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In Vitro Evaluation of Gastrointestinal Survivability and Functional Bio-Efficiency of Probiotic Strains with Antidiabetic and Antioxidant Potential

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ABSTRACT:

Objectives:

The purpose of this research was to separate and assess probiotic lactic acid bacteria from fermented foods according to their potential as antioxidants, antimicrobials, gastrointestinal survivors, and antidiabetic agents.

Materials and Methods:

A total of 27 bacterial strains were isolated from fermented foods, out of which 18 were considered presumptive strains of lactic acid bacteria. Five of these (L2, L4, L5, L8, and L9) were selected after safety tests and biochemical tests. Assessment of gastrointestinal survival was done at pH 2.0 and 3.0 and 0.3% bile salts. The functional assessment of strains was done by studying their antibacterial property, enzyme inhibition, and antioxidant property.

Results:

All the isolates chosen showed high resistance to simulated conditions of the gut. There were also high levels of antimicrobial activities shown to the most pathogenic bacteria such as Escherichia coli, Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa. L8 proved to be the most active against diabetes because it inhibited the α-amylase and α-glucosidase enzymes.

Conclusion:

The findings indicate that selected probiotic isolates, particularly L8, possess strong multifunctional bio-efficiency and potential for application in functional foods and management of metabolic disorders such as diabetes. Significant differences ($p < 0.05$) were observed among the selected probiotic isolates, with isolate L8 demonstrating the highest gastrointestinal survivability, antimicrobial, antidiabetic, and antioxidant activities.

1. INTRODUCTION

Diabetes mellitus has been defined as "a chronic metabolic disorder characterized by persistently raised levels of glucose in the blood, caused by defects in insulin secretion, insulin action, or possibly both." Diabetes mellitus is one of the most prevalent illnesses affecting people worldwide. Retinopathic, neuropathic, nephropathic, and cardiovascular diseases are among the disease's severe side effects. One of the primary causes of diabetes mellitus has been identified as oxidative stress, which is the imbalance between the generation of reactive oxygen species and the antioxidant system. The symptoms of the disease occur because of the damage caused by the imbalance. Diabetes mellitus symptoms eventually arise from cellular damage caused by the imbalance. There is an increased need to develop safe, natural, and effective methods to control and manage diabetes mellitus and oxidative stress.

Probiotics have attracted significant attention in the scientific and medical communities owing to their ability to confer health benefits when taken in sufficient quantities. The concept of "bio-efficiency" has also increased the evaluation and importance of probiotics, as the evaluation is now based on the actual efficiency of biological active compound functions rather than the mere presence of the compound [1]. Probiotics are now being considered as potential therapeutic agents, and the importance of probiotics is being realized, as the incidence of metabolic syndrome is on the rise. Probiotics are defined as "live microorganisms, when administered in adequate quantities, have health benefits to the host" [2]. The importance and potential of probiotics are being realized, and the potential is being utilized to develop new strains that have significant functions and benefits on the host's physiology.

Probiotics are considered as live organisms, and they are being utilized to maintain the balance of the intestinal microbial flora and inhibit the colonization of pathogenic organisms [3]. Probiotics have been found to affect and influence various conditions, and the importance and potential are being realized to develop new strains that have significant functions and benefits on the host's physiology.

However, the success of probiotics can greatly depend upon the specificity of the strain, its dose, and the ability of interaction with the host. Each strain of probiotics has different functions that make it necessary to evaluate its functionality specifically. The ability of probiotics to tolerate the harsh environment in the GI tract can be regarded as the most important condition of its effectiveness and viability. Probiotics have to overcome the high pH, bile salts, and enzymes present in the environment while passing the stomach and small intestines. To be effective, probiotics have to survive this harsh environment.

GI survivability is one of the factors affecting viability and functionality of probiotics. Research studies have shown that strains of Lactobacillus are capable of surviving the passage through the GI tract and exerting beneficial effects on the microbiota [4]. The development of probiotics in the form of encapsulated probiotics and the creation of probiotic-containing matrices have shown improved survivability in simulated GI conditions [5]. The advancements in probiotics have clearly shown that survivability is an important factor, not only from the technological point of view, but also as an important factor that determines the function and viability of probiotics.

