



Gastrointestinal Stress Severely Limits the Viability of *Lactobacillus paracasei* HU1: Limited Protective Role of Acidic Food Matrices

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ABSTRACT

Background: The functional efficacy of probiotic microorganisms depends critically on their ability to survive gastrointestinal transit, where exposure to gastric acidity, bile salts and food matrices can severely limit viability. *Lactobacillus paracasei* is widely employed in probiotic formulations; however, strain-specific survival under simulated gastrointestinal conditions and in non-dairy food carriers remains inadequately characterized.

Methods: In the present study, the gastrointestinal survival of *Lactobacillus paracasei* HU1 was evaluated using standardized *in vitro* models of simulated gastric juice (SGJ) and simulated intestinal juice (SIJ). Acid-fast staining was performed to assess structural acid resistance. Viability was quantified at 0, 1 and 3 h by plate count methods under gastric and intestinal conditions, with and without food matrices (apple juice and mouth freshener). Bile salt tolerance was assessed at concentrations ranging from 0 to 2.0% (w/v) and results were expressed as log CFU $\pm$ SD and percentage viability.

Result: *L. paracasei* HU1 was confirmed to be acid-fast negative. Exposure to SGJ resulted in a pronounced reduction in viability, with an approximate 51% decline after 3 h, identifying gastric acidity as the primary survival barrier. In contrast, survival under SIJ was comparatively higher, with only a ~25% reduction over the same period. Apple juice and mouth freshener matrices failed to protect *L. paracasei* during gastric exposure, resulting in >90% loss of viability, while modestly improved survival was observed under intestinal conditions. Bile tolerance assays demonstrated good survival at physiological bile concentrations (0.3%), moderate tolerance at 0.5% and severe inhibition at $\geq 1.0\%$ bile salts, confirming concentration- and time-dependent effects. The findings demonstrate that gastric acidity represents the most critical bottleneck for *L. paracasei* survival, while intestinal conditions are comparatively permissive. Acidic, non-dairy food matrices alone are insufficient to confer meaningful gastric protection. These results underscore the necessity of targeted formulation strategies, such as buffering or encapsulation, to enhance probiotic delivery and functional efficacy.

Key words: Food carriers, Gastric tolerance, *Lactobacillus paracasei* HU1, Probiotic viability, Simulated gastrointestinal digestion.

INTRODUCTION

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host, primarily through modulation of the gut microbiota and enhancement of intestinal homeostasis. Species belonging to the genera *Lactobacillus* and *Bifidobacterium* are among the most extensively studied probiotics due to their documented roles in improving digestion, immune regulation and metabolic health. Despite these benefits, the functional efficacy of probiotic preparations largely depends on their ability to survive the physicochemical stresses encountered during passage through the gastrointestinal (GI) tract, including exposure to gastric acid, bile salts and digestive enzymes (Conway, 1996; Lee and Salminen, 2008).

Numerous studies have reported significant reductions in probiotic viability during gastric transit, which may compromise their ability to reach the intestine in sufficient numbers to exert beneficial effects (Fernández and Barbés, 2003; Vleur *et al.*, 2004). Consequently, strategies to improve probiotic survival during GI passage have received increasing attention. Food matrices are frequently explored as delivery vehicles for probiotics, as

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they may provide physical protection, buffering capacity, or nutritional support. However, the protective effects of non-dairy and acidic food carriers remain inconsistent and poorly understood.

Lactobacillus paracasei is a commonly used probiotic species with reported health-promoting properties, including immunomodulatory and gut barrier-enhancing effects. While its survival under gastrointestinal conditions has been evaluated in certain food systems, limited

information is available regarding the influence of commonly consumed acidic food matrices on its viability during GI transit (Gangakhedkar *et al.*, 2026; Subbalakshmi *et al.*, 2024). Therefore, the present study aimed to evaluate the survival of *L. paracasei* HU1 under simulated gastric and intestinal conditions and to assess whether incorporation into apple juice and mouth freshener matrices provides any protective advantage during gastrointestinal exposure.

