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Original Article

Characterization of Probiotic Bacteria and Yeasts Isolated from Traditional Salt-Fermented Grains and Spice Seeds in Bangladesh

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ABSTRACT

Background: Traditional dried seeds are widely consumed as staple foods and spices in Bangladesh. Traditional salt-fermented grains and spice seeds are rich sources of diverse microorganisms, including lactic acid bacteria (LAB) and yeasts, many of them possess probiotic properties. The aim of this study is to characterize these microorganisms to identify suitable probiotic candidates for functional applications. **Methods:** Total twenty-seven varieties of dried seeds collected from local markets in Dhaka were fermented traditionally using 3% NaCl for 3–5 days at ambient temperature (25–35 °C). Isolates were obtained by enrichment in de Man, Rogosa and Sharpe (MRS) broth for bacteria and potato dextrose (PD) broth for yeasts, followed by streaking on MRS and PD agar plates. Distinct single colonies were identified through morphological observation, Gram staining, and short biochemical tests. All isolates were characterized for probiotic properties by acid and salt tolerance, temperature tolerance, sugar fermentation tests and antibacterial activity. **Results:** Total 27 lactic acid bacteria and 4 yeasts from 27 samples were isolated and were presumptively identified as *Lactobacillus* spp., *Lactococcus* spp., and yeasts. All identified LAB demonstrated strong survival under highly acidic (pH 1.5) and alkaline (pH 8.5) conditions, high salinity (up to 6% NaCl), and a wide temperature range (4–55 °C). The selected bacteria showed promising antibacterial properties against pathogenic bacteria. **Conclusion:** Traditional salt-fermented grains and spice seeds have been shown to be sources of diverse microorganisms, including lactic acid bacteria (LAB) and yeasts and have probiotic potential.

1. INTRODUCTION

Fermentation has long been an integral part of traditional food practices across many cultures,

including Bangladesh, where grains, pulses, and spice seeds are often preserved or enhanced through natural or salt-based fermentation. Such fermentation

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processes not only improve the texture, flavor and shelf life of foods but also serve as important reservoirs of beneficial microorganisms, particularly lactic acid bacteria (LAB) and yeasts (Tamang et al., 2017). LAB and yeast populations contribute to biochemical transformations that enhance nutritional quality, reduce antinutritional factors and suppress pathogenic organisms through acidification and antimicrobial metabolite production (Montet & Ray, 2017).

The global interest in probiotics has increased significantly in recent decades, driven by growing evidence of their roles in gut health, immune modulation, metabolic regulation, and pathogen inhibition (Hill et al., 2014; Sanders et al., 2019). Probiotics are formally defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO, 2002). Traditional fermented foods specially those undergoing spontaneous fermentation, are widely recognized as rich sources of novel probiotic strains due to the diverse indigenous microflora adapted to specific environmental conditions (Tamang et al., 2024).

Despite increased research on probiotic microbes from dairy, vegetable, and cereal-based fermented foods, the microbial diversity and probiotic potential of microorganisms present in fermented dried seeds and spice materials remain poorly understood. In Bangladeshi households, seeds such as mustard (*Brassica juncea*), cumin (*Cuminum cyminum*), coriander (*Coriandrum sativum*), fenugreek (*Trigonella foenum-graecum*), sesame (*Sesamum indicum*) and various pulses are stored in dried form and are occasionally salt-fermented to enhance flavor, reduce bitterness, and improve preservation (Rahman et al., 2021). These fermentation environments, characterized by high salinity, nutrient richness, and natural antimicrobial compounds, create selective ecological niches that may harbor stress-tolerant microorganisms.

Such environments are particularly relevant for the search for new probiotic candidates. To function effectively, probiotic strains must demonstrate robust survival under gastrointestinal (GI) conditions, including low pH (1.5–3.0), bile salts (0.3–2.0%), osmotic stress, and temperature fluctuations (Ayyash et al., 2021).

