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(Original Article)

Probiotic Attributes Potential of Some Yeast Strains

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Abstract

Due to their anti-fungal action, yeasts are becoming more and more well-liked as fresh sources for boosting food attributes including; flavor, color and vitamin content as well as agents for avoiding food spoilage. The purpose of this study was to investigate the probiotic attributes potential of some yeast strains. Ten yeast strains belong to five species (*Saccharomyces cerevisiae*, *Geotrichum candidum*, *Kluyveromyces lactis*, *Debaryomyces hansenii* & *Torulaspora delbrueckii*), and their probiotic characteristics were determined through several in vitro experiments. Except for two strains; *Debaryomyces hansenii* and *Torulaspora delbrueckii*, eight of these strains were capable of surviving in environments resembling the gastrointestinal tract with survival rates as high as 94-99% at pH 1.5-2.0. All strains can survive in an intestinal environment with a wide variety of temperatures, high NaCl concentrations, and bile salts of 0.5%. Additionally, after 48 h at 37°C, they were all able to digest cholesterol in the range of 18 to 44%.

Keywords: *Probiotic, Yeast strains, Cholesterol.*

Introduction

Yeasts, which are unicellular eukaryotes and members of the kingdom of fungi, have a variety of effects on the nutritional value and safety of food products. They are widespread, frequently contaminate; fruits, vegetables & other plant materials, and have an association with soil and insects (Bekatorou *et al*., 2006). They can grow well in milk and several kinds of cheese, such Toma Piemontese in Italy and Cantal in France, which are manufactured from raw milk (Chen *et al*., 2010). The influence of yeasts on products today goes beyond the traditional and well-known ideas of *Saccharomyces cerevisiae* fermentations of bread, beer and wine. Yeasts are becoming increasingly popular as new sources for enhancing food qualities like vitamin content, taste and color as well as preventing food from spoiling, due to their anti-fungal properties (Querol and Fleet, 2006). The relatively recent discovery of some yeasts' probiotic potential has increased interest in the study of these microorganisms in recent years. Probiotics are "living microbial preparations that, when consumed, alleviate, suppress, or cure a health disorder and act by altering the microbial balance in the gastrointestinal tract, and

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consequently altering microbial metabolism, and the interactions with order microorganisms (Playne, 2000; Ghindea *et al*., 2009). This contributes to the widespread interest in obtaining novel yeast strains with biotechnological potential from dairy products (Ghindea *et al*., 2009). *Saccharomyces*, *Debaryomyces*, *Kluyveromyces*, *Yarrowia* and *Issatchenkia* are the genera with the most frequently described strains despite the great range of yeasts recovered from dairy products. Numerous yeast species belong to the fungus kingdom genus *Saccharomyces*. Many members of the genus *Saccharomyces*, which derives its name from the Greek word for sugar, are crucial to the creation of food. It is sometimes referred to as baker's or brewer's yeast. They are saprophytic, unicellular fungi. *Saccharomyces cerevisiae*, which is used to make wine, bread and beer is one illustration (Sulieman *et al*., 2015).

Dairy products are particularly encouraging the growth of yeasts and the most prevalent isolated genera in these conditions include; *Candida*, *Debaryomyces*, *Mycoderma*, *Rhodotorula*, *Geotrichum candidum* and *Saccharomyces* (Neveen *et al*., 2011). Due to their ability to live in the gastrointestinal tract's low pH and bile milieu, some yeast strains that have been isolated from dairy products or human feces are thought to have probiotic potential, according to several published research (Psomas *et al*., 2001). The potential of yeasts to absorb cholesterol and act as antioxidants has recently attracted the attention of numerous experts. According to certain research, yeasts can eliminate cholesterol *in vitro*. However, only a small number of yeasts; outside of *S. cerevisiae*, *Kluyveromyces lactis* and *Pichia kudriavzevii* had their assimilation potential studied (Psomas *et al*., 2003). The chosen strain must be able to survive during the manufacturing process, produce large amounts of biomass easily, resist preservation techniques like lyophilization with a high shelf life in the finished product, be genetically stable, and not degrade the organoleptic properties of the final product. These requirements include; species identification, strain typing, safety testing and the ability to produce toxins, pathogens and hazardous metabolic activities (Petric and Putnik, 2020). Moreover, *Saccharomyces cerevisiae* have probiotic properties and there is strong evidence that probiotic and prebiotic yeast products can take the role of in-feed antibiotics in the production of broiler chickens (Ahiwe *et al*., 2021). *Geotrichum candidum* is a yeast like organism that can be found in raw milk, silage, grass, fruits, plants and soil. It is an important component of the microflora of artisanal ewe and goat raw milk cheeses as well as semi hard cheeses like St. Nectaire and soft cheeses like Camembert (Hayaloglu and Kirbag, 2007).