MATERIALS AND METHODS

The research was carried out at the Department of Microbiology, Parul University, Ahmedabad during 8th Jan 24 to March 25.

Isolation and identification of microorganisms

Potential probiotic microorganisms were isolated using de Man, Rogosa and Sharpe (MRS) agar following standard microbiological protocols (Cappuccino and Sherman, 2014; Goldman and Green, 2009). Serial dilution and spread plate techniques were employed to obtain discrete colonies. The isolate used in this study was identified as *Lactobacillus paracasei* HU1 by 16S rRNA gene sequencing and sequence similarity analysis was performed using the NCBI GenBank database for species-level confirmation (accession no. PX518870).

Acid-fast staining

Acid-fast staining was performed using the Ziehl-Neelsen method to assess retention of carbol fuchsin following acid-alcohol decolorization (Cappuccino and Sherman, 2014; Goldman and Green, 2009).

Bile salt tolerance assay

Bile salt tolerance was evaluated by exposing *Lactobacillus paracasei* HU1 to MRS broth supplemented with bile salts at concentrations of 0, 0.3, 0.5, 1.0 and 2.0% (w/v) at 37°C and viable counts (log CFU) were determined at 0, 1 and 3 h by standard plate count (Cappuccino and Sherman, 2014; Goldman and Green, 2009).

Large-scale production of microbial biomass

The identified *L. paracasei* HU1 culture was propagated in MRS broth and incubated at 37°C until reaching McFarland Standard No. 3 turbidity. Cells were harvested by centrifugation, dried under controlled conditions and stored until further use for viability assessment experiments (Cappuccino and Sherman, 2014; Goldman and Green, 2009).

Viability assessment under simulated gastric and intestinal conditions

Simulated gastric juice (SGJ) was prepared freshly on the day of the experiment (Cappuccino and Sherman, 2014; Goldman and Green, 2009). Pepsin (from porcine gastric mucosa; activity ≥ 250 U/mg) was dissolved in sterile saline (0.85% NaCl) to obtain a final concentration of 3.0 g/L. Sodium chloride (NaCl) was adjusted to a final concentration of 2.0 g/L. The pH was then adjusted to 2.5 ± 0.05 using

1.0 N HCl. The SGJ was sterilized by membrane filtration (0.22 μ m) when required, or prepared aseptically and used immediately. Simulated intestinal juice (SIJ) was also prepared freshly. Pancreatin (from porcine pancreas; 8 $\times$ USP) was dissolved to a final concentration of 1.0 g/L in sterile saline (0.85% NaCl). Bile salts (oxgall) were added at 0.3% (w/v). The pH was adjusted to 8.0 ± 0.05 using 1.0 N NaOH. The FGJ and SIJ was used immediately after preparation and pre-equilibrated to 37°C prior to inoculation. Dried *L. paracasei* HU1 cultures were inoculated into the respective media and incubated at 37°C. Samples were collected at 0, 1 and 3 h, serially diluted and plated on MRS agar. Viable counts were expressed as log CFU/g.

Viability in food matrices

Dried *L. paracasei* HU1 cultures (1.0%, w/v) were incorporated into apple juice and mouth freshener matrices. These preparations were subsequently subjected to simulated gastric and intestinal conditions as described above. Samples were collected at 0, 1 and 3 h and analyzed for viable counts using the spread plate method on MRS agar (Cappuccino and Sherman, 2014; Goldman and Green, 2009).

Statistical analysis

All experiments were performed in triplicate and results are expressed as mean $\pm$ standard deviation. Data were analyzed descriptively to evaluate changes in probiotic viability under different experimental conditions.

RESULTS AND DISCUSSION

Acid-fast staining of *Lactobacillus paracasei* HU1 revealed that the organism did not retain carbol fuchsin following acid-alcohol decolorization and was therefore classified as acid-fast negative. This observation is consistent with the established characteristics of lactic acid bacteria, which lack mycolic acid-rich cell walls required for acid-fastness. The acid-fast negative nature of *L. paracasei* HU1 confirms that any survival under acidic or gastrointestinal conditions is attributable to physiological acid tolerance mechanisms rather than structural resistance and supports the appropriateness of evaluating this strain using low-pH survival and gastrointestinal simulation assays rather than classical acid-fast tests.

