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Resistance of *Saccharomyces boulardii* to technological process, and its *in-vitro* probiotic properties

[1]Bornu, K. B., [1,2]Beyza, H. U., [1,2]*Fatma, K. Y.,
[1,2]Doruk, K. H. and [3]Victor, M.

[1]Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Near East University, Dikmen Road, 99100, Nicosia, Cyprus
[2]DESAM Research Institute, Near East University, Dikmen Road, 99100 Nicosia, Cyprus
[3]Department of Medical Biochemistry, Faculty of Medicine, Near East University, Dikmen Road, 99100 Nicosia, Cyprus

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Abstract

Saccharomyces boulardii has good functional benefits as probiotic bacteria. The objective of the present work was to examine the resistance of *S. boulardii* to technological processes such as high temperature, viability under cold storage (4 and -18°C), and the *in-vitro* probiotic properties that enable them to remain viable after passage through the acidic environment of the gastrointestinal tract, and resistance against bile salts. Results showed that *S. boulardii* growth was significantly higher ($p < 0.0001$) at 25°C compared to at 37°C for all the incubation times investigated, except 48 h. *Saccharomyces boulardii* count increased significantly ($p < 0.0001$) at 4°C during storage. However, at -18°C, no yeast survived beyond the first day of storage. It was significantly more stable ($p < 0.0001$) at a 1% salt ratio than other salt ratios (3 and 7%) at all the incubation times tested. *Saccharomyces boulardii* showed statistically significant tolerance ($p < 0.0001$) at pH 7.2 than at pH's 2.5 and 4.0 throughout the incubation times investigated. *Saccharomyces boulardii* tolerated 0.5% bile salt ratio significantly more ($p < 0.0001$) than the 1.5 and 2.5% bile salt ratios. In conclusion, the best conditions for *S. boulardii* were incubation at 25°C and storage at 4°C, but not freezing. It tolerated low pH (pH 2.5 and 4), high salt ratios (3 and 7%), and bile salts (0.5, 1.5, and 2.5%).

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Introduction

The struggle to maintain a healthy living faced by healthy and immune-compromised people has led many scientists to investigate and research the human microbiome. Several studies have revealed that the human microbiome does not only consist of bacteria, but also fungal and archaeal species (Hoffmann *et al.*, 2013). Other investigations also revealed major discoveries concerning the homeostatic environment *in-vitro* and *in-vivo* of human beings and other mammals, which highlighted the abundance of fungal genera such as *Saccharomyces*, *Malassezia*, and *Candida* (Nash *et al.*, 2017).

World Health Organization (WHO) defined probiotics as living microorganisms which when administered in the right quantity (1×10^9) should serve to provide certain functional benefits to consumers (FAO/WHO, 2006). Probiotics are also generally regarded as safe (GRAS), capable of surviving *in-vitro* and *in-vivo* harsh conditions, auto-aggregation, and can strengthen the epithelial cell walls (Gut *et al.*, 2018). In the past, more emphasis has been placed on the use of probiotic bacteria such as *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Bacillus*, and *Escherichia* (Bermudez-Brito *et al.*, 2012). However, several research studies have indicated that certain yeasts known as *Saccharomyces boulardii* also provide similar functional benefits as demonstrated by the probiotic bacteria (Czerucka *et al.*, 2007). This is evidenced by their immunostimulant properties such as β-glucans, proteases, and mannan-oligosaccharides, and also their powerful ability to release ethanol and carbon dioxide as compared to lactobacilli probiotic bacteria

*Corresponding author.
Email: fatma.kaya@neu.edu.tr

(Fooks and Gibson, 2002; Lokesh *et al.*, 2012; Chan *et al.*, 2021).

The history of *S. boulardii* can be traced back to the early 20th century when it was initially isolated from southeast Asian tropical fruits, lychee and mangosteen, by Henry Boulard (McFarland, 2010; Staniszewski and Kordowska-Wiater, 2021). With the continuous advancement in functional foods containing probiotics, and the growing focus on health and well-being, there has been an increasing trend in the inclusion of *S. boulardii* within various food formulations, with clinical evidence supporting its effectiveness in lyophilised form in improving various diarrhoeal conditions including antibiotic-associated diarrhoea, travellers' diarrhoea, acute diarrhoea, and also managing irritable bowel syndrome and treatment of *Helicobacter* infection (McFarland *et al.*, 2018). *Saccharomyces boulardii* have demonstrated survival and resistance to antibiotics, making them likely candidates for novel probiotics (Afolabi *et al.*, 2018).