Given these considerations, exploring the microbial communities of fermented dried grains and spice seeds represents a promising strategy for identifying novel bacterial and yeast strains with functional attributes suitable for incorporation into functional foods, nutraceuticals, and pharmaceutical

formulations. The probiotic potential of microorganisms associated with salt-fermented grain and spice seeds remains largely unexplored. This study therefore aimed to isolate and characterize bacteria and yeast from traditional salt-fermented dried seeds widely consumed in Bangladesh and evaluate their potential probiotic properties through a combination of cultural, morphological, biochemical, and stress-tolerance assays. The findings provide new insights into the microbial diversity of these culturally important food matrices and underscore their potential as valuable sources of new probiotic candidates.

2. METHODS

2.1 Sample collection and fermentation

A total of twenty-seven dried seed samples were collected from local markets in Dhaka, Bangladesh. Samples were china seed, paddy, millet rice, myrobalan plum, black pepper, areca nut, coriander, fennel, black mustard, sunflower seed, seam bean, cumin, carom seed, nigella seed, pignut, chickpea, dabli, corn, lily fruit seed, cardamom, pumpkin seed, nutmeg, black gram, jackfruit seed, flax seed, mung bean, and fenugreek. 10 g of each dried-seed sample was crushed and added into a sterile 250 mL bottle containing 90 mL of sterile 3% (w/v) NaCl solution for salt fermentation. Samples were thoroughly mixed and incubated at room temperature (25–35 °C) for 5 days to allow spontaneous salt fermentation. After the fermentation period, turbidity and gas were observed in each bottle, and aliquots were taken for microbiological analysis (Ghanbari et al., 2013). A flow diagram of the seed fermentation process is shown in Figure 1.

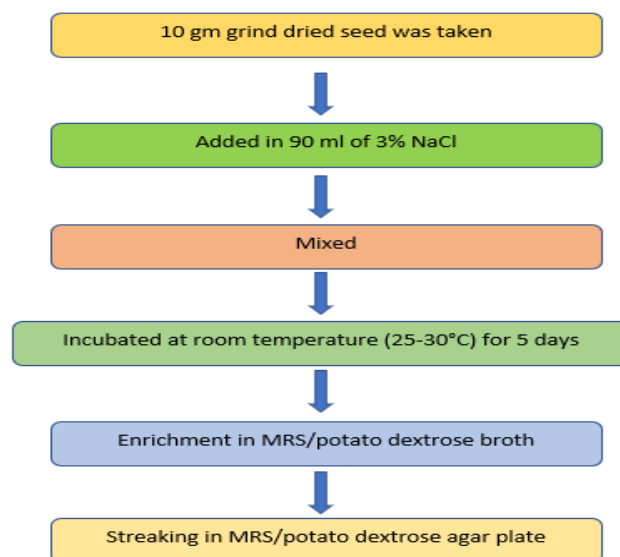


Figure 1: A flow diagram of the seed fermentation process.

