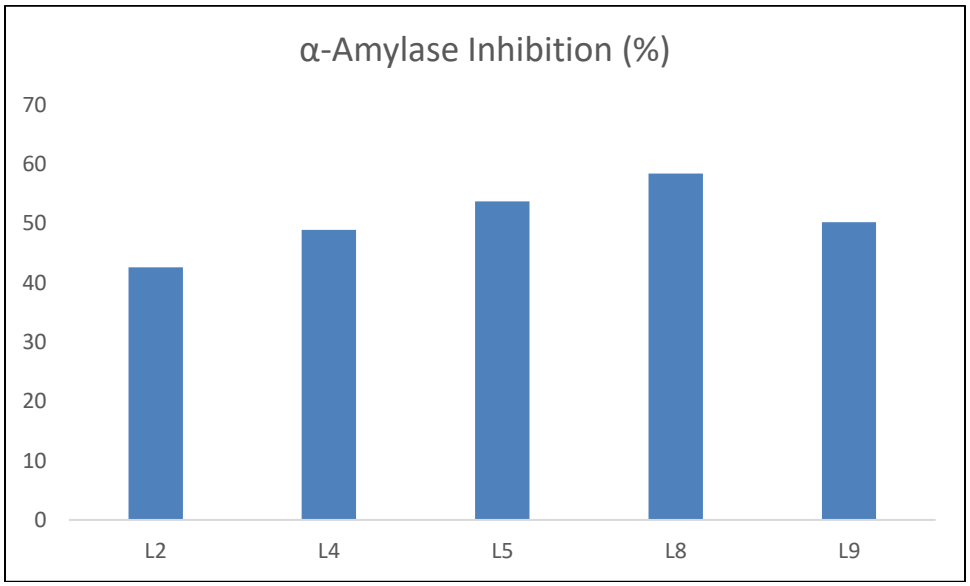
Figure 12: Comparative Antimicrobial Efficacy of Selected Isolates

3.6 In Vitro Antidiabetic Activity

The study demonstrated that all tested isolates reached significant levels of α-amylase inhibition which ranged from 42.6 to 58.4 percent and of α-glucosidase inhibition which ranged from 46.3 to 63.5 percent (Tables 15 and 16; Figures 13 and 14).

Table 15: α-Amylase Inhibitory Activity of Selected Probiotic Isolates

Isolate Code	α-Amylase Inhibition (%)
L2	42.6 ± 1.4
L4	48.9 ± 1.5
L5	53.7 ± 1.6
L8	58.4 ± 1.7
L9	50.2 ± 1.5



Values expressed as mean ± SD (n = 3)

Figure 13: α-Amylase Inhibitory Activity of Selected Probiotic Isolates

Table 16: α-Glucosidase Inhibitory Activity of Selected Probiotic Isolates

Isolate Code	α-Glucosidase Inhibition (%)
L2	46.3 ± 1.3
L4	52.1 ± 1.4
L5	57.8 ± 1.5
L8	63.5 ± 1.6
L9	54.6 ± 1.4

