International Journal of Community Medicine and Public Health
Kumar HKS et al. Int J Community Med Public Health. 2026 Jan;13(1):351-358
http://www.ijcmph.com
pISSN 2394-6032 | eISSN 2394-6040

DOI: https://dx.doi.org/10.18203/2394-6040.ijcmph20254448

Original Research Article

Comparative assessment of probiotic properties of seven commercially available probiotic strains: an *in vitro* study

Amrutha S. Raj, Anjana Baby, Hareeshma K. S., Nila Udayan, Harish Kumar K. S.*

Department of Medical Microbiology, School of Medical Education, Centre for Professional and Advanced Studies, Kottayam, Kerala, India

Received: 06 November 2025
Revised: 19 December 2025
Accepted: 20 December 2025

***Correspondence:**
Dr. Harish Kumar K. S.,
E-mail: drharishkumarks@gmail.com

ABSTRACT

Background: Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host. Their ability to survive harsh gastrointestinal conditions such as low pH, bile salts, and toxic compounds like phenol is crucial for colonization and activity. The present study was undertaken to evaluate the probiotic properties of commercially available probiotic strains including *Lactobacillus acidophilus*, *Limosilactobacillus reuteri, Lacticaseibacillus rhamnosus, Shouchella clausii, Weizmannia coagulans, Bacillus subtilis and Saccharomyces boulardii*.
Methods: All strains were germinated in brain heart infusion (BHI) broth and subjected to standard probiotic property evaluation. Acid, bile, and phenol tolerance were assessed using modified BHI broth adjusted to pH 2.0, 0.3% bile, and 0.4% phenol, respectively, with viable counts determined at specific time intervals. Antagonistic activity was tested against *E. coli* isolates by agar overlay method.
Results: Among tested strains, *Shouchella clausii* and *Weizmannia coagulans* exhibited highest tolerance to acidic, bile and phenolic stress. *Limosilactobacillus reuteri* showed moderate tolerance, while Lacticaseibacillus rhamnosus and Lactobacillus acidophilus demonstrated fair survival rates. *Saccharomyces boulardii* and *Bacillus subtilis* recorded comparatively lower tolerance. Most isolates showed significant inhibitory activity against coliforms.
Conclusions: The study highlights the potential of commercially available probiotic formulations as effective candidates for gastrointestinal health. The survival ability of Bacillus species under harsh gut conditions suggests their suitability as stable probiotics. Further studies focusing on molecular mechanisms and clinical efficacy are warranted.

Keywords: *Shouchella clausii, Limosilactobacillus reuteri, Lacticaseibacillus rhamnosus, Lactobacillus acidophilus, Weizmannia coagulans, Bacillus subtilis, Saccharomyces boulardii*

INTRODUCTION

Probiotics are defined by the world health organization as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host." The term "probiotic" originates from the Greek words meaning "for life," and describes a heterogeneous group of live microorganisms, including bacterial genera (*Lactobacillus*, *Bifidobacterium*, *Streptococcus, Bacillus*) and yeast species (*Saccharomyces*).[2] The beneficial effects of probiotics are mainly attributed to their ability to stabilize the intestinal microflora, stimulate host immune responses, and inhibit the growth of pathogenic microorganisms. Among them, *Lactobacillus, Bacillus*, and *Saccharomyces* are widely recognized for their diverse health-promoting effects.[2,3]

Probiotics exert a wide range of beneficial effects, not only in maintaining gastro intestinal health but also in the prevention of antibiotic-associated diarrhoea,

gastrointestinal infections, lactose intolerance, allergic diseases, and certain cancers.[8] The effective action of probiotic microorganisms depends on their ability to survive the harsh gastrointestinal environment, including exposure to gastric acidity, bile salts, and metabolic products such as phenol.[4,5] In addition, these microorganisms often exhibit antagonistic activity against enteric pathogens, particularly *E. coli*, through the production of organic acids and bacteriocins, thereby enhancing their therapeutic potential.[6,7] With growing commercial and scientific interest in probiotics, selecting strains that consistently deliver specific health benefits remains a major challenge. Probiotic properties are strain-specific, resulting in substantial functional diversity even within the same species.[8,9] Therefore, comparative understanding of their survivability and functional attributes under simulated gastrointestinal conditions is essential for validating their claimed benefits.[10] Recent taxonomic revisions by the international committee on systematics of prokaryotes have reclassified several probiotic species, with the former genus *Lactobacillus* now divided into multiple genera such as *Lacticaseibacillus rhamnosus, Limosilactobacillus reuteri,*and others, while former *Bacillus s*pecies such as *Bacillus clausii* and *Bacillus coagulans* have been reassigned to the genera *Shouchella* and *Weizmannia*, respectively.[11] The present study aimed to evaluate the acid, bile, phenol tolerance, antimicrobial activity, antibiotic susceptibility of seven commercially available probiotic strains-*L. acidophilus, Lacticaseibacillus rhamnosus, Limosilactobacillus reuteri, S. clausii, W. coagulans, B. subtilis. S. boulardii*

METHODS

The present cross-sectional study was conducted at school of medical education (SME), Kottayam, Kerala, India from June 2024 to June 2025.