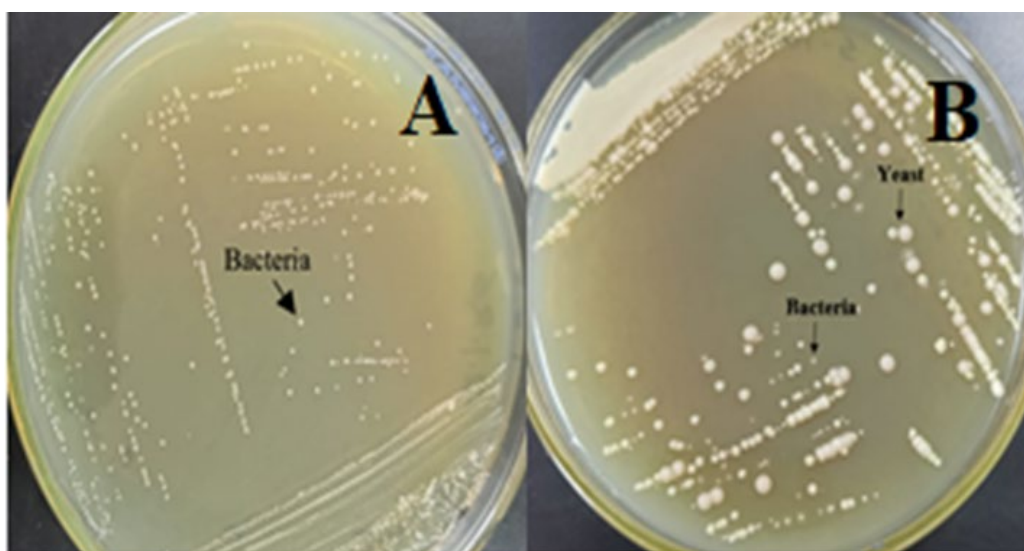


Figure 2: Plate A. bacterial colony (Sd 23) and Plate B. bacterial colony (Sd-13) & yeast colony (Sy-1).

2.2 Enrichment and isolation of lactic acid bacteria (LAB) and yeasts

Fermented seed was enriched in MRS broth at 35°C for 24 h (Ghanbari et al., 2013). After enrichment, the MRS cultures were streaked onto MRS agar plates and incubated at 35°C for 48 h and PD plates were used for yeast isolation. Distinct single colonies were isolated (Figure 2). Representative colonies from each sample were maintained on MRS and PD slants for short-term storage at 4°C. Long-term storage of purified isolates was maintained in 20% (v/v) glycerol stocks at -20 °C.

2.3 Biochemical tests

Isolated colonies were identified by Gram staining and short biochemical tests (MacFaddin, 2000; Bergey et al., 1994). All control strains and pathogenic bacteria for antibacterial test were obtained from laboratory stock.

2.4 Gram staining

Gram staining test was performed for all isolated colonies according to the standard procedure. Cell morphology was observed by light microscopy under oil immersion (100X). *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as positive and negative controls, respectively.

2.5 Spore Staining

Spore staining was performed according to the procedure of Leboffe and Pierce (2015). Briefly. Malachite Green was used for primary staining, and safranin was used for counter stained. Spore staining

was viewed with the microscope under the oil immersion objective (100X). *Bacillus subtilis* was used as a positive control.

2.6 Catalase test

3% hydrogen peroxide was added to a fresh culture on a sterile glass slide and mixed well. Producing bubbles or froth indicated catalase-positive, and no bubbles or froth indicated catalase-negative. *S. aureus* ATCC 25923 was used as a positive control.

2.7 Oxidase test

The oxidase test was performed with a 1% solution of N1N1-Tetramethyl-phenyldiamine-dihydrochloride, which was soaked in a piece of filter paper. A single colony was picked up and touched onto the paper with the impregnated reagent. A dark purple color development within 5-10 seconds was considered positive and no change of color was interpreted as a negative result for oxidase. *Pseudomonas aeruginosa* and *E. coli* ATCC 25922 were used as positive and negative controls, respectively.

2.8 Motility, Indole, and Urease (MIU) Test

MIU test was performed, and motility was evaluated by observing the pattern of bacterial growth from the stab line. Indole production was assessed by adding Kovac's reagent after incubation. Urease activity was determined by monitoring the phenol red indicator in the MIU medium; *Escherichia coli* and *Klebsiella pneumoniae* were used as positive control where uninoculated media were used as the negative control.

2.9 Probiotic potential tests

All the following tests were performed in triplicate.

2.10 pH tolerance test

pH tolerance test was performed using MRS broth adjusted to pH 1.5, 3.5, and 8.5. The pH of the media was adjusted with 10 N HCl or 1N NaOH with a calibrated pH meter. Fresh bacterial cultures were inoculated into each pH-adjusted MRS broth. Tubes were incubated at 37°C for 48 h. Uninoculated media at each pH served as the negative control. Bacterial growth was assessed at 24 h and 48 h by visual observation of turbidity compared to the negative control. Increased turbidity indicated tolerance and growth at the tested pH, while clear broth indicated absence of growth. No turbidity was observed in the negative controls.