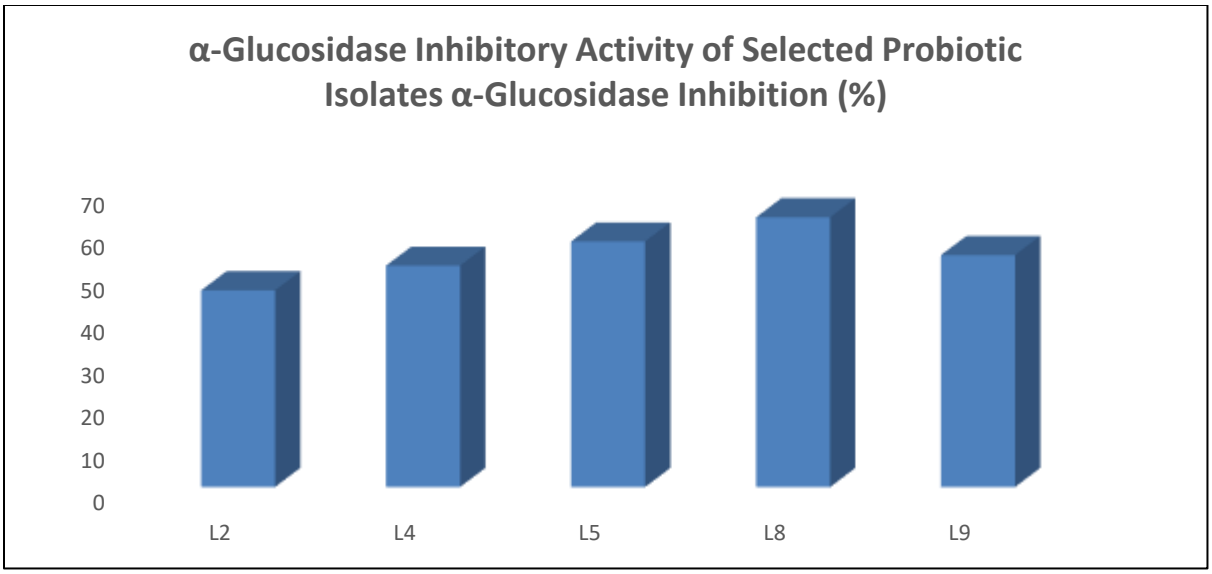


Figure 14: α-Glucosidase Inhibitory Activity of Selected Probiotic Isolates

The isolate L8 demonstrated the strongest inhibitory effect which other isolates L5 and L9 followed. The analysis showed that each strain had its own unique ability to fight diabetes because all tested strains showed stronger α-glucosidase inhibition than α-amylase inhibition (Table 17; Figure 15).

Table 17: Comparative Antidiabetic Enzyme Inhibition Profile of Selected Isolates

Isolate Code	α-Amylase Inhibition (%)	α-Glucosidase Inhibition (%)	Overall Antidiabetic Potential
L2	42.6	46.3	Moderate
L4	48.9	52.1	Moderate–High
L5	53.7	57.8	High
L8	58.4	63.5	Very High
L9	50.2	54.6	High

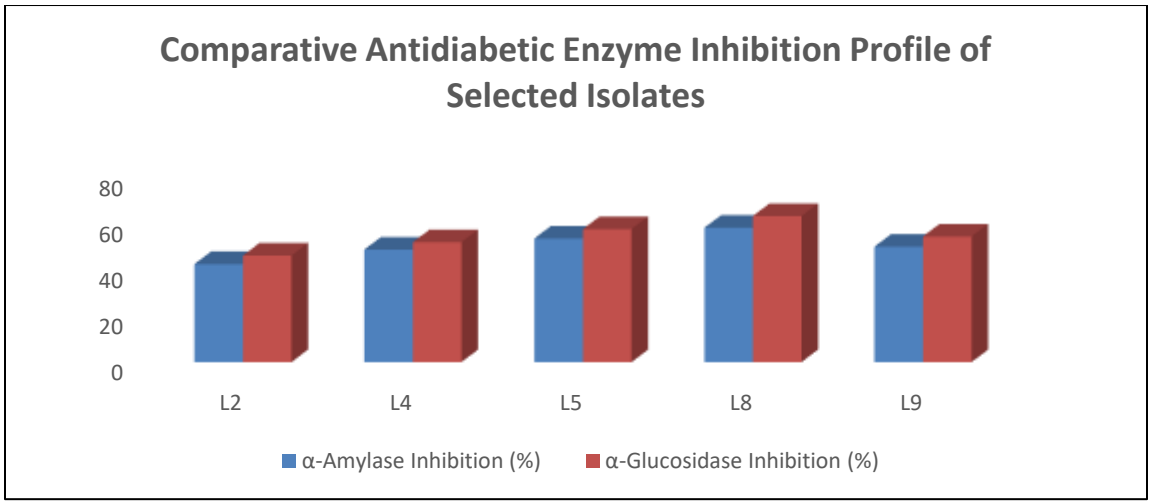


Figure 15: Comparative Antidiabetic Enzyme Inhibition Profile of Selected Isolates

3.7 Antioxidant Activity

The complete set of isolates showed excellent antioxidant performance during the DPPH test and ABTS test (Tables 18 and 19; Figures 16 and 17.

Table 18: DPPH Radical Scavenging Activity of Selected Probiotic Isolates

Isolate Code	DPPH Radical Scavenging Activity (%)
L2	45.8 ± 1.4
L4	51.2 ± 1.5
L5	56.7 ± 1.6
L8	61.9 ± 1.7
L9	53.6 ± 1.5

Values expressed as mean ± SD (n = 3)