Microbial strains

The lyophilized spore form of *Shouchella clausii* UBBC-07 and *Bacillus subtilis* (HU58*) were obtained as an oral suspension from Novogermina™ (Alkem Laboratories Ltd.) and Gutpro Mini Probiotic (JB Chemicals and Pharmaceuticals Ltd.) respectively. *Weizmannia coagulans* was produced from Velbiom Q-Gazz (Velbiom Probiotics Private Ltd.) *Limosilactobacillus reuteri* DSMZ 17648 (SONATA™LR) was obtained from Sun Pharmaceutical Industries Ltd., *Lacticaseibacillus rhamnosus* GG (ProGG) from ARISTO Pharmaceuticals Private Ltd., and *Lactobacillus acidophilus* MTCC 10307. A total of 25 *E. coli* clinical isolates were retrieved from the culture collection of the department of medical microbiology, SME, Kottayam and used for the present study.

Acid, bile, phenol tolerance tests were performed to evaluate the survival of probiotic strains under gastrointestinal stress, following the methodology described by Yadav et al while the antagonistic activity against *E. coli* was evaluated using the agar overlay method, based on the studies by Raj et al.[12,13]

Acid tolerance

To examine the effect of low pH on probiotic viability, isolates were incubated overnight in MRS /BHI broth at 37°C. Actively grown cells were harvested by centrifugation (7000 rpm, 4°C, 10 min). The pH of MRS/BHI broth was adjusted at pH 2.0 with 1N HCl. MRS broth adjusted to pH 6.5 was used as a control. Harvested cells were resuspended in MRS broth with acidic pH and incubated at 37°C. After a time, interval of 0-, 1-, and 2-hours samples were withdrawn and serially diluted in phosphate buffer saline (PBS). Samples were plated on MRS /MH agar plates and incubated at 37°C for 48 h. Cell viability was assessed by the plate count method and the results were expressed as log cfu/ml.

Bile tolerance

To evaluate the ability of probiotic strains to survive in the presence of bile salts, overnight precultures were harvested and resuspended in 5 ml of MRS/BHI medium supplemented with 0.3% Oxgall, and without as control After inoculation, samples were incubated at 37°C. After a time, interval of 0, 1, 2, hours samples were withdrawn and serially diluted using normal saline. Viable cell colonies were enumerated at 0, 1, and 2, h by plating 100 µl of cultures of appropriate dilutions onto MRS /MHA.

Resistance to phenol

Gut bacteria can deaminate aromatic amino acids, which are derived from dietary proteins and may lead to the formation of phenols. These phenol compounds can inhibit the growth of probiotics. Therefore, resistance to phenol by probiotics is important for their survival in the gastrointestinal tract. To determine the resistance of probiotic strains to phenolic compounds, the overnight grown cultures were inoculated in MRS/BHI broth with 0.4% phenol. After 0 and 24 h intervals, cultures were spread on MRS agar/MHA plates using serial dilution method. Cell viability was enumerated using plate count.

Agar overlay method for antagonistic activity

The antagonistic activity of seven commercially available probiotic strains against 25 different *E. coli* isolates was evaluated using the agar overlay method. Each probiotic strain was first inoculated as a 10 µL spot onto Mueller-Hinton Agar (MHA) plates and incubated overnight at 37°C to allow visible colony development. After incubation, a soft agar overlay (0.7% agar concentration) was prepared by mixing 100 µl of an overnight broth culture of *E. coli* with 5 mL of molten MHA (cooled to approximately 45°C). This mixture was then carefully overlaid on the surface of the probiotic-inoculated MHA plate. After solidification, the plates were incubated at

37°C for 24 hours. Zones of inhibition surrounding the probiotic colonies were measured in mm to assess the antagonistic effect against each *E. coli* strain. Results were categorized as sensitive, intermediate, or resistant based on zone diameter

Statistical analysis

All data and graphs were processed using Microsoft excel and appropriate statistical analysis were performed. The study was approved by the institutional ethical committee (IEC) at the School of Medical Education, Kerala, India.