Recent advancements in probiotics have led to the development of innovative technologies and strategies to enhance the viability and function of probiotics. The development of pH-responsive probiotic microspheres and hydrogel-based probiotic encapsulation is an important advancement in the development of probiotics, as they have shown improved survivability in the GI tract [6, 7, 8]. The application of CRISPR technology to enhance the adaptability and function of probiotics, especially in the case of metabolic disorders, is an important innovation in the development and application of probiotics [9].

The functional bio-efficiency of probiotics has gained much importance for assessing their effectiveness besides viability due to their therapeutic activities that can be quantified experimentally. Lactic acid probiotics are characterized by various biological activities, such as antimicrobial properties, enzyme inhibition, and antioxidant properties, which are very important in managing metabolic disorders such as diabetes. The primary mechanism through which diabetes can be managed is through the regulation of postprandial hyperglycemia through the inhibition of carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase. In most instances, it has been proven that strains of probiotics obtained from fermented foods inhibit these enzymes effectively.

Apart from the antidiabetic effects, probiotics also exert antioxidative effects, which are of great importance in lowering oxidative stress, which is one of the main reasons for the development and complication of diabetes mellitus. Lactic acid probiotics are known to secrete substances having antioxidant activity, improving the scavenging effect of radicals. Fermented foods are considered potent sources of these microorganisms that are functional in several ways. Thus, in vitro evaluation of the probiotic strains for their stability and functional bio-efficiency is required [19].

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2. METHODOLOGY

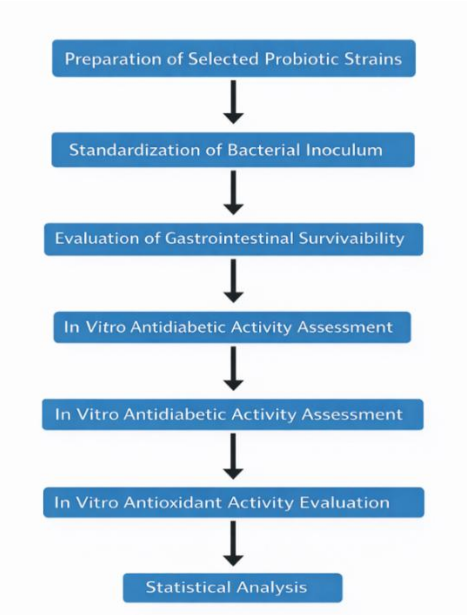

Figure 1: Process of sample collection, experimentation and representation of results

The present experiment was done in order to assess the gastric survivability and bio-efficiency in terms of function of chosen probiotics through an in vitro experimental design. The cultures were activated in de Man, Rogosa, and Sharpe broth for 24 hours at 37°C before being subjected to centrifugation and washing using sterile phosphate-buffered saline for removal of remaining broth. Standardization of bacterial count ranged from 10^8–10^9 CFU/mL by way of optical density at 600 nm and verified by plate count method on MRS agar as shown in figure 1. Assessment of gastric survivability of probiotics was performed through simulation of conditions including acidic pH (2.0 and 3.0), 0.3% bile salts, and enzymatic digestion via pepsin and pancreatin. By evaluating the inhibitory impact on α-amylase and α-glucosidase enzyme activity, antidiabetic potential was investigated. However, antioxidant potential was assessed using absorbance measurements at 517 nm and 734 nm, respectively, to determine DPPH and ABTS radical scavenging capabilities. When findings were statistically significant ($p < 0.05$), they were reported as mean ± SD. The experiments were conducted in triplicate. This research does not include testing on humans or animals.

3. RESULTS

The total number of bacterial isolates was 27 that were isolated from fermented foods such as curd, Yakult, mango pickle, carrot pickle, and idli batter with the help of selective MRS agar medium. Out of the 27 bacterial isolates, 18 isolates were selected based on their morphology, which is usually found in lactic acid bacteria and were named L1 to L18.