The current work's objective was to study some new potential probiotic yeasts physiological characteristics and to identify them utilizing some probiotic tests and also to study the potential of these probiotics for cholesterol absorption.

Materials and Methods

Yeast strains and growth media

Yeast strains used in this study were provided from the laboratories of food, microbiology and hygiene at the Egyptian universities of Azhar, Cairo and Ain Shams. The strain names and accession numbers are shown in Table 1.

Table 1. Yeast strains

Code	Strain name	Accession number
A	*Saccharomyces cerevisiae*	KY441458
B	*Saccharomyces cerevisiae*	MF380234
C	*Saccharomyces cerevisiae*	KY400198
D	*Saccharomyces cerevisiae*	KM504287
E	*Saccharomyces cerevisiae*	MF597761
F	*Geotrichum candidum*	MF383376
G	*Geotrichum candidum*	MF383368
H	*Kluyveromyces lactis*	DSM70799
I	*Debaryomyces hansenii*	DSM70238
J	*Torulaspora delbrueckii*	MF496102

Yeast strains were incubated at 25˚C for 24 h in yeast peptone dextrose (YPD) broth that contained of 0.5% (w/v) yeast extract, 1% peptone & 2% glucose. YPD agar, which prepared as YPD broth in addition to 1.8% agar, was used to preserve the colonies. The cultures were subculture at least three times and activated at 25˚C for 72 h before the experiment. About 10^8 cfu (ml^{-1}) of total viable cells were adjusted.

Physiological and biochemical characteristics of yeast strains

Fermentation and assimilation of carbon and nitrogen sources were carried out according to Kurtzman *et al*. (2011).

Stress tolerance of yeast strains

Thermotolerance

Yeast strains were checked for their thermotolerance at different temperatures (4, 25, 30, 37, 42 & 45˚C) on GPY agar medium (40 g glucose, 5 g peptone, 20 g agar & 5 g yeast extract; dissolved in 1 liter distilled water and autoclaved at 121˚C for 15 min) after 4 days, of incubation, according to Fakruddin *et al*. (2017).

Sodium chloride tolerance

Yeast strains resistance to sodium chloride was examined as the method of Fakruddin *et al*. (2013) with some modifications as follows:

Briefly, according to Fakruddin *et al*. (2017) YEGP broth (2% glucose, 0.5% yeast extract, 1% peptone heated to 30˚C for 20-24 h) was prepared containing 2, 4, 6, 8 and 10% of NaCl. Each tube contained 10 ml of YEGP liquid media with an appropriate concentration of salt and blank media was used as a control. Then

each was inoculated by half loopful of yeast cell and incubated at 30°C for 48 h. After 48 h the optical density was measured at 600 nm.

Probiotic properties

Tolerance of low pH

The growth at low pH was evaluated by inoculating (1% v/v) activated strains into YPD tubes that had been pH-adjusted to 1.5, 2.0 & 3.0 using 0.1 mol.L^{-1} HCl and 3 mol.L^{-1} H_2SO_4 (Chen *et al*., 2010). The samples were incubated at 37°C for 1, 2 & 3 h before the count plate method was used to calculate the number of viable cells. According to Williamson and Johnson (1981), the results are presented as the mean value of three replicates and represented as a percentage log survival as follows:

$$\text{Log survival percentage} = (\log N / \log N0) \times 100$$

Where: N0 is the count (cfu/ml) before incubation, and N is the count (cfu/ml) after incubation.