In 2020, *S. boulardii* obtained the qualified presumption of safety (QPS) status from the European Food Safety Authority (EFSA), establishing its safety for use as a probiotic (Pais *et al.*, 2020). The distinctive physiological characteristics of *S. boulardii* have positioned it as a prominent subject in recent reviews investigating its potential for technological applications in foods, and exploring its genomic and phenotypic traits, health benefits, and mechanism of action (Pais *et al.*, 2020; Ansari *et al.*, 2021). Common problems associated with probiotic viability are their low resistance to technological and various environmental conditions during production and storage (Paez *et al.*, 2012). During the technological processes, probiotics are subjected to various stress factors, which include changes in temperature, nutrient deficiency, exposure to toxic substances like hydrogen peroxide, build-up of ice crystals in the intercellular spaces of the cell due to freeze drying, dehydration, and osmotic shock (Bogosian *et al.*, 2000, Keer and Birch, 2003; Rittershaus *et al.*, 2013).

To maintain their viability during technological processes, probiotics should be able to tolerate adverse acidic conditions (pH 2 - 3), changes in temperature, low amounts of nutrients, high bile salts concentration, hydrolytic enzymes, pancreatic enzymes, and organic enzymes (Holzapfel *et al.*, 1998). Different scientific literature postulated that the required number of active probiotics required to obtain beneficial health effects should be around 10^6 CFU/mL in the small intestine, and 10^8 CFU/mL in the large intestine (Minelli and Benini, 2009). Therefore, probiotics must be able to maintain their viability by resisting the technological processes, and exhibiting its functional *in-vitro* properties which include engaging in syntrophic interactions with the resident microbiota, reinforcing the integrity of the epithelial barrier, modulating the immune system, producing antimicrobial substances, maintaining a long-term presence in the gut through colonisation, and forming biofilms (Bermudez-Brito *et al.*, 2012; Frese *et al.*, 2013; Segers and Lebeer, 2014; Li *et al.*, 2021).

Comparing *S. boulardii's* resistance to high temperature with *S. cerevisiae*, scientific investigations found that *S. boulardii* exhibits its best growth at the human body temperature of 37°C, whereas *S. cerevisiae* thrives optimally at 30°C. Moreover, *S. boulardii* demonstrates greater resistance to high temperatures, retaining 65% viability after 1 h at 52°C, whereas *S. cerevisiae*'s viability decreases to 45% (Fietto *et al.*, 2004). Bile salts, synthesised from cholesterol in the liver and released into the intestine to facilitate nutrient absorption, have detergent-like properties. These properties make bile salts potentially harmful to gastrointestinal microorganisms by disrupting the lipid bilayer structures of their cell membranes (Fietto *et al.*, 2004). Comparing the resistance of *S. boulardii* with the other probiotics such as *Lactobacillus* and *Bifidobacterium* spp. against bile salts in the intestine, *S. boulardii* possesses the ability to withstand degradation caused by hydrolytic enzymes and bile salts, and also demonstrates viability even when exposed to simulated gastric juice containing pepsin and hydrochloric acid (Kabluchko *et al.*, 2017). Similar scientific simulated findings also noted that even though *S. boulardii* survived the gastrointestinal milieu, their efficiency was improved for 2 h when encapsulated with a double layer of sodium alginate and gelatine (Du Le and Trinh, 2018). The increasing application of *S. boulardii* into foods and beverages is not surprising, especially during fermentation, given its advantageous technological characteristics in certain food compositions when compared to conventional probiotic bacteria. For instance, *S. boulardii* exhibits exceptional compatibility in beer due to its inherent resistance to hop iso-α-acids, and its superior ability to produce ethanol and carbon dioxide, surpassing that of probiotic lactobacilli

(Chan *et al.,* 2021). Furthermore, *S. boulardii* has the capability to maintain its viability (6 - 7 log CFU/mL) for several months longer than probiotic lactobacilli when incorporated into coffee and tea (Chan and Liu, 2022; Wang *et al.,* 2022).

According to FAO/WHO, it is mandatory to perform a preliminary *in-vitro* assessment of probiotic candidates (Hossain *et al.,* 2020). Therefore, the objective of the present work was to examine the probiotic potentials of *S. boulardii*, as well as its resistance to technological processes commonly applied on foods.

Materials and methods

Fresh sub-cultured colonies and the suspension of pure, activated colonies of *S. boulardii* were used for experimental trials as described below. All trials were performed in quadruplicate.