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**

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In all, 27 isolates of bacteria were obtained from different fermented food samples used in this study. These results show that fermented foods are a rich source of probiotic bacteria. From these isolates, 18 isolates were shortlisted for further screening and characterization of bacteria on the basis of their colony morphology, which resembles lactic acid bacteria. These isolates were designated as L1, L2, and so on, up to L18 and were retained for further microscopic and biochemical investigations in the following experiments. The reduction in the number of isolates from 27 to 18 is because preliminary screening criteria have been applied to select lactic acid bacteria colonies.

Table 2: Gastrointestinal Survivability and Antimicrobial Activity of Selected Probiotic Isolates

Isolate	**pH 2.0 (%)**	**pH 3.0 (%)**	**Bile 0.3% (%)**	**GI Status**	***E. coli* (mm)**	***S. aureus* (mm)**	***S. typhi* (mm)**	***P. aeruginosa* (mm)**	**Mean Inhibition (mm)**	**Activity Level**
L2	68.5 ± 1.6	85.2 ± 1.4	76.0 ± 1.4	Suitable	12.4 ± 0.6	14.2 ± 0.5	11.8 ± 0.4	10.6 ± 0.5	12.3 ± 0.5	Moderate
L4	72.1 ± 1.5	88.6 ± 1.3	81.4 ± 1.5	Suitable	14.1 ± 0.5	16.0 ± 0.6	13.5 ± 0.5	12.3 ± 0.4	14.0 ± 0.5	High
L5	70.6 ± 1.4	90.0 ± 1.2	83.8 ± 1.3	Highly Suitable	15.6 ± 0.6	17.8 ± 0.5	14.9 ± 0.6	13.7 ± 0.5	15.5 ± 0.6	High
L8	74.3 ± 1.3	91.4 ± 1.1	86.1 ± 1.2	Highly Suitable	17.2 ± 0.5	19.1 ± 0.6	16.4 ± 0.5	15.2 ± 0.4	17.0 ± 0.5	Very High
L9	69.8 ± 1.5	87.6 ± 1.4	79.6 ± 1.4	Suitable	14.8 ± 0.4	16.5 ± 0.5	13.9 ± 0.4	12.8 ± 0.5	14.5 ± 0.4	High
P value	0.06	0.02	<0.01		<0.01	<0.01	0.05	0.04	<0.01	

Values expressed as mean ± SD (n = 3)

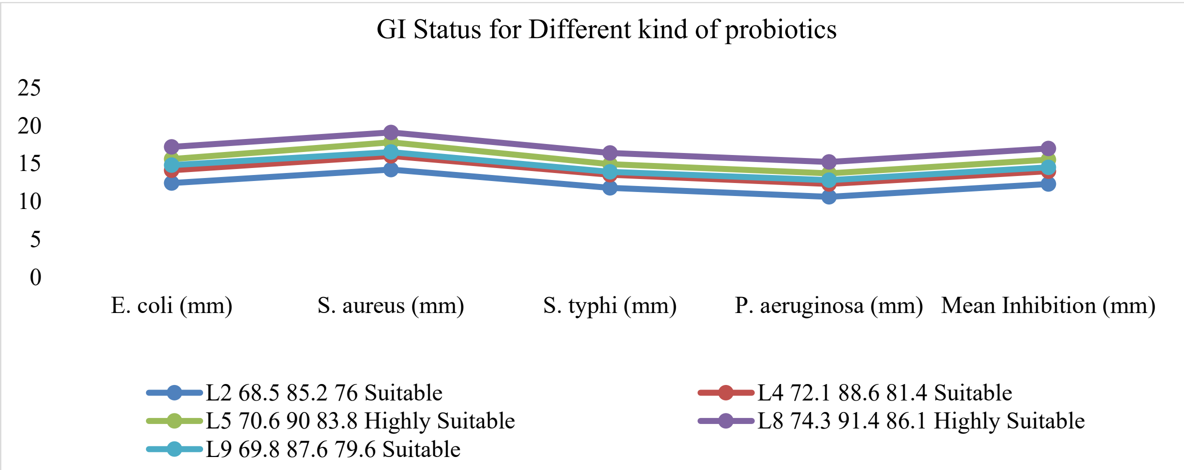

Figure 2: GI Status for Different kind of probiotics on Antimicrobial Activity

All isolates as showed in above table 2 and figure 2 have high gastrointestinal survival capacity, signifying that they may be effective probiotic strains. L8 was found to show highest acid and bile resistance compared to other isolates at pH 2.0 and 3.0, respectively, and is thus able to tolerate the harsh conditions of the gastrointestinal tract. All strains inhibited pathogenic bacteria and L8 proved to be the most effective with the highest zones of inhibition against pathogenic strains, whereas strain L5 also had significant antimicrobial activities but L2 was relatively less effective compared to others. Statistical analysis

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significant differences among the probiotic isolates in GI survivality and antimicrobial activity, stain L8 showed highest overall performance.