Bile tolerance

Freshly produced active yeast cells (1% w/v) and various concentrations of bile salts (0.1, 0.3, & 0.5%) were added to YPD broth. The decimal increase of yeasts was used to calculate the number of viable cells before and after incubation at 72 h on YPD agar plates, and this data was used to determine the resistance of the strain to bile (Psomas *et al*., 2001) by using the following equation

$$\text{Bile tolerance \%} = \frac{\text{increment of cells in broth with bile}}{\text{increment of cells in broth without bile}} \times 100$$

In vitro survival in the gastric and intestinal environment

An aqueous solution containing 3 g/1 pepsin (3260 U/mg) and 5 g/1 NaCl & pH 2.0, was used to imitate the stomach environment before activated cells and extracted by centrifugation at 3000 g for 10 min (Charteris *et al*., 1998). Aqueous solutions containing 1 g/1 pancreatin (903 U/mg) and 5 g/1 NaCl & pH 8.0 were also used to create yeast colonies. After 60, 120, 180 & 240 min of incubation at 37°C, the cell viability was assessed using the count plate method. The findings are shown as percentage log survival and the mean value of three replicates (Williamson and Johnson, 1981).

In vitro assimilation of cholesterol

The procedure of Pereira and Gibson (2002) was modified slightly to test the ability of yeast strains to decrease cholesterol levels *in vitro*. Briefly stated, yeast cells were grown at 37°C for 48 h in YEGP broth that was supplemented with 0.3% (w/v) Ox gall and 400 g ml^{-1} of cholesterol, according to Kourelis *et al*. (2010). 500 µl of the medium was taken at 48 h and centrifuged for 15 min at 4000 rpm. Using the cholesterol enzymatic colorimetric method, the cholesterol concentration in the medium supernatant was determined in 100 µl newly collected samples (kit).

Calculation

The total cholesterol concentration was calculated by using the following equation

$$\text{Cholesterol concentration (mg/dl)} = \frac{\text{A sample}}{\text{A standard}} \times \text{ standard concentrate.}$$

Where: A = Absorbance at 500 nm, standard concentrate = 200

$$\% \text{ Assimilation cholesterol} = \frac{(\%\, Cholesterol\ of\ control - \%\, Cholesterol\ of\ strain)}{\%cholesterol\ of\ control} \times 100$$

Results and Discussion

Since ancient times, yeast has been utilized in food fermentations. Numerous yeast strains can be found in dairy-related products or the digestive system. *Saccharomyces boulardii* consider one of the strains of the genus *Saccharomyces* that can be used as probiotic agents. Ten yeast strains from various genera were used for this study. Additionally, these strains underwent *in vitro* testing for a probiotic characteristic.

Physiological and biochemical characteristics of yeast strains

Yeast strains were selected from different Egyptian universities & research centers, and some physiological tests were performed on them to verify their properties. The data presented in Table 2 showed that; A, B, C, D, E, H & J strains could catabolize glucose and sucrose by fermentation. The strain I was the only strain that could ferment lactose, while the rest of the strains couldn't. However, the obvious disparity between the tested strains could be explained by the fact that various yeast strains showed great variability in their ability to grow on different carbon sources.

Table 2. Physiological and biochemical characteristics of yeast strains

Strains	Fermentation test					Assimilation of carbon and nitrogen compounds												
	Glucose	Galactose	Sucrose	Lactose	D-Mannitol	Glucose	Galactose	Sucrose	Lactose	Starch	Maltose	D-Mannitol	Ethanol	Methanol	Glycerol	D-xylose	NO_2	NO_3
A	+	-	+	-	-	+	w	+	-	-	+	-	+	-	-	-	-	-
B	+	-	+	-	-	+	+	+	-	-	+	-	+	-	-	-	-	-
C	+	-	+	-	-	+	w	+	-	-	+	-	+	-	-	-	-	-
D	+	-	+	-	-	+	w	+	-	-	+	-	+	-	-	-	-	-
E	+	-	+	-	-	+	+	+	-	-	+	+	+	-	+	-	-	-
F	-	-	-	-	-	+	-	+	-	-	w	+	+	-	+	+	-	-
G	-	-	-	-	-	+	-	+	-	-	w	+	+	-	+	+	-	-
H	+	-	+	+	-	+	w	+	w	-	+	+	+	-	+	-	-	-
I	-	-	-	-	-	+	w	+	-	w	+	+	+	-	+	+	-	-
J	+	-	+	-	-	+	-	+	-	w	+	+	+	-	+	-	-	-

Symbols: (+) Positive; (-) Negative; (w) Weak positive

As for the ability to assimilate carbon and nitrogen compounds, obvious differences between tested strains were detected, it could be gathered from the

obtained results that all strains assimilated glucose, sucrose, maltose and ethanol. Our finding are in agreement with those reported by Khater *et al*. (2019). In contrast, the strains were unable to assimilate lactose, starch and methanol, only strain H could slowly consume lactose and likewise I and J strains for starch. Moreover, all yeast strains failed to assimilate either nitrate or nitrite as a sole source of nitrogen.