2.11 NaCl Tolerance Test

The salt tolerance test was evaluated in MRS broth with different concentrations of sodium chloride (1% and 6% w/v). Fresh overnight cultures were inoculated (1% v/v) into 5 mL of each NaCl-adjusted MRS broth and incubated at 37°C for 48 h. Only media containing the corresponding NaCl concentrations served as negative controls and showed no growth. Bacterial growth was assessed after 24 h and 48 h by observing turbidity compared to the negative control. Increased turbidity indicated active growth and salt tolerance at the tested NaCl concentrations, while clear broth indicated no growth.

2.12 Temperature tolerance test

The temperature tolerance test was performed for the isolated LAB by inoculating bacteria into MRS broth and incubating at temperatures of 4°C, 45°C, 55°C and 60°C. Results were evaluated by observing the turbidity of bacterial growth after 24 and 48 h. The presence or absence of growth in the media ensured the survival or non-survival of LAB at different temperatures. Only the medium, without bacterial inoculation, served as the negative control.

2.13 Kliger's Iron Agar (KIA) test

All isolates were tested for the KIA test to determine the mode of glucose and lactose utilization. Fresh culture was inoculated by stabbing the butt and streaking the slant. After incubation at 37°C for 24 h, results were recorded for color changes of the butt or slant, H₂S or other gas production. Production of hydrogen sulfide caused blackening of the medium, and gas production gave rise to bubble formation in

the tube. *E. coli* ATCC 25922 and *Salmonella typhi* were used as the positive control, where the medium without inoculation was the negative control.

2.14 Antibacterial activity test

The antibacterial assay was performed on selected pathogenic bacteria by the agar well diffusion method according to the standard procedure. *Salmonella typhi*, *Salmonella paratyphi*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Shigella dysenteriae* were selected as test pathogenic bacteria. Culture supernatant of each isolate was prepared by growing the isolates in MRS broth at 37°C and centrifuged at 4,000 x g for 10 min. 100 µL culture supernatant of each isolate was placed in a well on Mueller Hinton agar (MHA) plate, which was seeded (1% v/v) with the test microorganism. The plates were incubated at 37°C for 24 h, and zones of inhibition were measured (mm). Duplicate test was done and average results were taken for each organism.

3. RESULTS

A total of twenty-seven bacteria designated as Sd-1 to Sd-27 were presumptively identified as Gram-positive lactic acid bacteria, primarily belonging to *Lactobacillus* spp. and *Lactococcus* spp. respectively, based on macroscopic, microscopic characteristics and biochemical tests (Figure 3; Tables 1 and 2). However, four yeasts were identified and designated as SY-1 to SY-4, and results of yeast characterization tests are in progress and not included here. *In vitro* stress tolerance assays were conducted to evaluate their probiotic potential. All isolates demonstrated strong resistance to extreme pH conditions, surviving both highly acidic (pH 1.5) and alkaline (pH 8.5) environments, and exhibited salt tolerance up to 6% NaCl (Table 3). In addition, the isolates showed broad thermal tolerance, maintaining viability across a temperature range of 4°C to 55°C (Table 3). However, preliminary studies of antibacterial activities revealed promising antibacterial activity against pathogenic bacteria, with results shown in Figure 4.

4. DISCUSSION

In this study, microbial isolates were obtained from traditional fermented grains and spice seed sources and identified as Gram-positive lactic acid bacteria (LAB), along with yeasts. The isolation of diverse LAB from plant-derived substrates is consistent with previous reports demonstrating that cereals, seeds, and spices serve as natural reservoirs of beneficial microorganisms with probiotic potential