Saccharomyces boulardii activation and culture solution preparation

Saccharomyces boulardii CNCM I-745 from capsules of 250 mg Reflor® was homogenised (1:9, v/v) in Maximum Recovery Diluent (MRD), and then cultured on Sabouraud Dextrose Agar (SDA) for 48 h at 30°C. For activation, pure colonies were sub-cultured in 25 mL of Yeast Peptone Dextrose (YPD-yeast extract, 1%; peptone, 2%; and dextrose, 2%) broth which was prepared as described by Martins *et al.* (2009). After incubation, centrifugation at 8,000 *g* was performed for 20 min. The pellets were separated from the supernatant, and suspended in sterile Phosphate-Buffered Saline (PBS, 0.85%). CFU/mL values in suspensions were adjusted approximately to 3×10^9 CFU/mL (1 MFU) by McFarland turbidity test, and confirmed by enumeration on the SDA plate. These suspensions were immediately used in related analyses. The number of colonies that were enumerated on SDA was used as the initial number of colonies (initial load).

Resistance to technological processes

Incubation temperature

To determine *S. boulardii* survival at different temperatures, the prepared and enumerated yeast solution in YPD broth was used. The tubes were incubated for 24, 48, and 72 h at 25 and 37°C. After incubation, the tubes were enumerated again to obtain the survived viable cell value following different treatments.

Storage temperature

To determine the viability of *S. boulardii* under a cold chain, the prepared and enumerated yeast solution in YPD broth was used. These tubes were stored at 4 and -18°C for 1, 5, 7, and 15 d. After incubation, the tubes were enumerated again to obtain the survived viable cells value following different treatments.

Salt

Saccharomyces boulardii was tested for tolerance against different NaCl concentrations. The growth rate of yeast culture in the 10 mL of YPD broth containing different levels (1, 3, and 7%) of NaCl was determined. Briefly, 1 mL of prepared and enumerated culture suspension was added to sterile tubes containing 9 mL of salt ratio adjusted YPD broth, and incubated at 30 ± 2°C. Sampling was performed at 12, 24, 48, and 72 h of incubation, and cultured on SDA in order to see the viability following different treatments.

In-vitro probiotic activity analyses

Acid and alkaline tolerance

This experiment was performed by inoculating 1 mL of prepared and enumerated culture suspension to sterile tubes containing 9 mL of YPD broth which were adjusted to different pH's (2.5, 4.0, and 7.2), and incubated at 37 ± 2°C. Sampling was performed at 12, 24, 48, and 72 h of incubation and cultured on SDA in order to see the viability following different treatments.

Bile salt tolerance

The growth rate of *S. boulardii* was determined in 9 mL of YPD broth supplemented with different levels of bile salt concentrations (0.5, 1.5, and 2.5%). Next, 1 mL of prepared and enumerated culture suspension was added to each tube containing bile salt after sterilisation. The broth was then incubated at 37 ± 2°C. Sampling was performed at 12, 24, 48, and 72 h of incubation and cultured on SDA to see the viability following different treatments.

Statistical analysis

All analytical tests were conducted in quadruplicates, and the results were presented in mean ± standard deviation (SD). The CFU/mL was converted to $\log_{10}$ CFU/mL. A Two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test was performed to

determine the significant differences, and draw relevant conclusions. The data analyses were performed using GraphPad Prism version 6.0. for Windows (GraphPad Software, Boston, Massachusetts, USA).

Results

Activated pure colonies of *S. boulardii* were screened to determine the survival at different incubation and storage temperatures, and tolerance against different NaCl concentrations. To determine the probiotic activities, *in-vitro* conditions, acid-alkaline tolerance, and bile salt tolerance tests were performed to mimic the conditions in the gut and gastric medium environment. The results of all analyses were expressed in mean ± standard deviation (n = 4), and presented at the related tables and figures.

Discussion

The ability of a microorganism to be resistant to technological processes and be viable during simulated gastrointestinal tract conditions is a factor required for probiotic microorganism selection (Sarao and Arora, 2015). In accordance with the suggestion of FAO/WHO (FAO/WHO 2002; Palma *et al.*, 2015), *S. boulardii* was investigated for some technological and *in-vitro* probiotic properties, including proper incubation time, low pH, salt tolerance, bile tolerance, and storage temperatures. *S. boulardii* showed greater resistance to environmental changes, and the acid tolerance ability of *S.* var. *boulardii* has been regarded as an important value due to its ability to lower bacterial contamination in probiotic products obtained (Singh *et al.*, 2005).