Stress tolerance of yeast strains

One of the most crucial elements influencing yeast growth, reproduction and other activities is temperature. These ten strains were chosen since a probiotic organism must be capable of surviving and growing at human body temperature, which is 37°C. Similarly to this, a study by Rajkowska and Kunicka-Styczyska (2010) found that yeast strains obtained from kefir and chicken excrement could grow at 37ºC. Additionally, it has been said that *Saccharomyces cerevisiae's* growth at 37ºC is a changeable trait.

Data presented in Table 3 and Fig 1 illustrate the yeast strains that can survive in a variety of temperature ranges and NaCl concentrations. All strains can grow at temperatures between 25 and 37ºC. However, only F and G strains were capable of growth at 4°C, whereas the other strains were unable to. While no strains grew at a temperature of 45ºC, the A, B, C, D, E & H strains' growth was sluggish at 42ºC. These experiments are consistent with those published by Fakruddin *et al*. (2013), who found that all strains tested had optimal conditions ranging in temperatures between 25 and 30°C.

Table 3. Growth temperature of the yeast strains

Strains \ IT*	4	25 ºC	30 ºC	37 ºC	42 ºC	45 ºC
A	-	+	+	+	w	-
B	-	+	+	+	w	-
C	-	+	+	+	w	-
D	-	+	+	+	w	-
E	-	+	+	+	w	-
F	+	+	+	+	-	-
G	+	+	+	+	-	-
H	-	+	+	+	w	-
I	-	+	+	+	-	-
J	-	+	+	+	-	-

IT*: Incubation temperature

To observe the effect of different concentrations of NaCl on the ability to the survive of selected yeast strains, five different concentrations (2, 4, 6, 8 & 10% of NaCl) were added to the growth media (Fig 1).

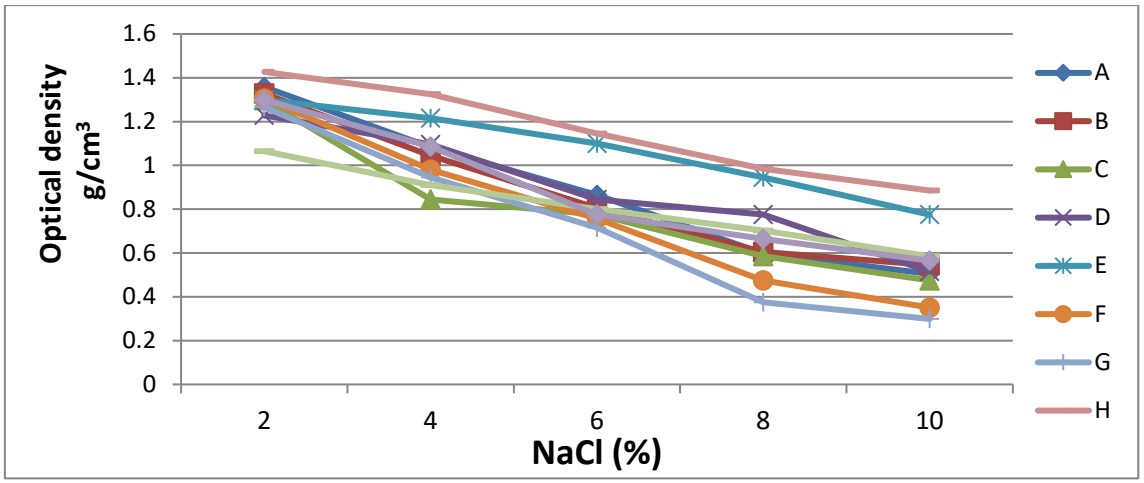

Fig 1. Effect of NaCl concentrations on the viability of yeast strains

The results demonstrate that all strains are capable of withstanding a variety of high NaCl concentrations. In general, strains H and E exhibited more NaCl resistance and possessed the highest survival after the incubation period at 10% concentrate. On contrary, the sensitive strains to NaCl concentration were G strains, followed by F strain, at the same concentration.