Table 3: Antidiabetic and Antioxidant Activity of Selected Probiotic Isolates

Isolate	α-Amylase (%)	α-Glucosidase (%)	DPPH (%)	ABTS (%)	Overall Functional Potential
L2	42.6 ± 1.4	46.3 ± 1.3	45.8 ± 1.4	48.6 ± 1.3	Moderate
L4	48.9 ± 1.5	52.1 ± 1.4	51.2 ± 1.5	54.9 ± 1.4	Moderate–High
L5	53.7 ± 1.6	57.8 ± 1.5	56.7 ± 1.6	60.3 ± 1.5	High
L8	58.4 ± 1.7	63.5 ± 1.6	61.9 ± 1.7	65.8 ± 1.6	Very High
L9	50.2 ± 1.5	54.6 ± 1.4	53.6 ± 1.5	57.1 ± 1.4	High
p vlaue	<0.01	<0.01	<0.01	<0.01	

Values expressed as mean ± SD (n = 3)

It can be observed from the results of this above table 3 that all isolates exhibit significant anti-diabetic and antioxidant properties, thereby making them functionally efficient. In particular, isolation L8 exhibited a relatively high inhibition towards the α-amylase and α-glucosidase enzymes. This makes it a more potent substance for regulation of hyperglycemia after meals. Moreover, it is highly effective as an antioxidant as indicated by its high activities in the DPPH and ABTS methods of determining free radicals. The second best isolates were L5, L4, and L9. On the other hand, isolate L2 was relatively less active among all isolates. Therefore, it can be concluded that isolates L8 and L5 have promising properties based on the statistical analysis where p values were <0.05 showing significant variations

4. DISCUSSION

The present study also points to the tremendous potential associated with fermented food materials, considering the fact that 27 strains of bacteria were isolated as described in table 1, out of which 18 strains displayed morphological features characteristic of lactic acid bacteria. This finding corroborates previous studies, which emphasized the fact that traditional fermented foods are rich in a variety of functional and therapeutic probiotic bacteria [23]. Further screening and characterization studies resulted in the identification of five strains, namely, L2, L4, L5, L8, and L9, which displayed essential characteristics associated with probiotic bacteria, such as Gram-positive nature, non-motility, negativity for catalase, and non-hemolytic activity. This has been emphasized in previous studies, considering such characteristics to be essential markers for food and therapeutic uses of such bacteria [24]. The reduction of strains from 27 strains to 18 strains also suggests that there should be more research on the need for screening and characterization in order to identify strains that have probiotic properties.

As described in table 2 and figure 2 the function of a probiotic microorganism is greatly determined by its survival capability in the gastrointestinal tract. The isolates tested in this study all have the ability to tolerate bile salt (0.3%) and acidic conditions (2.0, 3.0). In comparison to isolate L5, which similarly had a positive survivorship rate in acidic environments, the highest survivability rate was observed for isolate L8 out of all the isolates tested, with a survivorship rate of 74.3% at a pH of 2.0 and 91.4% at a pH of 3.0. It can be deduced from this experiment that the survivability rate is important in determining the survivability capability of a probiotic bacterium [25]. Since these bacterial strains have shown a high rate of survivability, they possess a positive capability of surviving in adverse conditions of the gut [23]. The ability of isolate L8 and isolate L5 to withstand harsh conditions also confirms a positive ability to withstand harsh conditions, thus qualifying them to be categorized under "highly suitable strains," a factor that makes them potentially applicable in functional food and therapeutic formulations.