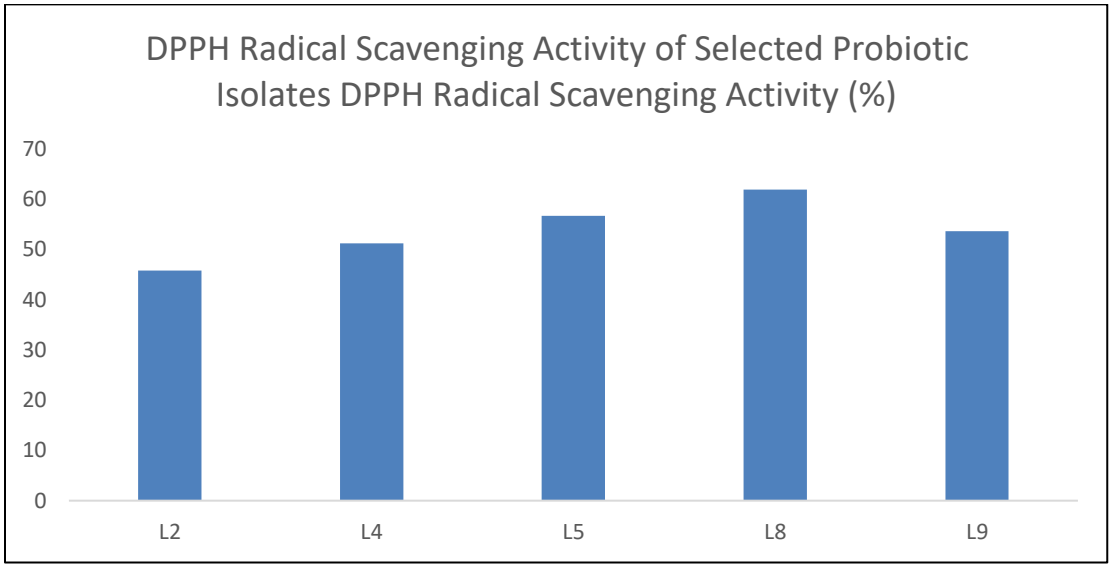


Figure 16: DPPH Radical Scavenging Activity of Selected Probiotic Isolates

Table 7: ABTS Radical Scavenging Activity of Selected Probiotic Isolates

Isolate Code	ABTS Radical Scavenging Activity (%)
L2	48.6 ± 1.3
L4	54.9 ± 1.4
L5	60.3 ± 1.5
L8	65.8 ± 1.6

L9	57.1 ± 1.4

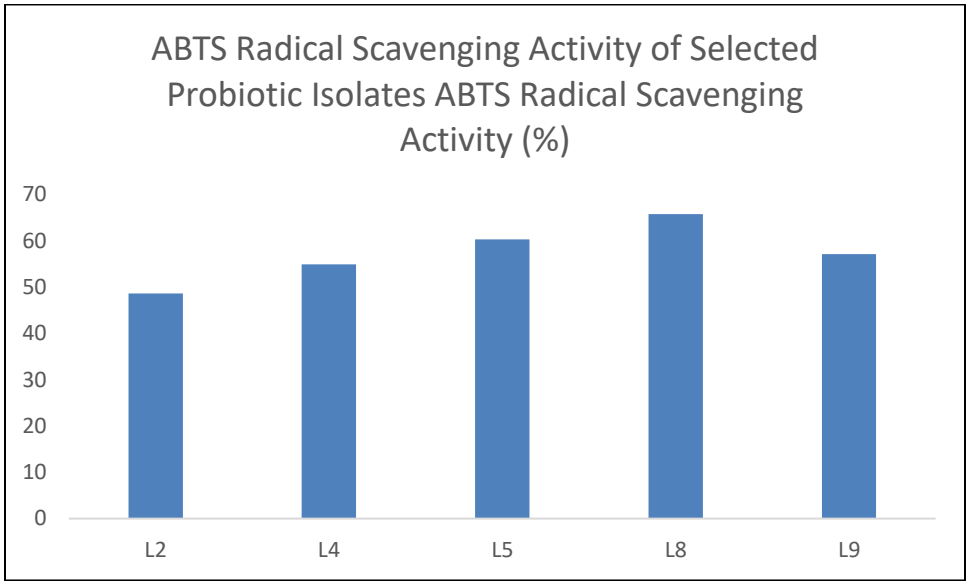


Figure 17: ABTS Radical Scavenging Activity of Selected Probiotic Isolates

The assay results showed that isolate L8 achieved the highest scavenging efficiency across both tests which L5 and L9 followed. The evaluation found L8 to possess extremely high antioxidant ability which established its importance as a probiotic strain that boosts health benefits (Table 20; Figure 18).