From the results obtained in the present work, *S. boulardii* colonies regularly increased within 3 d at 25°C and 37°C, reaching its peak at 48 h, and started to decline afterwards (Table 1, Figures 1A and 1B). The steady increase at 25°C agreed with previous report that temperatures ranging between 25 - 30°C are more suitable for cell growth and viability (Aktan and Kalkan, 2000). In conformity with the human body temperature which ranges between 35 - 37°C, the incubation temperature showed the growth of *S. boulardii* at 37°C, reaching its maximum growth at 48 h before declining afterwards. These findings agreed with a previous report that *S. boulardii* can grow and adapt to human body temperature at 37°C

Table 1. *Saccharomyces boulardii* viability at different incubation temperatures.

Incubation time (h)	Viability (log CFU/mL)	
	25°C	37°C
6	6.91 ± 0.01	5.98 ± 0.04
24	6.95 ± 0.01	6.38 ± 0.10
48	7.04 ± 0.03	7.10 ± 0.15
72	7.23 ± 0.06	6.51 ± 0.04

Values are mean ± standard deviation (n = 4).

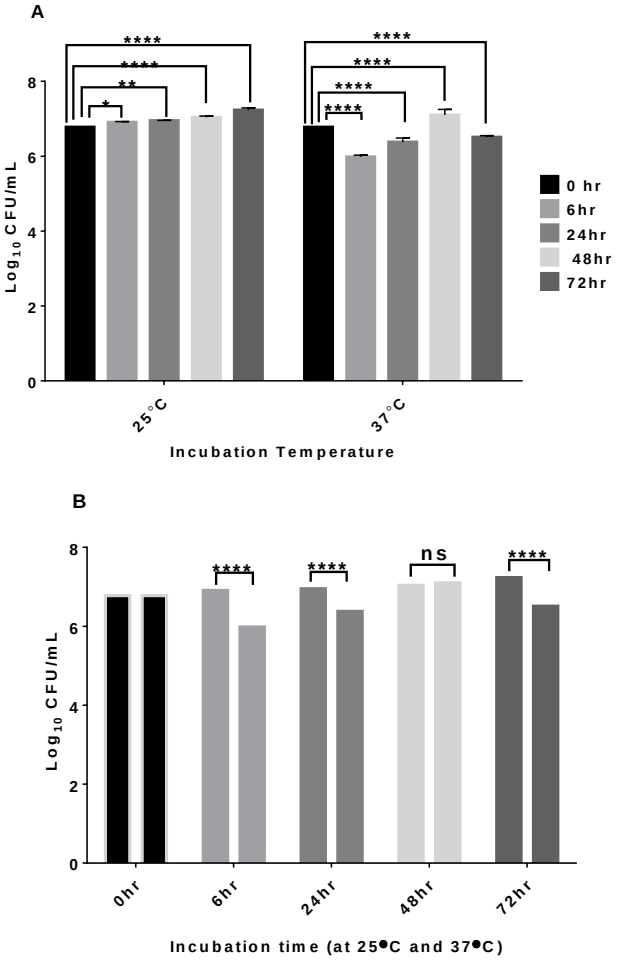

Figure 1. Growth of *Saccharomyces boulardii* at different temperatures. **(A)** *Saccharomyces boulardii*'s CFU/mL at 25 and 37°C, and their comparison with the initial load of 6×10^6 CFU/mL. **(B)** Comparison of *Saccharomyces boulardii*'s growth between incubation temperatures (25 and 37°C) per time in hours (h); the grouped bars are presented in order of increasing temperatures, 25 and 37°C, respectively. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, and ns: not significant. Values are mean ± standard deviation (n = 4).

(Gil-Rodríguez *et al.*, 2015). According to Chelliah *et al.* (2021) on the thermo-tolerant ability of *S. boulardii*, the report indicated that it is a thermophile with enzymatic properties that enables it to survive high temperatures at 95°C for 2 h, and 121°C for 15 min. Therefore, *S. boulardii* is a thermo-tolerant yeast that reproduces fully even at 37°C (physiological temperature of the host), while *S. cerevisiae* reproduces at 30°C (Rajkowska and Kunicka-Styczynska, 2010).