The ability of the selected isolates to demonstrate antimicrobial activity also confirms a positive ability to be applicable in functional food and therapeutic formulations. All of the chosen strains in this study demonstrated a positive ability to inhibit pathogenic bacteria, including Escherichia coli, Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa, among many other pathogenic bacteria. The zone of inhibition was recorded to be greatest in isolate L8 at 19.1 mm, while that of L5 and L4 isolates was smaller than the one in L8 as depicted in table 2 and figure 2. This ability can be attributed to the production of organic acids, bacteriocins, hydrogen peroxide, and other antimicrobial metabolites, which play a critical role in inhibiting pathogenic bacteria, thus confirming previous studies that emphasized a positive ability to inhibit pathogenic bacteria, thereby reducing the risk of infection and maintaining a balance in gut bacteria, a factor that makes them potentially applicable in functional food and therapeutic formulations [26].

Another aspect of the study, which proved to be very important, relates to the antidiabetic potential as described in table 3 of the selected probiotic strains, particularly in relation to the inhibitory effects on carbohydrate-hydrolyzing enzymes, including α-amylase and α-glucosidase. Inhibitory effects were observed in all the tested probiotic strains, which were very high in the case of α-glucosidase compared to α-amylase. The maximum inhibitory effect was observed in the case of L8, followed by L5 and L9, which possessed an inhibitory effect of 63.5% on α-glucosidase and 58.4% on α-amylase. These results are consistent with recent research that found probiotic lactic acid bacteria isolated from fermented food sources have comparable inhibitory actions on carbohydrate hydrolyzing enzymes [27]. Since α-glucosidase is crucial to the delayed absorption of glucose, its inhibitory action is more critical. Additionally, the considerable preservation of glucose metabolism in the presence of metformin suggests that these strains may be used in conjunction with metformin to treat diabetes. It has been shown that probiotics are crucial for the treatment of diabetes.

Antioxidant activity as described in table 3 and figure 4 is an essential functional characteristic of probiotic bacteria because oxidative stress plays a critical role in the onset, progression, and consequences of diabetes. DPPH and ABTS tests verified that all of the isolates in this investigation had strong antioxidant activity. Among the free radicals, isolate L8 had the greatest scavenging capability, followed by isolates L5 and L9 for DPPH and ABTS free radicals, respectively, with values of 61.9 and 65.8. This study has validated the probiotic bacteria's capacity to generate metabolites that can scavenge free radicals and shield the host from the negative effects of oxidative stress.

Similar results have been seen with lactic acid bacteria, where the amount of oxidative stress resistance is increased by the formation of peptides, exopolysaccharides, and antioxidant enzymes [28]. One important characteristic of probiotic bacteria is their strong antioxidant potential, which has demonstrated the bacteria's capacity to prevent and lessen oxidative stress issues.

Overall, the evaluation of the gastrointestinal survivability of the probiotic bacteria, antimicrobial potential, antidiabetic potential, and antioxidant potential of the probiotic bacteria provides a detailed understanding of the bio-efficiency of the selected strains of probiotics. Out of all the strains, L8 exhibited the best performance in each activity, followed by L5. Such strain-specific variations in the bio-functional efficiency of probiotics have been well established in previous studies, underscoring the need to use strains with high bio-functional efficiency for specific therapeutic applications [27]. The multi-functional properties of the selected strains of probiotics highlight the potential of these strains to be used in the development of functional foods with the ability to manage metabolic disorders such as diabetes.

CONCLUSION

This study shows that fermented foods are important sources of probiotic lactic acid bacteria with promising functional capabilities. From the list of isolates, five strains (L2, L4, L5, L8, and L9) met the necessary qualities of being a probiotic, which include safety and stability. All chosen isolates had great tolerance to acidic and bile environments in the intestine, meaning they have great potential to colonize it. Furthermore, all the isolates were effective in killing harmful bacteria, hence balancing the gut flora. They had strong anti-diabetes qualities through inhibiting enzymes such as alpha amylase and alpha glucosidase with more effectiveness on alpha glucosidase. This indicates their importance in preventing post-prandial hyperglycemia. Functional characteristics of the selected probiotics showed significant difference ($p<0.05$), with the isolate L8 possessing the best multifunctional probiotics capability in the use of functional food products and metabolic disorders management.

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