RESULTS

Commercial probiotic strains of *Lactobacillus acidophilus*, *Lacticaseibacillus rhamnosus*, *Limosilactobacillus reuteri*, *Shouchella clausii, Weizmannia coagulans, Bacillus subtilis*, and *S.boulardii.* were germinated in BHI broth by overnight incubation, and subcultured on MRS agar/MHA for further assays. All isolates were then subjected to *in vitro* property testing including acid tolerance, bile salt tolerance, phenol resistance, and antagonistic activity against clinical strains of *E. coli.*

Detection of acid tolerance of probiotic strains

The Table 1 summarizes the acid tolerance of seven strains of probiotics by testing their survival rate after being left in an acidic pH (2.0) for up to 2 hours. Results are presented as log cfu/ml (Colony forming units per milliliter) and as a survivability percentage. *Limosilactobacillus reuteri* was the most acid-tolerant, with 76.5% survivability after 2 hours. *W. coagulans* and *S. clausii* were also found to be highly acid tolerant with survivability of 83.9% and 85% respectively after 2 hours. The survivability of the remaining strains, *Lacticaseibacillus rhamnosus* (63.0%), *Lactobacillus acidophilus* (60%), *B. subtilis* (59%), and *S. boulardii* (46.4%), was found to be less in 2 hours with *S. boulardii* showing the least percentage of survival. In short, the research established that the most acid-resistant strains among those examined are *Limosilactobacillus reuteri, W. coagulans, and S. clausii*, while the remaining ones had a more pronounced decline in viability upon exposure to an acidic condition

Detection of bile tolerance of probiotic strains

The Table 2 gives an overview of the bile tolerance of seven probiotic strains by determining their survivability after the exposure of bile up to 2 hours. The results are given as log cfu/ml and percentage survivability. *S. clausii* had the best bile tolerance, with a survivability rate of 83% after 2 hours. *W. coagulans* and *Limosilactobacillus reuteri* also exhibited high tolerance to bile with survivability rates of 77.7% and 67% respectively after 2 hours. The other strains exhibited a more significant drop in viability. The survivability rates after 2 hours were: *Lacticaseibacillus rhamnosus* (65.7%), *S. boulardii* (61.5%), *L. acidophilus* (61.4%), and *B. subtilis* (57.8%). Overall, the study suggests that *S. clausii*, *W. coagulans,* and *Limosilactobacillus reuteri* are most tolerant to bile among the strains tested.

Detection of phenol tolerance of probiotic strains

The Table 3 shows the outcome of a study determining the tolerance of seven probiotic strains to phenol after 24 hours of exposure. The results are determined using a viability percentage. *S. clausii* registered the highest tolerance to phenol at 71% viability after 24 hours. *W. coagulans* and *Limosilactobacillus reuteri* had significant tolerance, with survivability rates of 67% and 62.8% respectively. The others had less viability percentages: *Lacticaseibacillus rhamnosus* (58.5%), *B. subtilis* (58.2%), *L. acidophilus* (55%), and *S. boulardii* (52%). In summary, *Shouchella clausii* was the most tolerant to phenol among the strains, followed by *W. coagulans* and *Limosilactobacillus reuteri*. The remaining strains had a greater reduction in viability after exposure to phenol

Antagonistic activity of probiotic strains against E. coli

B. subtilis and *L. acidophilus* recorded the highest mean antagonistic activity with 4.16 and 3.84, respectively. The remaining strains-*Lacticaseibacillus rhamnosus, Limosilactobacillus reuteri, S. clausii*, and *W. coagulans*-recorded much lower mean values ranging from 2.12 to 2.28. *S. boulardii* had no antagonistic activity with a mean of 0. ANOVA (Analysis of variance) The ANOVA table indicates a p value (Sig.) of 0.048. Because the p value is less than 0.05, there is a statistically significant difference in the mean antagonistic activity against *E. coli* between the seven probiotic strains (Table 5). This indicates that at least one of the strains has a significantly different mean antagonistic activity from the others. In summary, the findings reveal that although there was a statistically significant difference in the efficacy of the tested probiotics in inhibiting *E. coli, B. subtilis* and *L. acidophilus* were more effective. However, *S. boulardii* had no effect.

The multiple comparisons table 6 (Tukey HSD) indicates which particular probiotic strains are significantly different from each other in their antagonistic activity against *E. coli*. Significance is indicated by the p value, with a value below 0.05 indicating a statistically significant difference between the two groups being compared. Key findings: The single statistically significant difference was between *B. subtilis* and *S. boulardii*. The p value for the comparison in this case is 0.028, which is below 0.05. This indicates that *B. subtilis* exhibits a significantly greater antagonistic activity against *E. coli* than does *S. boulardii*. None of the other combinations of probiotic strains demonstrated a statistically significant difference between their antagonistic activity (all other p values are above 0.05). Overall, although the ANOVA test overall showed

significant difference between the groups, the Tukey HSD post-hoc test identifies that the main cause of this difference lies in the higher antagonistic activity exhibited by *B. subtilis* when compared to *S. boulardii*. None of the other strains were found to be statistically different from one another.