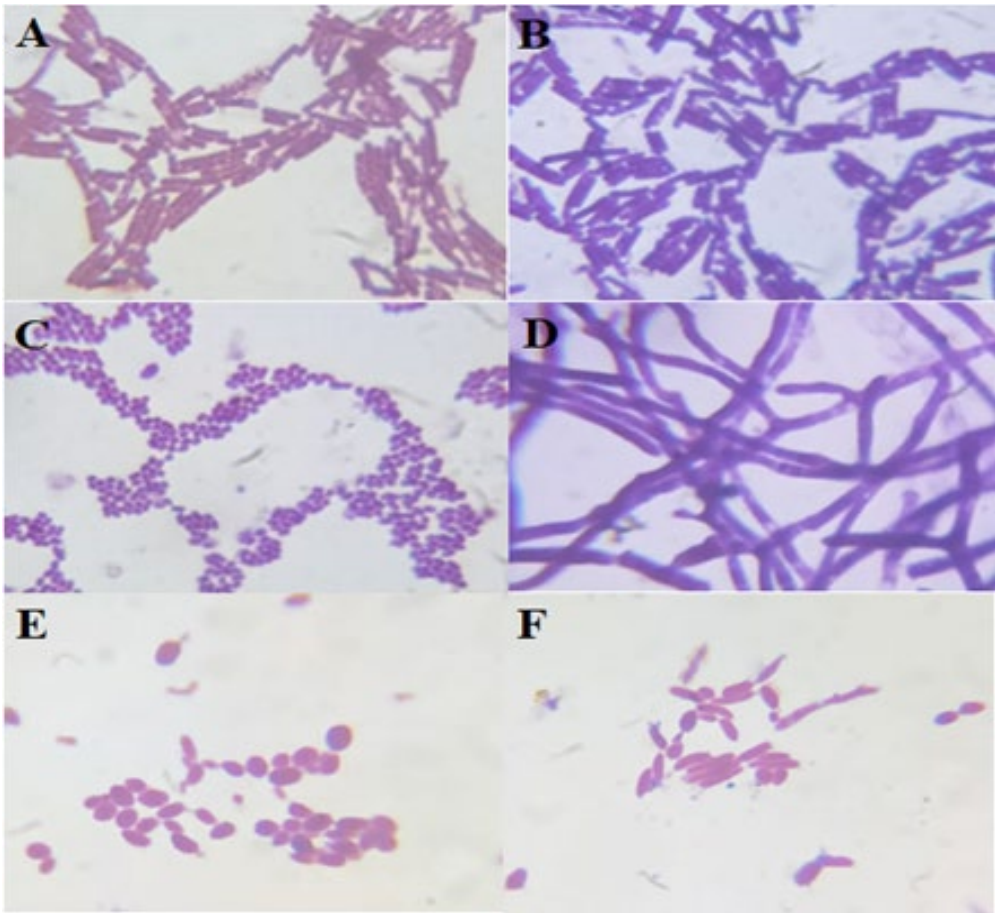


Figure 3: Microscopic images of Gram staining bacteria and yeast. A. Gram-positive Lactobacilli (Sd -24), B. Gram-positive Lactobacilli (Sd-20), C. Gram-positive Lactococci (Sd-13), D. Gram-positive Lactobacilli (Sd-25), E. Yeast (Sy-1), and F. Yeast (Sy-3).