To survive in a low-temperature conditions, yeast must feed on a substrate in the form of a carbon source which provides energy for its survival. Given this, the yeast produces a variety of extracellular hydrolytic enzymes capable of breaking down complex sugars into assimilable forms (Liszkowska and Berlowska, 2021). In the present work, *S. boulardii* was investigated for its resistance to low storage temperatures ranging between 4°C to -18°C (Table 2, Figures 2A and 2B). The results obtained showed that *S. boulardii* growth at low temperature of 4°C slightly increased at day 1, and remained stable during 15-day storage. The results also showed that *S. boulardii* growth was significantly higher than day 1 at -18°C. These findings agreed with previous scientific reports that although ideal temperatures for yeast strains are around 25 - 30°C, they can remain viable when stored under both industrial and laboratory conditions at temperatures of around 4°C, retaining viability for a long period (Crawford and Pavitt, 2019). At day 1 of freezing temperature (-18°C), the result showed that *S. boulardii* slightly survived with less or no visible growth seen when compared to *S. boulardii* at day 1 at 4°C, during 15-day storage. The resistance to low temperature during freezing/thawing and the impact of reducing water activity on *S. boulardii* cells in different batch growth phases has been investigated. At batch 1 (20 h) and batch 2 (4 h), freezing cycles (at -20°C) on the viability of the yeast cells (7×10^5 CFU/mL) were harvested during the exponential and stationary phases. The survival percentages after each freezing cycle demonstrated that the cells in the stationary phase were more resistant to freezing than those in the exponential phase (Ishihama, 1997; Khroustalyova *et al.*, 2001). In the present work, the number of viable cells at the freezing point was under the detectable level after day 1.

The viability of *S. boulardii* inoculated in YPD broth as seen in Table 3 and Figure 3A was evaluated

Table 2. *Saccharomyces boulardii* viability at different storage temperatures.

Storage time (d)	Viability (log CFU/mL)	
	4°C	-18°C
1	7.36 ± 0.04	6.57 ± 0.03
5	7.16 ± 0.18	ND
7	7.12 ± 0.08	ND
15	7.22 ± 0.17	ND

Values are mean ± standard deviation (n = 4). ND: not detected.

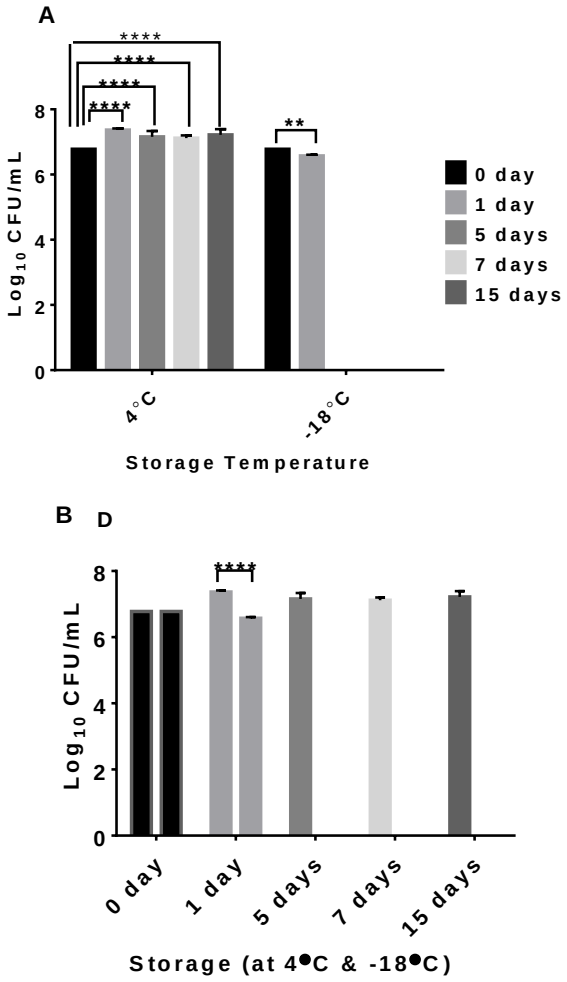

Figure 2. Viability of *Saccharomyces boulardii* at different temperatures. **(A)** *Saccharomyces boulardii*'s CFU/mL at 4 and -18°C, and their comparison with the initial load of 6×10^6 CFU/mL at 0 h. **(B)** Comparison of *Saccharomyces boulardii*'s viability between storage temperatures, 4 and -18°C, per time in days; the grouped bars are presented in order of increasing temperature, 4 and -18°C, respectively. $^{**}p < 0.01$, and $^{****}p < 0.0001$. Values are mean ± standard deviation (n = 4).

Table 3. *Saccharomyces boulardii* viability at different salt concentrations.

Incubation time (h)	Viability (log CFU/mL)		
	1%	3%	7%
6	6.80 ± 0.01	6.54 ± 0.04	6.31 ± 0.06
24	6.88 ± 0.01	6.49 ± 0.01	ND
48	6.92 ± 0.02	6.55 ± 0.05	ND
72	6.94 ± 0.03	6.49 ± 0.01	ND

Values are mean ± standard deviation (n = 4). ND: not detected.