Table 1: Acid tolerance of probiotic strains at pH 2.0 over 0, 1 and 2 hours.

Probiotics	Time point	1/11	1/33	1/99	1/297	1/891	Cfu/ml	Log cfu/ml	Survivability
Limosilactobacillus reuteri	0	Confluent	Confluent	340	117	40	336,600	5.53	100%
	1	Confluent	Confluent	287	93	27	284,130	5.45	84.4%
	2	Confluent	Confluent	260	80	21	257,400	5.41	76.5%
Lacticaseibacillus rhamnosus	0	Confluent	Confluent	330	105	33	326,700	5.51	100%
	1	Confluent	Confluent	248	85	25	245,520	5.39	75.2%
	2	Confluent	Confluent	208	62	20	205,920	5.31	63.0%
L. acidophilus	0	Confluent	Confluent	310	108	32	306,900	5.49	100%
	1	Confluent	Confluent	226	70	18	223,740	5.35	72.9%
	2	Confluent	Confluent	186	58	15	184,140	5.27	60%
S. clausii	0	Confluent	Confluent	120	45	18	118,800	5.07	100%
	1	Confluent	Confluent	108	34	10	106,920	5.03	90%
	2	Confluent	Confluent	102	28	9	100,980	5.00	85%
W. coagulans	0	Confluent	Confluent	280	89	26	277,200	5.44	100%
	1	Confluent	Confluent	246	80	24	243,540	5.39	87.9%
	2	Confluent	Confluent	235	75	20	232,650	5.37	83.9%
B. subtilis	0	Confluent	Confluent	200	70	28	198,000	5.30	100%
	1	Confluent	Confluent	138	48	12	136,620	5.14	69%
	2	Confluent	Confluent	118	34	9	116,820	5.07	59%
S. boulardii	0	Confluent	Confluent	222	78	20	219,780	5.34	100%
	1	Confluent	Confluent	132	48	16	130,680	5.12	59.4%
	2	Confluent	Confluent	103	39	10	101,970	5.01	46.4%

Table 2: Bile tolerance of probiotic strains at 0.3% oxgall over 0, 1 and 2 hours.

Probiotic name	Time point	1/11	1/33	1/99	1/297	891	Cfu/ml	Log cfu/ml	survivability
Limosilactobacillus reuteri	0	Confluent	Confluent	300	95	26	297,000	5.47	100%
	1	Confluent	Confluent	234	80	23	231,660	5.36	78%
	2	Confluent	Confluent	200	71	19	198,000	5.30	67%
Lacticaseibacillus rhamnosus	0	Confluent	Confluent	280	100	28	277,200	5.44	100
	1	Confluent	Confluent	207	64	18	204,930	5.31	74%
	2	Confluent	Confluent	184	58	13	182,160	5.26	65.7%
L. acidophilus	0	Confluent	Confluent	290	103	31	287,100	5.46	100%
	1	Confluent	Confluent	203	73	20	200,970	5.30	70%
	2	Confluent	Confluent	178	52	13	176,220	5.25	61.4
S. clausi	0	Confluent	Confluent	100	36	15	99,000	5.000	100%
	1	Confluent	Confluent	88	26	10	87,120	4.94	88%
	2	Confluent	Confluent	83	22	6	82,170	4.91	83%
W. coagulans	0	Confluent	Confluent	260	89	32	257,400	5.41	100%
	1	Confluent	Confluent	221	76	28	218,790	5.34	85%
	2	Confluent	Confluent	202	66	19	199,980	5.30	77.7%
B. subtilis	0	Confluent	Confluent	230	80	28	227,700	5.36	100%
	1	Confluent	Confluent	156	44	12	154,440	5.19	67.8%
	2	Confluent	Confluent	133	38	9	131,670	5.12	57.8%
S. boulardii	0	Confluent	Confluent	200	71	26	198,000	5.30	100%
	1	Confluent	Confluent	138	42	15	136,620	5.13	69%
	2	Confluent	Confluent	123	35	9	121,770	5.09	61.5%

Table 3: Phenol tolerance of probiotic strains in 0.4% phenol over 0 hour and 24 hours.

Probiotic name	Time point	1/11	1/33	1/99	1/297	1/891	Cfu/ml	Log cfu/ml	Viability
Limosilactobacillus reuteri	0	Confluent	Confluent	320	100	14	316,800	5.50	100%
	24	Confluent	Confluent	201	65	22	198,990	5.30	62.8%
Lacticaseibacillus rhamnosus	0	Confluent	Confluent	270	93	24	267300	5.43	100%
	24	Confluent	Confluent	158	48	12	156420	5.19	58.5%
L. acidophilus	0	Confluent	Confluent	300	107	30	297,000	5.47	100%
	24	Confluent	Confluent	165	58	15	163,350	5.21	55%

Continued.