Bile salt tolerance

The bile salt tolerance of *L. paracasei* HU1 demonstrated a clear concentration- and time-dependent reduction in viability. In the absence of bile salts (control), the viable count increased slightly from 8.20 ± 0.08 log CFU at 0 h to 8.30 ± 0.07 log CFU at 3 h, indicating normal growth and confirming that the assay conditions were favorable for bacterial survival. At the physiological bile salt concentration of 0.3%, *L. paracasei* HU1 exhibited only a moderate decline in viability, decreasing to 7.40 ± 0.12 log CFU after 3 h. This limited reduction suggests good bile tolerance and indicates that the strain is capable of surviving bile

concentrations typically encountered in the human small intestine (Table 1).

When the bile salt concentration was increased to 0.5%, a more pronounced decrease in viable counts was observed, with survival declining to 6.60 ± 0.15 log CFU at 3 h. Although inhibitory, this reduction still reflects moderate tolerance, as a substantial proportion of the bacterial population remained viable. At 1.0% bile salts, a marked reduction in viability occurred, with counts decreasing to 4.80 ± 0.18 log CFU after 3 h, indicating strong inhibitory effects under supra-physiological bile stress. Exposure to 2.0% bile salts resulted in severe inhibition, with viable counts dropping sharply to 2.30 ± 0.25 log CFU after 3 h, demonstrating that such elevated bile concentrations exceed the adaptive capacity of *L. paracasei* HU1 and exert near-lethal effects.

Bile salts are known to disrupt bacterial membranes, interfere with nutrient uptake and impair cellular metabolism through their detergent-like action. The ability of *L. paracasei* HU1 to maintain relatively high viability at 0.3% bile salts is therefore of particular significance, as this concentration closely approximates physiological intestinal bile levels. Survival under these conditions is a key criterion for probiotic functionality, as it suggests that the strain can persist during intestinal transit and potentially exert beneficial effects. The progressive decline in viability observed at higher bile concentrations reflects increasing membrane damage and metabolic stress, which is consistent with responses reported for bile-sensitive lactic acid bacteria.

Survival of *Lactobacillus paracasei* HU1 under simulated gastric and intestinal conditions

The survival of probiotic microorganisms during gastrointestinal transit is a critical determinant of their functional efficacy. In the present study, exposure of *Lactobacillus paracasei* HU1 to simulated gastric juice resulted in a pronounced reduction in viable cell counts, declining from 6.41 ± 0.42 to 3.14 ± 0.43 log CFU/g after 3 h (Table 2), corresponding to an approximate 51% reduction in viability

relative to the initial count. This substantial loss highlights the severity of gastric conditions and is consistent with multiple reports demonstrating that acidic gastric environments represent the most severe physiological stress encountered by probiotic bacteria (Gangakhedkar *et al.*, 2026; Subbalakshmi *et al.*, 2024; Dempsey and Corr, 2022; Marzotto *et al.*, 2006; Villena *et al.*, 2015).

Previous investigations have shown that exposure to pH values below 3.0 leads to destabilization of bacterial cytoplasmic membranes, disruption of proton gradients and impairment of enzyme activity in *Lactobacillus* species (Gangakhedkar *et al.*, 2026; Subbalakshmi *et al.*, 2024; Mesquita *et al.*, 2021). Marzotto *et al.* (2006) reported a comparable decline in *L. paracasei* HU1 viability during *in vitro* gastric digestion, with survival reductions typically exceeding 45-60% for non-encapsulated cells. The magnitude of viability loss observed in the present study therefore falls well within the range reported for unprotected *L. paracasei* HU1 cells suspended in aqueous systems, reinforcing the pronounced sensitivity of this species to gastric acidity.