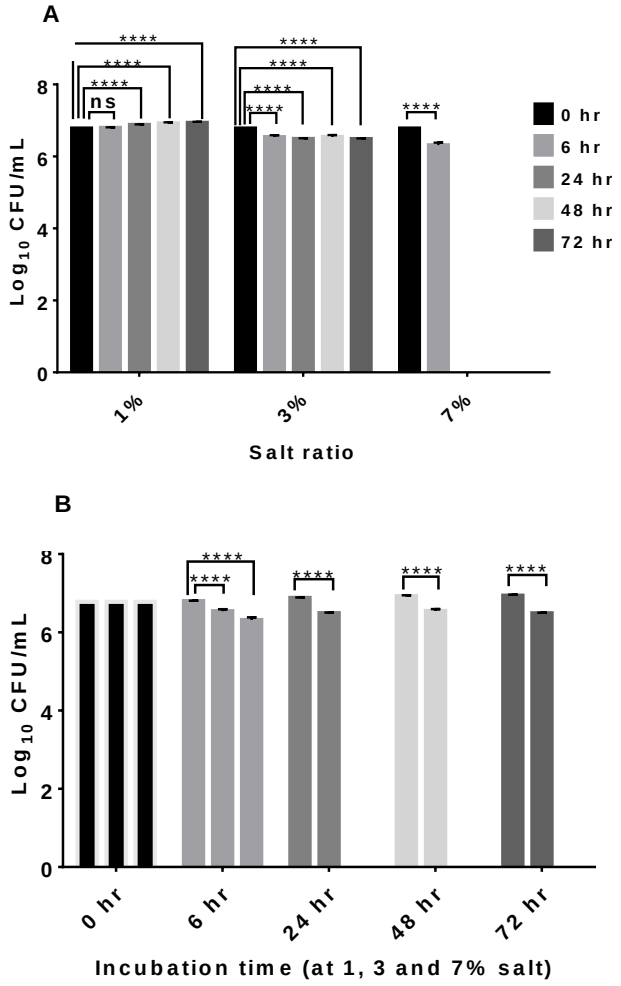

Figure 3. Salinity stability of *Saccharomyces boulardii*. **(A)** *Saccharomyces boulardii*'s CFU/mL at different salt ratios and their comparison with the initial load of 6×10^6 CFU/mL. **(B)** Comparison of *Saccharomyces boulardii*'s salinity stability at different salt ratios per time (in hours); the grouped bars are presented in order of increasing salt ratio 1, 3, and 7%, respectively. ****$p < 0.0001$, and ns: not significant. Values are mean ± standard deviation (n = 4).

at different salt concentrations ranging from 1, 3, and 7% to determine its resistance and viability at different salt ratios. The incubation temperature was set at 25°C, and was observed at different concentrations per time (in hours) as seen in Figure 3B. Results showed that at 1% salt concentration, the growth curve of *S. boulardii* was significant except for the first 6 h when they were adjusting to the environment. The growth at 1% salt concentration in YPD broth was higher when compared to the growth in solution without any salt at the same 25°C. At 3% salt concentration, we observed a gradual decrease in the number of yeast, but they remained stable throughout the 72 h period of observation, while at 7% salt concentration, except for the first 6 h, the yeast cells were all inactivated at the end of 24 h. Our observation also showed that at 25°C, 1% salt concentration was favourable for the growth of *S. boulardii* throughout the entire 72 h, and it adapted properly to the osmotic stress produced by the ionic solutes. These findings agreed with previous scientific literature which reported that *S. boulardii* exhibited a gradual growth until 8.0% salt concentration, but was greatly reduced when introduced into 3.0% salt concentration (Palma *et al*., 2015).

One of the major hurdles for a probiotic is the *in-vitro* passage through the stomach which exposes the probiotics to low pH/high acid (< 3), and a significant concentration of pepsin can cause the probiotic cell rupturing (Derrien and Van Hylckama Vlieg, 2015). We investigated *S. boulardii* at different pH's namely 2.5, 4.0, and 7.2 at 25°C incubation temperatures per time (in hours) as seen in Table 4 and Figures 4A and 4B to simulate the *in-vitro* passage of the probiotic through the acidic gastric juice of the stomach. Results revealed that *S. boulardii* displayed a high level of tolerance at pH 7.2; even though they were affected at 6, 48, and 72 h, their growth curve was significantly higher than at pH 4.0 and 2.5 throughout the incubation temperature of 25°C and time tested. At pH 2.5, we observed a significant reduction in the growth of *S. boulardii* with the highest impact observed at 24 h of incubation at 25°C, and when compared to pH 4.0, it was still significantly lowered but no inactivation of the probiotic cell occurred at pH 2.5 throughout the time (hours) tested. Our findings agreed with other scientific investigations that *S. boulardii* is highly resistant to high acidic pH and various degrees of high

Table 4. *Saccharomyces boulardii* viability at different pH's.