Probiotic properties

Any microorganisms used as probiotics must be able to withstand the pressures present inside the human body. Growing across a wide variety of temperatures, low pH, tolerance to bile salt, high NaCl concentration, simulated gastric juice, intestinal environment α- amylase, trypsin, lysozyme, assimilation of cholesterol and antibiotic resistance are only a few examples (Fakruddin *et al*., 2017). A distinguishing advantage for yeast to be used as a probiotic over bacteria is that it has a higher resistance to antibiotics. Vitamins also play a critical part in many metabolic processes of the body and yeasts have been found to be able to create vitamins, especially vitamin B complex (Syal and Vohra, 2014).

Tolerance of low pH

Probiotics must withstand the harsh physiological circumstances of the gastrointestinal tract after consumption, including the highly acidic environment and detergent-like effects of bile secretions. Human stomach pH ranges from 1.5 to 3.5, while small intestine bile salt concentrations are physiologically between 0.2 & 2.0% (Sahadeva *et al*., 2011). Therefore, yeast strains in the study were examined for their tolerance to low pH (1.5, 2.0 & 3.0). The obtained data in Table 4 observed that pH 1.5 was more harmful to the tested yeast strains. It was obvious, that B, F, A, G & H strains exhibited more acid resistance at pH 1.5, and were ranked the highest figure for survival percentages, actually 94.78, 94.35, 89.36, 85.45 & 84.58%, respectively, after 180 min of exposure to acidic condition. While C, D & E strains showed the least acid tolerance and lost about 20% of their viable cell under the same conditions. Moreover, I & J strains could not resist incubation for 180 min at pH 1.5, there was no growth completely.

Table 4. The viability of yeast strains on pH 1.5

Strains	Inculpation time (h)			
	BI*	1	2	3
	(Log cuf/ml)	Survival (%)		
A	7.14± 0.03	98.88	94.82	89.36
B	6.13± 0.00	98.53	96.90	94.78
C	6.65± 0.01	92.48	88.57	79.55
D	6.65± 0.01	94.44	88.72	80.45
E	6.60± 0.02	92.88	87.42	78.03
F	6.02± 0.08	97.84	96.84	94.35
G	6.05± 0.09	96.86	89.59	85.45
H	6.81± 0.00	95.74	89.87	84.58
I	6.48± 0.04	81.02	0.00	0.00
J	6.95± 0.02	97.27	78.99	0.00

BI*: Before incubation, cfu: colony forming unit.

Strong oxidizers include acids like HCl in the human stomach. As a result, while going through reduction, it can oxidize several significant biomolecular substances in the cells and damage them. Fatty acids, proteins, cholesterol and DNA are some of the vital biological substances that acid can destroy (Pan *et al*., 2009). The growth and metabolism of microorganisms are assumed to be inhibited in low pH conditions, which lowers the viability of the probiotics. Moreover, it might be gathered from the obtained data that pH 2.0 showed a moderate effect on the most examined strains after 180 min (Table 5). However, I & J strains did not show any resistance to the same conditions of incubation for 180 min.

Table 5. The viability of yeast strains on pH 2.0

Strains	Inculpation time (h)			
	BI	1	2	3
	(Log cuf/ml)	Survival (%)		
A	7.20± 0.00	99.03	96.53	92.50
B	6.29± 0.00	99.68	99.68	99.52
C	6.74± 0.01	96.29	88.87	86.50
D	6.60± 0.01	97.42	92.42	87.88
E	6.61± 0.01	94.70	89.11	82.15
F	6.12± 0.04	98.86	98.37	96.41
G	6.01± 0.06	98.50	97.67	90.68
H	6.81± 0.00	95.74	89.87	84.58
I	6.48± 0.04	81.02	0.00	0.00
J	6.95± 0.02	97.27	78.99	0.00

BI*: Before incubation, cfu: colony forming unit.

Results in Table 6 revealed that all yeast tested strains survived better at pH 3.0 up to 180 min of incubation. The survival percentage varied from 96.80 & 100.42%. Continuously, it was interesting to notice that the behavior of both B and A strains showed an increase in numbers throughout the incubation period (60, 120 & 180 min). Furthermore, the obtained data revealed that I & J strains were the most acid sensitive among all tested yeast strains. In conclusion, from the foregoing results, it could be stated that all tested strains retained high viability (survival percent, varied from 98.76% to 100.79%) for 60 min, which is the time required for stomach content to empty nearly completely (Mainville *et al*., 2005).