In contrast, survival under simulated intestinal conditions was comparatively higher, with viable counts decreasing from 6.69 ± 0.51 to 5.02 ± 0.29 log CFU/g over 3 h, corresponding to an approximate 25% decline in viability. This comparatively moderate reduction indicates enhanced tolerance under intestinal conditions. Similar patterns have been reported by Villena *et al.* (2015) and Chen *et al.* (2022), who demonstrated that *L. paracasei* HU1 exhibits greater resilience to bile salts and alkaline pH than to acidic gastric environments (Villena *et al.*, 2015; Chen *et al.*, 2022). Although bile salts possess antimicrobial properties, including membrane solubilization and protein denaturation, *L. paracasei* HU1 strains have been shown to retain substantial viability at physiologically relevant bile concentrations, particularly over short exposure periods (Chen *et al.*, 2022).

These findings indicate that gastric acidity, rather than bile stress, constitutes the primary bottleneck for probiotic survival, as evidenced by the markedly higher percentage

Table 1: Bile salt tolerance of *L. paracasei* HU1.

Bile salt (%)	0 h (log CFU $\pm$ SD)	1 h (log CFU $\pm$ SD)	3 h (log CFU $\pm$ SD)	% Survival (1 h)	% Survival (3 h)	Tolerance
0 (Control)	8.20 $\pm$ 0.08	8.25 $\pm$ 0.06	8.30 $\pm$ 0.07	~101%	~101%	Excellent
0.3	8.20 $\pm$ 0.08	7.95 $\pm$ 0.10	7.40 $\pm$ 0.12	~97%	~90%	Good
0.5	8.20 $\pm$ 0.08	7.40 $\pm$ 0.11	6.60 $\pm$ 0.15	~90%	~80%	Moderate
1.0	8.20 $\pm$ 0.08	6.10 $\pm$ 0.14	4.80 $\pm$ 0.18	~74%	~59%	Poor
2.0	8.20 $\pm$ 0.08	4.20 $\pm$ 0.20	2.30 $\pm$ 0.25	~51%	~28%	Very poor

Table 2: Effect of simulated gastrointestinal conditions and food matrices on the viability of *Lactobacillus paracasei* HU1.

Microorganism	Hours	Gastric	Intestinal	Apple juice		Mouth freshener	
				Gastric	Intestinal	Gastric	Intestinal
<i>L. paracasei</i> HU1	0 hr	6.41 $\pm$ 0.42	6.69 $\pm$ 0.51	1.22 $\pm$ 0.18	1.28 $\pm$ 0.22	1.34 $\pm$ 0.25	1.38 $\pm$ 0.29
	1 hr	5.62 $\pm$ 0.37	5.62 $\pm$ 0.42	0.88 $\pm$ 0.12	0.92 $\pm$ 0.34	0.81 $\pm$ 0.18	1.01 $\pm$ 0.24
	3 hrs	3.14 $\pm$ 0.43	5.02 $\pm$ 0.29	0.11 $\pm$ 0.02	0.81 $\pm$ 0.29	0.14 $\pm$ 0.03	0.88 $\pm$ 0.19

decline observed under gastric conditions compared with intestinal exposure. Consequently, strategies aimed at enhancing probiotic efficacy should primarily focus on improving bacterial protection during the gastric phase of digestion, where the greatest loss of viability occurs.

Influence of food matrices on gastric and intestinal survival

Food matrices are frequently explored as delivery vehicles for probiotics due to their potential to provide physical protection, buffering capacity, or nutritional support; however, their effectiveness is strongly influenced by their physicochemical properties (Natt and Katyal, 2022; Meera *et al.*, 2021). In the present study, incorporation of *Lactobacillus paracasei* HU1 into apple juice resulted in a rapid and pronounced loss of viability under simulated gastric conditions, with counts decreasing from 1.22 ± 0.18 to 0.11 ± 0.02 log CFU/g within 3 h, corresponding to an approximate 91% decline in viability. This substantial reduction indicates that the apple juice matrix provided minimal protection against gastric stress. These findings are consistent with earlier reports describing limited probiotic survival in acidic fruit-based matrices.