Probiotic name	Time point	1/11	1/33	1/99	1/297	1/891	Cfu/ml	Log cfu/ml	Viability
S. clausii	0	Confluent	Confluent	100	34	15	99,000	5.0	100%
	24	Confluent	Confluent	71	26	6	70,290	4.85	71%
W. coagulans	0	Confluent	Confluent	230	80	23	227,700	5.36	100%
	24	Confluent	Confluent	154	48	13	152,460	5.18	67%
B. subtilis	0	Confluent	Confluent	220	70	25	217,800	5.34	100%
	24	Confluent	Confluent	128	44	13	126,720	5.10	58.2%
S. boulardii	0	Confluent	Confluent	230	83	30	227,700	5.36	100%
	24	Confluent	Confluent	120	37	8	118,800	5.07	52%

Table 4: Antagonistic activity of probiotic strains against *E. coli.*

Probiotic name	N	Mean	SD	Std. error	95% CI for mean	
					Lower bound	Upper bound
L. acidophilus	25	3.84	5.86	1.17	1.42	6.26
Lacticaseibacillus rhamnosus	25	2.16	4.42	0.88	0.33	3.99
Limosilactobacillus reuteri	25	2.12	4.34	0.87	0.33	3.91
S. clausii	25	2.28	4.67	0.93	0.35	4.21
W. coagulans	25	2.28	4.67	0.93	0.35	4.21
B. subtilis	25	4.16	5.71	1.14	1.80	6.52
S. boulardii	25	0	0.00	0.00	0.00	0.00
Total	175	2.41	4.71	0.36	1.70	3.11

Table 5: One-way ANOVA for antagonistic activity against *E. coli.*

Groups	Sum of squares	Df	Mean square	F	P value
Between groups	277.39	6	46.23	2.17	0.048
Within groups	3574.80	168	21.28		
Total	3852.19	174			

Table 6: Tukey HSD post-hoc multiple comparison analysis.

(I) *E. coli* strain	(J) *E. coli* strain	Mean difference (I-J)	Std. error	P value*	95% CI	
					Lower bound	Upper bound
L. acidophilus	*L. rhamnosus*	1.68	1.30	0.86	-2.21	5.57
	Limosilactobacillus reuteri	1.72	1.30	0.84	-2.17	5.61
	S. clausii	1.56	1.30	0.90	-2.33	5.45
	W. coagulans	1.56	1.30	0.90	-2.33	5.45
	B. subtilis	-0.32	1.30	1.00	-4.21	3.57
	S. boulardii	3.84	1.30	0.06	-0.05	7.73
Lacticaseibacillus rhamnosus	*L. acidophilus*	-1.68	1.30	0.86	-5.57	2.21
	Limosilactobacillus reuteri	0.04	1.30	1.00	-3.85	3.93
	S. clausii	-0.12	1.30	1.00	-4.01	3.77
	W. coagulans	-0.12	1.30	1.00	-4.01	3.77
	B. subtilis	-2	1.30	0.73	-5.89	1.89
	S. boulardii	2.16	1.30	0.65	-1.73	6.05
Limosilactobacillus reuteri	*L.acidophilus*	-1.72	1.30	0.84	-5.61	2.17
	Lacticaseibacillus rhamnosus	-0.04	1.30	1.00	-3.93	3.85
	S.clausii	-0.16	1.30	1.00	-4.05	3.73
	W. coagulans	-0.16	1.30	1.00	-4.05	3.73
	B. subtilis	-2.04	1.30	0.71	-5.93	1.85
	S. boulardii	2.12	1.30	0.67	-1.77	6.01
S. clausii	*L. acidophilus*	-1.56	1.30	0.90	-5.45	2.33
	L. rhamnosus	0.12	1.30	1.00	-3.77	4.01
	Limosilactobacillus reuteri	0.16	1.30	1.00	-3.73	4.05
	W. coagulans	0	1.30	1.00	-3.89	3.89
	B. subtilis	-1.88	1.30	0.78	-5.77	2.01
	S. boulardii	2.28	1.30	0.59	-1.61	6.17

Continued.