Table 6. The viability of yeast strains on pH 3.0

Strains	Inculpation time			
	BI (Log cuf/ml)	1	2	3
		Survival (%)		
A	7.08± 0.01	100.14	100.14	100.42
B	6.31± 0.07	100.79	101.74	102.22
C	6.70± 0.03	98.96	98.81	99.70
D	6.61± 0.03	99.24	99.09	99.24
E	6.59± 0.03	99.24	99.24	99.09
F	6.07± 0.07	99.34	99.18	98.68
G	6.03± 0.11	99.50	98.67	96.85
H	6.79± 0.01	99.41	99.41	99.41
I	6.43± 0.03	98.76	97.82	97.51
J	6.88± 0.01	98.98	98.55	96.80

BI*: Before incubation, cfu: colony forming unit.

Bile tolerance

One of the critical characteristics necessary for the probiotic organism's high survival rate is also thought to be bile tolerance. There is disagreement over the precise concentration that the chosen strains should be tolerant it. The small intestine's physiological concentration of bile salts ranges from 0.2 to 2.0% (Gunn, 2000). Therefore, probiotics must have the ability to tolerate bile. Bile acids cause cellular homeostasis to break down, causing the lipid bilayer and integral protein of the cell membranes to separate. This leaking of bacterial content leads to cell death (Sahadeva *et al*., 2011). Thus, the obtained data in Table 7 show two mimic approximate levels in the intestinal tract. It could be noted that all tested yeast cultures exhibited excellent bile tolerance. However, tolerance to bile varied among the tested strains and bile concentrations. In this respect, Rajkowska and Kunicka-Styczyńska (2010) reported that the addition of 0.1 & 1.0% bile did not restrict the viability of microorganisms.

In general, D, G, B, F & C strains exhibited more bile resistance than other tested yeast strains (Table 7). In contrast, A strain was the most sensitive to bile acid concentrations at 0.5%, exhibiting less growth of 29.95%. Numerous *in vitro* investigations with similar findings have shown that yeasts from *Saccharomyces*, *Debaryomyces* and *Kluyveromyces* species are very tolerant to low pH and high bile salt concentrations (up to 1% ox gall) (Sourabh *et al*., 2011).

Table 7. Effect of bile salt concentrations on the growth of yeast strains (Log cuf/ml)

Strains	Bile concentration % (w/v)	Incubation time (h)		Increase (%)
		BI	72 h	
A	0.3	5.96±0.01	8.02±0.02	34.56
	0.5	6.21±0.01	8.07±0.00	29.95
B	0.3	5.72±0.04	7.83±0.04	36.89
	0.5	5.74±0.03	7.73±0.03	34.67
C	0.3	5.73±0.03	7.78±0.05	35.78
	0.5	5.78±0.05	7.89±0.05	36.51
D	0.3	5.73±0.03	8.00±0.03	39.62
	0.5	5.77±0.03	8.06±0.01	39.69
E	0.3	5.76±0.05	7.71±0.06	33.85
	0.5	5.75±0.01	7.61±0.03	32.35
F	0.3	5.34±0.04	7.29±0.04	36.52
	0.5	5.34±0.03	7.20±0.05	34.83
G	0.3	5.19±0.03	7.16±0.03	37.96
	0.5	5.16±0.04	7.15±0.07	38.57
H	0.3	5.81±0.03	7.80±0.03	34.25
	0.5	5.77±0.03	7.82±0.01	35.53
I	0.3	5.38±0.08	7.22±0.06	34.20
	0.5	5.48±0.04	7.31±0.04	33.39
J	0.3	5.97±0.02	7.98±0.01	33.67
	0.5	6.02±0.03	8.02±0.04	33.22

BI*: Before incubation, cfu: colony forming unit.

In agreement with the literature, all tested yeast strains exhibited excellent bile tolerance. Notably, most yeast strains showed enhanced growth in media containing either 0.3 or 0.5% bile acids at the end of incubation time. In this respect, Erkkilä and Petäjä (2000) stated that microorganisms that survive in the acidic condition of the stomach also have to survive in intestinal secretion and the bile salts in the duodenum. The strong growth of the strains at 0.3 or 0.5% bile may indicate that these bacteria have mechanisms for adapting to stress, which would be a plausible explanation for the growth that occurs with extended incubation times. Despite significant variances in design, a recurring finding in these investigations is that acid and bile have both individual and combined impacts on strain growth. Moreover, Lin *et al*. (2006) proposed that sub lethally wounded microorganisms might have a varied and unpredictable resistance to new stress factors because bile stress occurs following pH stress in the stomach. The probiotic strains were found to have exceptional bile tolerance. The bile salt hydrolase (BSH) activity, which is responsible for bile salt resistance, is a further crucial component. Some strains have been found to cause BSH to hydrolyze conjugated bile, which lessens its bactericidal action (Du Toit *et al*., 1998). This could account for some strains' sensitivity that lacked this BSH activity.