Table 20: Comparative Antioxidant Activity Profile of Selected Probiotic Isolates

Isolate Code	DPPH Scavenging (%)	ABTS Scavenging (%)	Overall Antioxidant Potential
L2	45.8	48.6	Moderate
L4	51.2	54.9	Moderate–High
L5	56.7	60.3	High
L8	61.9	65.8	Very High
L9	53.6	57.1	High

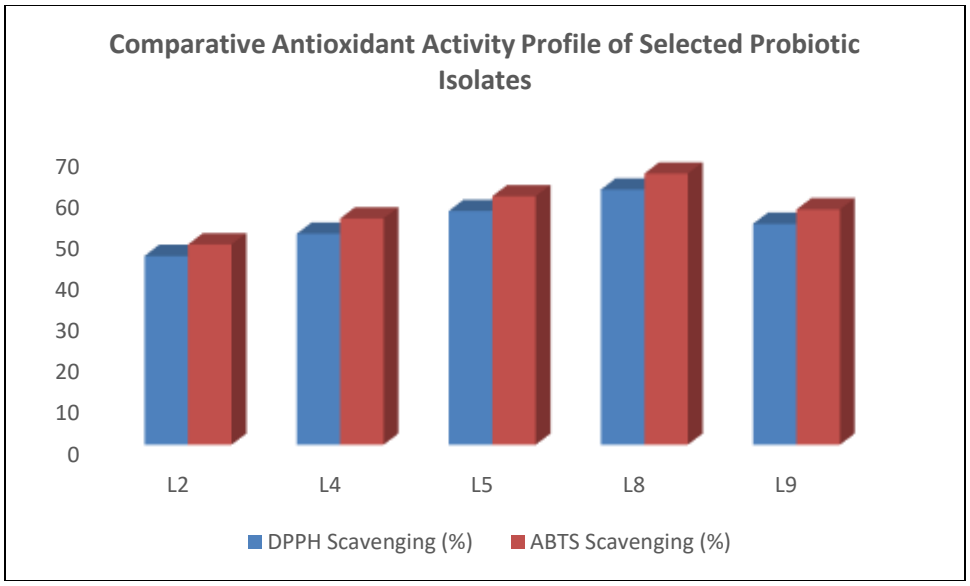


Figure 18: Comparative Antioxidant Activity Profile of Selected Probiotic Isolates

DISCUSSION

In the current study, the isolation, characterization, and functional evaluation of probiotic lactic acid bacteria (LAB) from fermented food products have been demonstrated, showing considerable strain-specific variations in their metabolic, antimicrobial, antioxidant, and antidiabetic activities. A total of 27 isolates were obtained from fermented food products, and five strains, i.e., L2, L4, L5, L8, and L9, were selected for further study based on their probiotic potential. This selection strategy is in accordance with previous studies, where a number of isolates from fermented foods were screened to obtain a few potent strains showing antidiabetic potential (21,22).

In the current study, the colony morphology and biochemical characteristics of the isolates confirmed their identity as lactic acid bacteria, as demonstrated by their Gram-positive, rod-shaped, non-motile, and non-hemolytic characteristics. These results are in accordance with previous reports, where lactic acid bacteria isolated from fermented foods were found to be Gram-positive, rod-shaped, non-motile, and non-hemolytic, and safe for probiotic applications (22).

The molecular characterization by sequencing the 16S rRNA gene confirmed that the selected isolates were predominantly from the genus Lactobacillus. Moreover, the presence of key genes involved in glycolysis, namely glk, pfk, and ldh, in all the selected isolates indicates their high metabolic potential for glucose metabolism and lactic acid production. Similar results have been found in LAB strains with hypoglycemic potential, where efficient carbohydrate metabolism is an essential factor for glucose metabolism (23).

The results from the metabolic profiling showed high glucose metabolism ranging from 76.0% to 84.5%, with the highest glucose metabolism by isolate L8, i.e., 84.5%. This indicates that the probiotic strains can play an important role in decreasing blood glucose levels by efficiently metabolizing the available carbohydrates. Similar results have been found where LAB strains significantly improved glucose metabolism, thereby decreasing blood glucose levels (22). Moreover, the lactic acid produced by the isolates varied from 7.8 to 9.3 g/L, with a corresponding decrease in pH to as low as 3.8. This indicates the high metabolic potential of the isolates for lactic acid production. The superior metabolic potential of isolate L8 can be due to its high glycolytic activity.

The effect of metformin on glucose metabolism revealed that despite a slight decrease in glucose utilization in the presence of the drug, all isolates showed a high level of metabolic activity. Notably, isolate L8 showed a glucose utilization of 80.8% even under treatment with metformin. This suggests that probiotic strains could be used in combination with existing anti-diabetic treatment regimens, as revealed in previous studies on the synergistic effects of probiotics and anti-diabetic drugs (22).