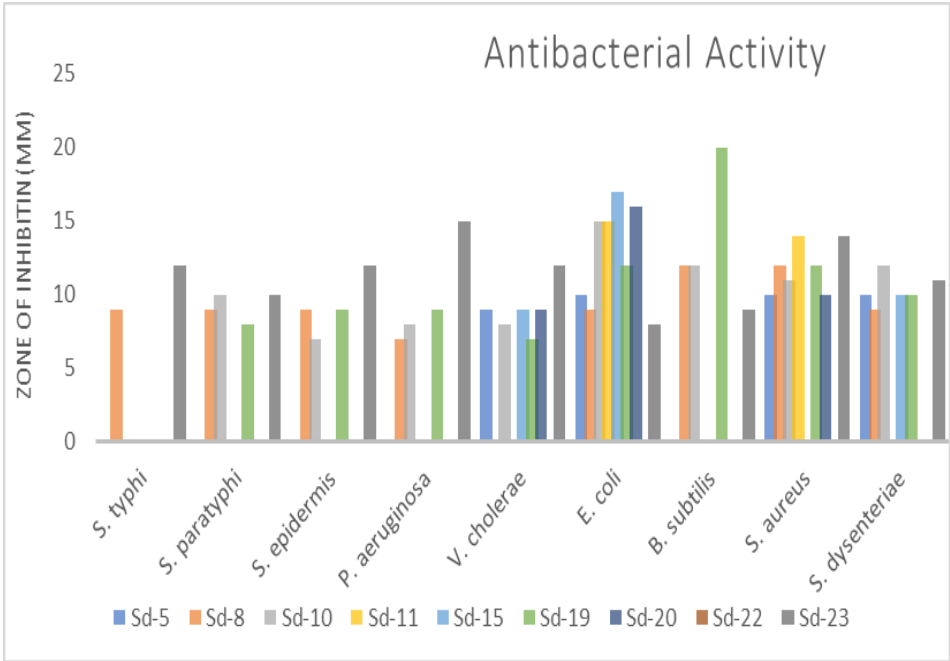


Figure 4: Antibacterial activity of nine selected lactic acid bacteria against pathogenic bacteria.

Table 1: Colony morphology of isolated bacteria and yeast from fermented seeds.

Name (local) of seeds	Isolate no (Bacteria)	Morphology of colony	Gram staining/identification of bacteria & yeast	Spore formation
Chia seed	Sd-1	Small, circular, regular and white	Gram positive Lactococci	Negative
Paddy	Sd-2	Large, circular, irregular and white	Gram positive Lactococci	Negative
Millet Rice	Sd-3	Small, circular, regular and white	Gram positive Lactococci	Negative
Myrobalan	Sd-4	Medium, circular, regular and white	Gram positive Lactococci	Negative
Black paper	Sd-5	Small, circular, regular and white	Gram positive Lactococci	Negative
Areca nut	Sd-6	Small, circular, regular and white	Gram positive Lactococci	Negative
Coriander	Sd-7	Small, circular, regular and white	Gram positive Lactococci	Negative
Sweet coriander	Sd-8	Small, circular, regular and white	Gram positive Lactococci	Negative
Mustard	Sd-9	Small, circular, regular and white	Gram positive Lactococci	Negative
Sunflower	Sd-10	Small, circular, regular and white	Gram positive Lactococci	Negative
Green beans	Sd-11	Medium, circular, regular and white	Gram positive Lactococci	Negative
Cumin	Sd-12	Small, circular, regular and white	Gram positive Lactococci	Negative
Black Cumin	Sd-13	Small, circular, regular and white	Gram positive Lactococci	Negative
	SY-1	Large, oval with elongated budding and white	Yeast	Not done
Radhuni	Sd-14	Small, circular, regular and white	Gram positive Lactococci	Negative
Pignut	Sd-15	Small, circular, regular and white	Lactococci	Negative
Pulses	Sd-16	Small, circular, regular and white	Gram positive Lactococci	Negative
Dabli	Sd-17	Large, circular, irregular and white	Gram positive Lactococci	Negative
	SY-2	Large, oval with elongated budding and white	yeast	Not done
Corn	Sd-18	Medium, circular, regular and white	Gram positive Lactococci	Negative
Lily fruit seeds	Sd-19	Medium, circular, irregular and white	Gram positive Lactobacilli	Negative
Cardamom	Sd-20	Small, circular, regular and white	Gram positive Lactobacilli	Negative
	SY-3	Large, oval with elongated budding and white	Yeast	Not done
Pumpkin seed	Sd-21	Small, circular, regular and white	Gram positive Lactococci	Negative
Nutmeg	Sd-22	Small, circular, regular and white	Gram positive Lactococci	Negative
Black gram	Sd-23	Small, circular, regular and white	Gram positive Lactococci	Negative
Jackfruit seed	Sd-24	Small, circular, regular and white	Gram positive Lactobacilli	Negative
Linseed	Sd-25	Small, circular, regular and white	Gram positive Lactobacilli	Negative
	SY-4	Large, oval with elongated budding and white	Yeast	Not done
Mugdal	Sd-26	Small, circular, regular and white	Gram positive Lactococci	Negative
Fenugreek	Sd-27	Small, circular, regular and white	Gram positive Lactococci	Negative

Table 2: Results of biochemical tests of isolated bacteria.