Incubation time (h)	Viability (log CFU/mL)		
	2.5	4.0	7.2
6	6.44 ± 0.36	6.26 ± 0.03	6.93 ± 0.02
24	5.38 ± 0.19	6.05 ± 0.04	7.03 ± 0.04
48	5.37 ± 0.08	6.01 ± 0.02	6.93 ± 0.03
72	4.19 ± 0.24	5.01 ± 0.02	6.74 ± 0.05

Values are mean ± standard deviation (n = 4).

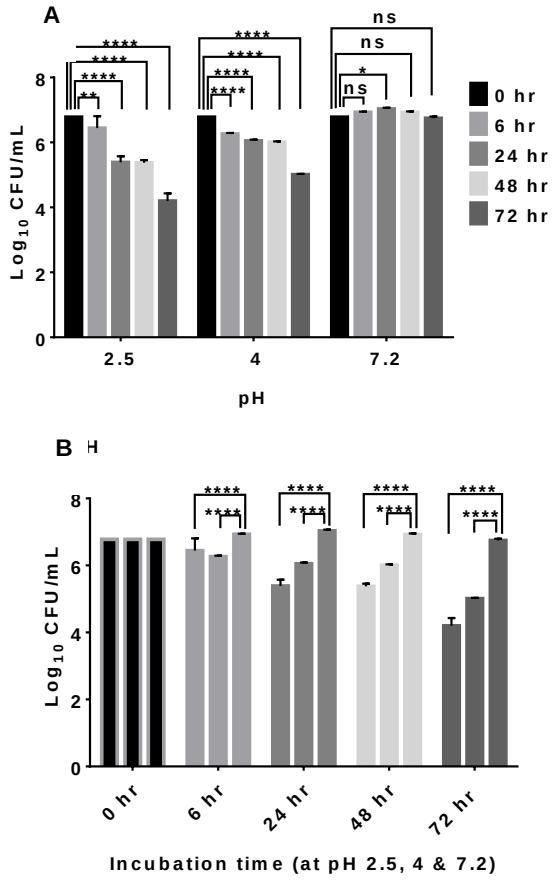

Figure 4. Acid and alkaline tolerance of *Saccharomyces boulardii*. **(A)** *Saccharomyces boulardii*'s CFU/mL at different pH's and their comparison with the initial load of 6 × 10^6 CFU/mL **(B)** Comparison of *Saccharomyces boulardii*'s tolerance of different pH per time (in hours); the grouped bars are presented in order of increasing pH, 2.5, 4.0, and 7.2, respectively. *p < 0.05, **p < 0.01, ****p < 0.0001, and ns: not significant. Values are mean ± standard deviation (n = 4).

temperature than *S. cerevisiae*, and viable at pH as low as 2.0 (Moradi *et al.*, 2018). Chelliah *et al.* (2021) discussed the ability of six probiotic strains of *S. boulardii* to survive at pH values ranging from 1 - 7. The results showed that *S. boulardii* survived at pH 2.0, and remained viable up to pH 7.0. The present work confirmed the ability of *S. boulardii* to survive the high acidic/low pH of the gastric juice of the gastrointestinal tract at varying degrees of temperature per time.

According to FAO/WHO (2006), the probiotic response to the breaking down of bile salts is one of the major features when selecting a probiotic strain. This is because the *in-vitro* passage of the probiotic strain through the dark green to yellowish-brown detergent-like fluid that constitutes bile acids, cholesterol phospholipid, and pigment biliverdin of the small intestine duodenal loop, has the ability to alter the cell membrane lipids of the yeast cell, thereby causing adverse effect to the cell permeability, and distorting the cell membrane response to its environment (Taranto *et al.*, 2003; Ilango *et al.*, 2016). In the present work, 0.5, 1.5, and 2.5% concentrations of bile salt solution were compared against the initial yeast load of 6 × 10^6 CFU/mL as seen in Table 5 and Figure 5A, and per time as seen in Figure 5B. The incubation for all the test was set at 37°C to determine the effect of bile salts at various concentrations on *S. boulardii* at body temperature. At 0.5% bile salts, we observed exponential growth of *S. boulardii* with the highest significance occurring at 48 h of the incubation period, except for the first 6 h, where the yeast cell was believed to be adjusting to the environment. At 1.5 and 2.5% concentrations of bile salts, we observed a gradual decrease in the growth curve of *S. boulardii*, but no record of inactivation occurred even at the end of 72 h. Our findings revealed that even at 37°C, *S. boulardii* was highly resistance to bile salts at 0.5%, maintaining a constant growth and was also stable at 1.5 and 2.5% bile salts without any inactivation of the yeast cells. Another scientific literature revealed that *S. boulardii* displayed a high level of tolerance to bile salts at 2.0% concentration, and also produced maximum growth rate (Czerucka *et al.*, 2007).