Survivability in the gastrointestinal tract is a fundamental criterion for the effectiveness of probiotics. In this regard, this study revealed that all the selected isolates showed a high level of survival under acidic conditions with a pH of 2.0-3.0 and bile salt concentrations of 0.3%. Notably, isolate L8 showed the highest survival rate of 74.3% at a pH of 2.0, 91.4% at a pH of 3.0, and 86.1% tolerance to bile salts. This is in agreement with previous studies revealing that for probiotics to be effective, they must be able to survive under harsh gastrointestinal conditions, as revealed in previous studies (24,21).

The antimicrobial activity of the selected isolates against various pathogenic bacteria such as E. coli, Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa further confirms their functional importance. The antimicrobial activity of the selected isolate L8 was the highest, with a mean inhibition zone of 17.0 mm, followed by

L5 and L4. The antimicrobial activity of the selected LAB may be due to the production of organic acids, bacteriocins, and inhibitory compounds. Such antimicrobial properties have been widely reported in various LAB strains isolated from various fermented food products (25).

The antidiabetic potential of the selected LAB isolates has been clearly demonstrated by their ability to inhibit α-amylase and α-glucosidase enzymes. The inhibitory activity of the selected LAB against α-amylase and α-glucosidase enzymes varied from 42.6 to 58.4% and 46.3 to 63.5%, respectively, with isolate L8 showing the highest inhibitory activity in both cases. The selected LAB have also been found to have higher inhibitory activity against α-glucosidase than α-amylase, which is desirable in the management of postprandial hyperglycemia. These results are in agreement with earlier findings that LAB have been reported to have inhibitory activity against carbohydrate-hydrolyzing enzymes and thereby lower blood glucose levels (24,21).

When the results obtained in this study regarding α-glucosidase inhibition (63.5%) are compared to those in literature, it has been observed that, although it is slightly above the 13-40% inhibition observed in some LAB isolates (24), it is slightly lower than the maximum inhibition observed in some strains (up to 85%) (22). In addition, some strains exhibited even higher levels of enzyme inhibition (up to 98%), indicating that enzyme inhibition is a highly strain-specific characteristic (26), confirming again that isolate L8 exhibited the highest results in all parameters tested.

The mechanism of action of LAB for the management of diabetes has been associated primarily with the inhibition of enzyme activities such as α-glucosidase and α-amylase, thereby slowing carbohydrate metabolism and glucose uptake in the intestine. In addition to this, extracellular polysaccharides (EPS), also produced by LAB, can also contribute to enzyme inhibition (27)

In addition to this, the chosen isolates have demonstrated potent antioxidant activity. The DPPH scavenging activity varied from 45.8% to 61.9%, whereas ABTS scavenging activity varied from 48.6% to 65.8%, with isolate L8 again displaying the highest antioxidant activity. This is in accordance with previous studies where LAB have been reported to produce bioactive compounds with antioxidant activity, which have the ability to scavenge free radicals and reduce oxidative stress in the body (22). As oxidative stress plays a crucial role in the progression of diabetes, the antioxidant activity of the chosen isolates further adds to their potency.

Thus, the results of this study have demonstrated the multifunctional attributes of probiotic LAB strains, with potent metabolic activity, enzyme inhibition, antimicrobial activity, and antioxidant activity. Among all the isolates, isolate L8 consistently demonstrated superior activity for all the parameters. This further indicates the potency of isolate L8 for the development of antidiabetic formulations. As demonstrated in this study, the variability in the strains of probiotics is in accordance with previous reports emphasizing the point that probiotic functionality varies significantly among different strains of LAB (21,23).

CONCLUSION

The present study showed that fermented food sources are a good reservoir for probiotic lactic acid bacteria. Among the 27 isolates, five strains (L2, L4, L5, L8, and L9) were selected as good candidate microorganisms based on their morphology, biochemical, and safety characteristics. Molecular analysis confirmed that they belonged to lactic acid bacteria, which possessed the genes for the glycolytic pathway, thereby showing efficient metabolic activity.

All the selected strains showed high glucose utilization (76.0–84.5%) and lactic acid production (7.8–9.3 g/L). Among the selected strains, strain L8 showed better activity. All the selected strains showed high tolerance to acidic and bile environments, showing their ability to survive in the gastrointestinal tract. All the selected strains showed high antimicrobial activity against pathogenic microorganisms.

All the selected strains showed high antidiabetic activity. The selected strains showed high inhibition of α-amylase (42.6–58.4%) and α-glucosidase (46.3–63.5%), as well as antioxidant activity. Among the selected strains, strain L8 showed the highest activity.

Overall, the present study showed the multifunctional properties of probiotic lactic acid bacteria, thereby showing their potential for their application as a drug for the management of diabetes.

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