Isolate no	Oxidase test	Catalase test	KIA test	MIU test
Sd-1	+	-	alkaline/acid	M (-), I (-), U (Yellow)
Sd-2	+	-	acid/acid	M (-), I (-), U (Yellow)
Sd-3	+	-	acid/acid	M (-), I (-), U (Yellow)
Sd-4	-	-	alkaline/alkaline	M (-), I (-), U (Yellow)
Sd-5	-	-	alkaline/acid	M (-), I (-), U (Yellow)
Sd-6	-	-	alkaline/alkaline	M (-), I (-), U (Yellow)
Sd-7	-	-	acid/acid	M (-), I (-), U (Yellow)
Sd-8	-	-	acid/acid	M (-), I (-), U (Yellow)
Sd-9	-	-	acid/acid	M (-), I (-), U (Yellow)
Sd-10	+	-	alkaline/alkaline	M (-), I (-), U (Yellow)
Sd-11	+	-	alkaline/acid	M (-), I (-), U (Yellow)
Sd-12	-	-	alkaline/alkaline	M (-), I (-), U (Yellow)
Sd-13	-	-	acid/acid	M (-), I (-), U (Yellow)
Sd-14	-	-	alkaline/alkaline	M (-), I (-), U (Yellow)
Sd-15	-	-	acid/acid	M (-), I (-), U (Yellow)
Sd-16	+	+	alkaline/alkaline	M(-), I (-), U(Yellow)
Sd-17	+	-	alkaline/alkaline	M (-), I (-), U (Yellow)
Sd-18	-	+	acid/acid	M (-), I (-), U (Yellow)
Sd-19	-	-	alkaline/acid	M (-), I (-), U (Yellow)
Sd-20	-	-	alkaline/alkaline	M (-), I (-), U (Yellow)
Sd-21	-	-	acid/acid	M (-), I (-), U (Yellow)
Sd-22	-	-	acid/acid	M (-), I (-), U (Yellow)
Sd-23	-	-	alkaline/acid	M (-), I (-), U (Yellow)
Sd-24	-	-	alkaline/alkaline	M (-), I (-), U (Yellow)
Sd-25	-	-	alkaline/acid	M (-), I (-), U (Yellow)
Sd-27	-	-	alkaline/alkaline	M (-), I (-), U (Yellow)
+ = positive; - = negative; KIA: Slant/Butt, M= motile, I= indole; U= urease				

(Tamang et al., 2017; Rezac et al., 2018). LAB are particularly adapted to nutrient-rich and fluctuating environments, enabling them to exhibit functional attributes that are favorable for survival in the gastrointestinal tract (Gaggia et al., 2011).

The isolates in this study showed strong tolerance to highly acidic (pH 1.5) and alkaline (pH 8.5) conditions (Table 3). Acid tolerance is a primary selection criterion for potential probiotic strains because microorganisms must endure gastric acidity during oral consumption (Dunne et al., 2001). Several studies have also shown that *Lactobacillus* and *Lactococcus* species typically survive pH levels as low as 2.0, confirming their suitability for gastrointestinal

passage (Begley et al., 2005; Lin et al., 2007). The observed alkaline tolerance further suggests resilience in the intestinal environment, where pH ranges from 7.5 to 8.5.

Salt tolerance up to 6% NaCl observed in all isolates is also consistent with properties reported in LAB originating from fermented plant foods (Angmo et al., 2016). Salt tolerance enhances microbial survivability during fermentation and storage, making such strains valuable for functional food and pharmaceutical applications. All isolates survived within a broad temperature range (4°C-55°C), indicating significant physiological adaptability (Table 3). Previous studies have similarly reported wide

Table 3: Results of NaCl, pH and temperature tolerance test of isolates.