(I) *E. coli* strain	(J) *E. coli* strain	Mean difference (I-J)	Std. error	P value*	95% CI	
					Lower bound	Upper bound
W. coagulans	*L.acidophilus*	-1.56	1.30	0.90	-5.45	2.33
	Lacticaseibacillus rhamnosus	0.12	1.30	1.00	-3.77	4.01
	Limosilactobacillus reuteri	0.16	1.30	1.00	-3.73	4.05
	S. clausii	0	1.30	1.00	-3.89	3.89
	B. subtilis	-1.88	1.30	0.78	-5.77	2.01
	S. boulardii	2.28	1.30	0.59	-1.61	6.17
B. subtilis	*L. acidophilus*	0.32	1.30	1.00	-3.57	4.21
	Lacticaseibacillus rhamnosus	2	1.30	0.73	-1.89	5.89
	Limosilactobacillus reuteri	2.04	1.30	0.71	-1.85	5.93
	S. clausii	1.88	1.30	0.78	-2.01	5.77
	W. coagulans	1.88	1.30	0.78	-2.01	5.77
	S. boulardii	4.16000*	1.30	0.03	0.27	8.05
S. boulardii	*L. acidophilus*	-3.84	1.30	0.06	-7.73	0.05
	Lacticaseibacillus rhamnosus	-2.16	1.30	0.65	-6.05	1.73
	Limosilactobacillus reuteri	-2.12	1.30	0.67	-6.01	1.77
	S. clausi	-2.28	1.30	0.59	-6.17	1.61
	W. coagulans	-2.28	1.30	0.59	-6.17	1.61
	B. subtilis	-4.16000*	1.30	0.03	-8.05	-0.27

*p< 0.05 considered statistically significant (Tukey HSD test).

DISCUSSION

In the present study, *S. clausii* and *W. coagulans* exhibited the highest acid tolerance, maintaining viability above 80% after 2 hours of incubation at pH 2.0. *B. subtilis* also showed good tolerance, with survival rates around 60%. Among the lactobacilli, *Limosilactobacillus reuteri* demonstrated relatively higher survival (76%), whereas *Lacticaseibacillus rhamnosus* and *L. acidophilus* showed moderate-to-lower tolerance (63% and 60%, respectively). *S. boulardii* exhibited the lowest survivability under acidic conditions (46%). These findings are consistent with previous reports indicating that spore-forming *Bacillus* species exhibit greater resistance to gastric acidity than non-spore-forming bacteria as shown by Cutting et al and Yadav et al.[3,12] The moderate survival of *Limosilactobacillus reuteri* and *Lacticaseibacillus rhamnosus* aligns with earlier studies suggesting that acid resistance among *Lactobacillus spp*. is strain-dependent and influenced by factors such as membrane fatty-acid composition, proton-pumping systems (F1F0-ATPase), and production of stress response proteins as described by Ramose et al and Begley et al.[5,14] The comparatively low survivability of *S. boulardii* observed in this study supports prior observations that some yeast probiotics, despite their beneficial metabolic activities, may exhibit sensitivity to prolonged exposure to very low pH depending on inoculum density and environmental conditions as noted by Edwards-Ingram et al.[15]

Compared with acid tolerance pattern, bile tolerance followed a similar trend in which *S. clausii* again the demonstrated the highest survivability (83%) after 2 hours of exposure to 0.3% bile, followed by *W. coagulans* (77.7%). *Limosilactobacillus reuteri* and *Lacticaseibacillus rhamnosus* demonstrated moderate survival (67% and 65.7%, respectively), while *L. acidophilus* maintained 61.4% viability. In contrast, *B. subtilis* and *S. boulardii* displayed comparatively lower tolerance (57.8% and 61.5%). These observations indicate that spore-forming *Bacillus* species are inherently more resistant to bile stress, consistent with the findings of Cutting et al and Yadav et al who noted that endospore formation and membrane lipid alterations enhance survivability in intestinal conditions.[3,12] The moderate bile tolerance observed in *Lactobacillus* strains agrees with reports by Begley et al and Ramose et al where species such as *Lacticaseibacillus rhamnosus* and *Limosilactobacillus reuteri* exhibited strain-specific resistance depending on bile-salt-hydrolase (BSH) activity and cell-surface adaptation mechanisms. Similarly, reduced survival of *S. boulardii* aligns with the results of Gilliland et al and Guo et al who suggested that non-bacterial probiotics lacking BSH enzymes are more susceptible to bile-induced membrane disruption.[5,14,16,17] Overall, the findings of this study reinforce previous evidence that bile tolerance is a strain-dependent trait influenced by physiological factors such as bile salt hydrolase (BSH) activity, membrane integrity, and stress-response proteins, which collectively determine the ability of probiotics to survive intestinal transit.