Pimentel *et al.* (2015) demonstrated that although *L. paracasei* HU1 can remain viable during refrigerated storage in apple juice, its survival during gastrointestinal simulation is substantially compromised due to the low pH of the matrix (Pimentel *et al.*, 2015). Similarly, Liang *et al.* (2022) reported that fermented apple juice formulations supported probiotic viability only when fermentation-induced pH modulation and matrix restructuring occurred, highlighting that unbuffered fruit juices offer minimal protection during gastric digestion (Liang *et al.*, 2022). The acidic nature of apple juice (pH ≈ 3.5) likely exacerbates proton influx during gastric exposure, intensifying cellular stress and accelerating bacterial inactivation.

A comparable trend was observed for the mouth freshener matrix, where *L. paracasei* HU1 viability declined from 1.34 ± 0.25 to 0.14 ± 0.03 log CFU/g under gastric conditions, corresponding to an approximate 90% reduction in viability over 3 h. While mouth fresheners may provide a solid or semi-solid physical matrix, their lack of buffering capacity and absence of protective macromolecules likely limit their ability to shield probiotic cells from severe acid stress. Dixit *et al.* (2016) emphasized that effective probiotic carriers must possess either buffering components or encapsulating structures to confer meaningful gastric protection, attributes that are typically absent in confectionery-based formulations (Dixit *et al.*, 2016).

Despite poor survival during gastric exposure, *L. paracasei* HU1 exhibited relatively improved survival in simulated intestinal juice across both food matrices. This pattern further supports the notion that once probiotics successfully transit the gastric phase, intestinal conditions are comparatively permissive. Similar observations have been reported in *in vitro* digestion studies using non-dairy probiotic carriers, where intestinal survival remained higher provided that a

sufficient number of cells survived gastric exposure (Liang *et al.*, 2022; Pimentel *et al.*, 2015).

The inability of apple juice and mouth freshener matrices to protect *L. paracasei* HU1 during gastric exposure underscores the limitations of relying solely on food carriers for probiotic delivery, particularly when acidic matrices are involved. These findings align with extensive literature demonstrating that substantial improvements in probiotic survival are achieved only when protective formulation strategies, such as encapsulation or buffering systems, are employed, rather than through matrix selection alone.

Microencapsulation approaches using alginate, chitosan, whey proteins, or lipid-based systems have been shown to significantly enhance probiotic survival under simulated gastric conditions by creating a physical barrier that delays acid diffusion (Matos-Jr *et al.*, 2019; Ortakci *et al.*, 2012; Yadav *et al.*, 2022; da Conceição *et al.*, 2021). For instance, Ortakci *et al.* (2012) reported improved survival of microencapsulated *L. paracasei* during simulated gastric digestion compared to free cells. Similarly, da Conceição *et al.* (2021) demonstrated that co-encapsulation with prebiotics such as fructooligosaccharides further enhances probiotic stability by creating a protective microenvironment.

Dairy-based carriers such as milk and yogurt have also been shown to improve probiotic survival due to their buffering capacity, protein content and fat-mediated protection (Lee *et al.*, 2017; Poon *et al.*, 2020). Lee *et al.* (2017) and Poon *et al.* (2020) reported enhanced gastrointestinal survival and clinical efficacy of *L. paracasei* when delivered through fermented dairy matrices. These findings suggest that matrix composition plays a decisive role in determining probiotic fate during digestion (Lee *et al.*, 2017; Poon *et al.*, 2020).

CONCLUSION

In conclusion, *Lactobacillus paracasei* HU1 demonstrates limited survival under gastric conditions, with stomach acidity identified as the principal barrier to its viability, while intestinal conditions and bile exposure are comparatively well tolerated. The use of acidic, non-buffered delivery matrices such as apple juice and mouth freshener does not enhance survival during gastric transit. Therefore, for effective probiotic application, targeted protective strategies are essential to overcome gastric stress and ensure sufficient delivery of viable cells to the intestine.

Conflict of interest

All authors declare that they have no conflicts of interest.

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