Sample no.	1% NaCl	6% NaCl	pH 1.5	pH 3.5	pH 8.5	4°C	45°C	55°C	60°C
Sd-1	++	+	+	+	+	+	+	+	-
Sd-2	++	+	+	+	+	+	++	+	-
Sd-3	+	+	+	+	+	+	+	+	-
Sd-4	++	+	—	+	+	+	+	-	-
Sd-5	+	+	+	+	+	+	++	+	-
Sd-6	+	—	+	+	+	+	+	-	-
Sd-7	++	+	+	+	+	+	+	+	-
Sd-8	++	+	+	+	+	+	+	+	-
Sd-9	+	+	+	+	+	+	+	+	-
Sd-10	++	+	+	+	+	+	+	+	-
Sd-11	++	+	+	+	+	+	+	-	-
Sd-12	+	+	+	+	+	+	+	-	-
Sd-13	++	+	+	+	+	+	+	-	-
Sd-14	++	+	—	+	+	+	++	+	-
Sd-15	+	+	+	+	+	+	+	+	-
Sd-16	++	+	+	+	+	+	+	+	-
Sd-17	++	+	+	+	+	+	+	+	-
Sd-18	++	++	+	+	+	+	+	+	-
Sd-19	++	+	+	+	+	+	++	+	-
Sd-20	+	+	+	+	+	+	++	+	-
Sd-21	++	+	+	+	+	+	++	+	-
Sd-22	++	+	+	+	+	+	+	+	-
Sd-23	++	+	+	+	+	+	+	-	-
Sd-24	+	++	+	+	+	+	+	-	-
Sd-25	+	+	+	+	+	+	+	-	-
Sd-26	++	+	+	++	+	+	+	+	-
Sd-27	++	+	+	+	+	+	++	+	-
+ = positive and - = negative									

thermal tolerance among LAB, which is advantageous for industrial processing and formulation stability (Hossain et al., 2019).

The presence of both *Lactobacillus* and *Lactococcus* species is significant because these genera are well-documented for their probiotic benefits, including modulation of host immunity, enhancement of gut barrier function, and antagonistic activity against pathogens (Hill et al., 2014; Lebeer et al., 2008). Yeast isolates, though less frequently studied than bacterial

probiotics, also offer substantial functional advantages such as toxin binding, vitamin synthesis, and tolerance to gastrointestinal conditions (Kumura et al., 2004). In this study, the effectiveness of the antibacterial effect against pathogenic bacteria was found to be promising. One of the most important therapeutic potentials of probiotics is their antibacterial activity against pathogenic microorganisms. Probiotics can produce various antimicrobial compounds, such as organic acids, bacteriocins, hydrogen peroxide, and other

bioactive metabolites, which inhibit the growth of harmful bacteria. The identification and characterization of these effective antibacterial compounds may contribute to the development of improved treatment strategies for infectious diseases and help address the growing challenge of antimicrobial resistance. Our study showed consistency results with others study of probiotic potential strains. Both isolated bacteria and yeast have characteristic properties that make them gut-friendly and potential probiotics.

5. CONCLUSION

In this study, primarily Gram-positive lactic acid bacteria belonging to *Lactobacillus* and *Lactococcus* spp., along with four yeast strains, were identified from fermented dried seeds. The isolates demonstrated strong tolerance to acidic and alkaline pH, high salt concentration, and a wide temperature range, indicating their robust physiological adaptability. These characteristics align with key selection criteria for probiotic candidates. Results of this study revealed that the isolated LAB are suitable to survive in the environment of the human gastrointestinal tract and showed antibacterial potential in inhibiting pathogenic bacteria. These findings suggest that dried seeds may represent promising natural sources of microorganisms with probiotic potential.

List of abbreviations

MRS	de Man, Rogosa and Sharpe broth
broth	
PD	Potato dextrose
LAB	Lactic acid bacteria
GI	Gastrointestinal
MIU	Motility, Indole, and Urease
MHA	Mueller Hinton agar

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Conflict of Interest

None declared.

Ethical Statement

There is no ethical approval is required.

Declaration of Generative AI

No artificial intelligence (AI) tools were used in the preparation of this manuscript.

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