Tolerance to phenolic compounds showed marked variability among the evaluated probiotic strains. *S. clausii* again demonstrated the highest survivability

(approximately 71%) after 24 hours of exposure to 0.4% phenol, followed closely by *W. coagulans* (67%). *Limosilactobacillus reuteri* maintained moderate resistance (around 62.8%), while *Lacticaseibacillus rhamnosus*, *B. subtilis* and *L. acidophilus* showed comparatively lower survival rates (58.5%, 58.2 and 55%, respectively). *S. boulardii* was the most sensitive, retaining only about 52% viability. These findings are in line with reports of Zheng et al and Ramose et al who noted that *Bacillus* species withstand phenolic toxicity due to their robust spore coat and efficient oxidative stress defenses.[11,14] The intermediate resistance in *Limosilactobacillus reuteri* and *Lacticaseibacillus rhamnosus* is consistent with Begley et al and De Angelis et al which emphasized that phenol tolerance in lactic acid bacteria depends on enzymatic detoxification mechanisms, membrane-bound efflux pumps, and strain-specific stress protein expression.[5,18] The comparatively lower survival of *S. boulardii* aligns with the findings of Panda et al suggesting that phenolic compounds can disrupt yeast cell wall integrity and interfere with normal metabolic activity.[19] Collectively, these observations support that phenol tolerance is a multifactorial trait influenced by cell wall architecture, stress adaptation capacity, and the ability to neutralize toxic aromatic intermediates, all of which contribute to the persistence of probiotic strains under intestinal stress conditions.

The antagonistic activity of probiotic microorganisms is a critical determinant of their therapeutic use, as it reflects the ability to inhibit enteric pathogens such as *E. coli* through secretion of antimicrobial compounds, bacteriocins, and organic acids. In this study the comparative antagonistic effectiveness of seven commercial probiotic strains demonstrated a distinct variance among isolates, supported by ANOVA analysis ($p=0.048$), confirming a statistically significant in their mean inhibition zones. Among the tested strains, *B. subtilis* and *L. acidophilus* exhibited highest antagonistic activity, forming inhibition zones averaging 4.16 mm and 3.84 mm respectively, while *S. boulardii* showed no observable antagonism (0 mm). The post hoc Tukey test further indicated that the significant difference lay especially between *B. subtilis* and *S. boulardii* ($p=0.028$), understanding the effectiveness of bacterial spore-forming species over the yeast-based probiotics. The remaining strains-including *L. rhamnosus*, *L. reuteri, S. clausii, and W. coagulans*-demonstrated moderate inhibition with mean values between 2.12 and 2.28 mm, but without statistically distinct differences from one another. The superior antagonistic effect of *B. subtilis* likely arises from its recognised ability to produce diverse antimicrobial peptides, such as subtilin and bacilysin, that can suppress Gram-negative bacterial growth as demonstrated by Cutting et al and Corr et al.[3,6] Similarly, *L. acidophilus*' moderate inhibitory effect can be attributed to lactic acid and bacteriocin production, consistent with reports from Dunne et al and Sanders et al where *Lactobacillus* strains effectively restricted pathogen proliferation.[2,4] The relatively weak inhibition by *S. clausii*, *Lacticaseibacillus rhamnosus*, and *W. coagulans* suggests strain-specific variability in secondary metabolite production, as supported by Hill et al who emphasized that probiotic functionality is often species- and even strain-dependent.[8]

Importantly, the yeast *S. boulardii* exhibited no inhibitory activity in this model, concordant with prior findings by Ouwehand et al suggesting that its probiotic benefits are primarily immunomodulatory rather than directly bactericidal.[20] Such results indicate limited efficacy of *S. boulardii* in direct antagonism assays but highlight its complementary mechanism of action within the gut ecosystem.

This study has certain limitations. All evaluations were conducted under *in vitro* conditions, which may not fully reflect the complex environment of the human gastrointestinal tract, as factors such as host–microbe interactions, immune modulation, mucosal adherence, and competition with resident microbiota were not assessed. The probiotic strains were derived from commercially available formulations rather than freshly isolated human-origin cultures; thus, processing and storage conditions may have influenced strain viability and metabolic activity. Additionally, strain-level molecular identification was not performed, limiting precise attribution of observed effects. Finally, the study assessed only selected probiotic properties, namely tolerance to acid, bile, and phenol, and antagonistic activity against *E. coli*. Other functional traits, including epithelial adhesion, immunomodulatory potential, and long-term colonization ability, warrant further investigation through molecular and *in vivo* studies.

CONCLUSION

In conclusion, the present study demonstrates that spore-forming probiotics, such as *S. clausii* and *W. coagulans*, exhibit superior tolerance to acidic, bile and phenolic conditions compared with non-spore-forming bacteria and yeast. *Lactobacillus* strains showed moderate, strain-dependent survivability, while *S. boulardii* was the most sensitive under tested conditions. *B. subtilis* and *L. acidophilus* displayed the strongest antagonistic activity against *E. coli*, highlighting the functional diversity among commercial probiotic strains. These findings underscore the importance of selecting strains based on their survival and functional characteristics, as resilience under gastrointestinal stress is critical for probiotic efficacy.