Table 5. *Saccharomyces boulardii* viability at different bile salt concentrations.

Incubation time (h)	Viability (log CFU/mL)		
	0.5%	1.5%	2.5%
6	6.80 ± 0.01	6.58 ± 0.01	6.49 ± 0.01
24	6.83 ± 0.02	6.29 ± 0.03	6.33 ± 0.03
48	6.96 ± 0.01	6.25 ± 0.02	6.28 ± 0.02
72	6.73 ± 0.02	6.05 ± 0.06	6.01 ± 0.02

Values are mean ± standard deviation (n = 4).

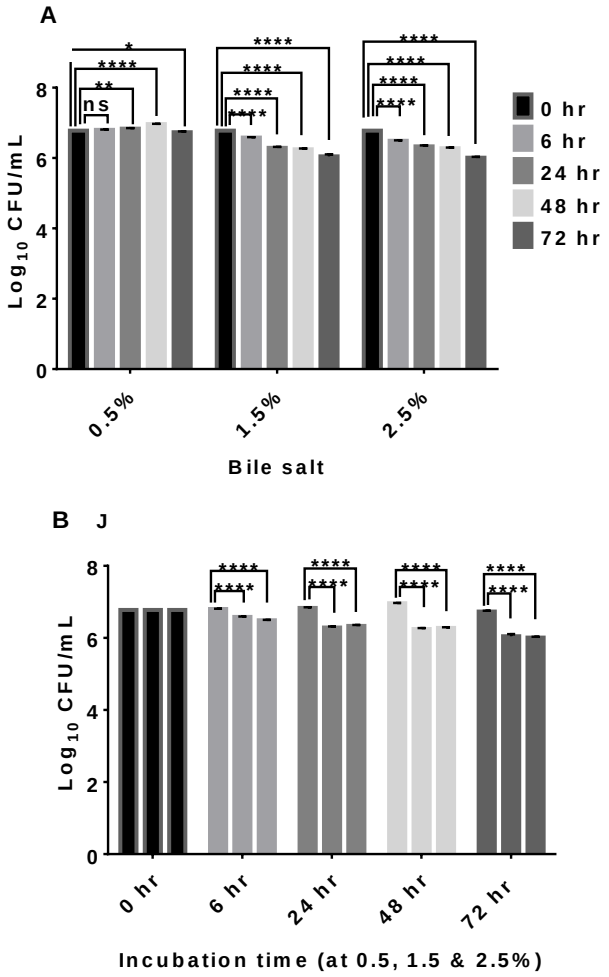

Figure 5. Bile salt tolerance of *Saccharomyces boulardii*. **(A)** *Saccharomyces boulardii*'s CFU/mL at different bile salt ratios and their comparison with the initial load of 6×10^6 CFU/mL. **(B)** Comparison of *Saccharomyces boulardii*'s tolerance of different bile salt ratios per time (in hours); the grouped bars are presented in order of increasing bile salt ratio 0.5, 1.5, and 2.5%, respectively. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, and ns: not significant. Values are mean ± standard deviation ($n = 4$).

Conclusion

In recent years, *S. boulardii* has become the focus of research due to its probiotic potential, and as an alternative to functional food technology. In this context, preserving its probiotic character under various conditions, and maintaining its viability throughout food processing are important research questions. In the present work, we investigated the survival and growth characteristics of *S. boulardii* against possible conditions that it may encounter in food. The best conditions for *S. boulardii* were incubation at 25°C and storage at 4°C. Freezing was seen to decrease the number of cells. It tolerates low pH (pH 2.5 and 4), high salt ratios (3 and 7%), and bile salts (0.5, 1.5, and 2.5%). Due to tolerance against acid and bile salt, it could be considered as a good probiotic.

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