While this study was limited to *in vitro* assessment and did not include molecular characterization or additional functional properties such as adhesion or immunomodulation, the results provide valuable insights for future research. Further *in vivo* studies and genomic analyses are warranted to comprehensively evaluate the therapeutic potential of commercially available probiotic formulations.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. FAO/WHO. Guidelines for the evaluation of probiotics in food. Report of a joint FAO/WHO Working Group on drafting guidelines for the evaluation of probiotics in food, London, Ontario, Canada. Food and Agriculture Organization of the United Nations and World Health Organization. 2002. Available at: https://isappscience.org/wp-content/uploads/2019/04/probiotic_guidelines.pdf. Accessed on 20 October 2025.
2. Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastall RA. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nat Rev Gastroenterol Hepatol. 2019;16(10):605-16.
3. Cutting SM. Bacillus probiotics. Food Microbiol. 2011;28(2):214-20.
4. Dunne C, O'Mahony L, Murphy L, Thornton G, Morrissey D, O'Halloran S, et al. *In vitro* selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. Am J Clin Nutr. 2001;73(2):386S-92S.
5. Begley M, Hill C, Gahan CGM. Bile salt hydrolase activity in probiotics. Appl Environ Microbiol. 2006;72(3):1729-38.
6. Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CGM. Bacteriocin production as a mechanism for the anti-infective activity of *Lactobacillus salivarius* UCC118. Proc Natl Acad Sci U S A. 2007;104(18):7617-22.
7. Ouwehand AC, Salminen S, Isolauri E. Probiotics: an overview of beneficial effects. Int J Food Microbiol. 2002;78(1-2):99-105.
8. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11(8):506-14.
9. Marco ML, Sanders ME, Gänzle M. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on fermented foods and health. Nat Rev Gastroenterol Hepatol. 2021;18(3):196-208.
10. Charteris WP, Kelly PM, Morelli L, Collins JK. Development and application of an *in vitro* methodology to determine the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in the upper human gastrointestinal tract. J Appl Microbiol. 1998;84(5):759-68.
11. Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, et al. A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. Int J Syst Evol Microbiol. 2020;70(4):2782-858.
12. Yadav R, Puniya AK, Shukla P. Probiotic properties of *Lactobacillus plantarum* RYPR1 from an indigenous fermented beverage Raabadi. Front Microbiol. 2016;7:1683.
13. Raj A, Kumar HKS, Anil A, Azhar MM, Kuriakose N. Anti-*Staphylococcus aureus* activity of *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* on clinical isolates: an *in vitro* study. J Med Res. 2024;10(1):5.
14. Ramos CI, Farias-Rodrigues JL, Araujo GR, et al. *in vitro* assessment of acid and bile tolerance of lactic acid bacteria isolated from traditional fermented foods. Fermentation. 2022;8(10):529.
15. Edwards-Ingram L, Gitsham P, Burton N, Warhurst G, Clarke I, Hoyle D, et al. Genotypic and physiological characterization of *Saccharomyces boulardii*, the probiotic strain of *Saccharomyces cerevisiae*. Appl Environ Microbiol. 2007;73(8):2458-67.
16. Gilliland SE, Staley TE, Bush LJ. Importance of bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct. J Dairy Sci. 1984;67(12):3045-51.
17. Guo X, Chen DD, Peng KS, Cao Y, Tan ZY. Comparative evaluation of acid and bile tolerance in *Saccharomyces boulardii* and *Lactobacillus* species. Food Sci Nutr. 2019;7(8):2619-27.
18. De Angelis M, Calasso M, Siragusa S. Comparative transcriptomics and functional characterization of *Lactobacillus plantarum* and *Lactobacillus rhamnosus* under stress conditions. Front Microbiol. 2019;10:1203.
19. Panda AK, Rao SS, Gurumurthy P, Reddy V. Probiotic yeast *Saccharomyces boulardii*-mechanisms of action and clinical implications. Int J Probiotics Prebiotics. 2008;3(1):1-10.
20. Ouwehand AC, Kirjavainen PV, Grönlund MM, Isolauri E, Salminen SJ. Adhesion of probiotic micro-organisms to intestinal mucus. Int Dairy J. 2002;9(9):623-30.

Cite this article as: Raj AS, Baby A, Hareeshma KS, Udayan N, Kumar HKS. Comparative assessment of probiotic properties of seven commercially available probiotic strains: an *in vitro* study. Int J Community Med Public Health 2026;13:351-8.