Generally, from the foregoing results, it could be concluded that all the examined strains showed normal growth at bile concentrations up to 0.5% (w/v). However, bile resistance is a crucial factor to consider when choosing a culture as a dietary adjunct, since it may enable the growth of the ingested probiotic bacterium in the intestine (Walker and Gilliland, 1993).

In vitro survival in the gastric and intestinal environment

Each day, approximately 2.5 liters of gastric juice with a pH of 2.0 and a salt concentration of at least 0.5% w/v are secreted (Hill, 1990). During digestion, these secretions act as a pH, enzymatic and bile barrier to prevent ingested microbes from surviving. The obtained data in Table 8 show, how the ten tested strains would fare in the presence of simulated gastric juice. From these results, it could be noticed that gastric juice exerted a moderate influence on the growth of most yeast strains. In general, F, B, G, A, E & D strains exhibited more gastric juice resistance and possessed the highest survival percent, being 91.09, 89.46, 87.83, 86.87, 80.33 & 78.04% after incubation for 240 min of exposure, respectively. On contrary, the sensitive strains to gastric juice were H strain, followed by C strain, they ranked survival percent of 58.91 & 72.80% after 240 min of exposure, respectively. While I & J strains could not resist gastric juice, as they did not present any growth during the different incubation periods under the same conditions. This finding confirms the result of Mathara *et al*. (2008), who stated that tolerance to gastric juice was observed among strains that showed much higher acid tolerance.

Table 8. Survival percentages of yeasts in simulated gastric juice

Strains	Incubation time (min)				
	BI	60	120	180	240
	Log cfu/ml	Survival (%)			
A	7.16±0.01	97.49	92.74	87.43	86.87
B	7.12±0.02	98.60	95.79	92.28	89.46
C	6.95±0.00	92.52	90.65	89.64	72.80
D	6.65±0.01	94.29	90.23	84.96	78.04
E	6.61±0.03	94.55	89.71	83.96	80.33
F	6.29±0.05	96.03	93.80	92.37	91.09
G	6.41±0.06	95.16	91.73	89.39	87.83
H	6.84±0.01	76.02	72.95	65.94	58.91
I	6.51±0.03	77.11	0.00	0.00	0.00
J	6.81±0.01	0.00	0.00	0.00	0.00

BI*: Before incubation, cfu: colony forming unit.

Intestinal juice

Each day, 0.7 liters of pancreatic juice with a pH of about 8.0 and a salt content of at least 0.5% w/v is secreted into the proximal intestine **(**Keele and Neil, 1965). A little under 0.2 ml of each washed cell suspension was combined with 1.0 ml of intestinal juice (pH 8.0, & 0.1% pancreatin). The liquids were briefly vortexed and then incubated at 30ºC for 60, 120, 180 & 240 min to determine the yeast count. The obtained data in Table 9 revealed that B, F, A, G, E, D & C strains were better with regard to intestinal juice tolerance than that of other strains. Where their survival percent were 90.96, 89.61, 89.02, 87.40, 86.27, 85.26 & 84.79% after 240 min of exposure to intestinal juice, respectively. In contrast; H, J & I strains ranked the lowest figure for survival percent, with percentages of 79.39, 72.85 & 69.82% under the same conditions, respectively. Our data concluded that most tested strains retained approximately normal viability during growth in simulated small intestinal juice and are considered intrinsically tolerant to intestinal transit.

In this respect, the majority of probiotic strains were reported to be innately resistant to simulated pancreatic juice (Charteris *et al*., 1998). The previous results mean that most tested yeast strains were considered tolerant to intestinal juice and could be successfully functioning effectively in the intestinal tract.

Table 9. Survival percentages of yeasts in simulated intestinal juice

Strains	Incubation time (min)				
	BI	60	120	180	240
	Log cfu/ml	Survival (%)			
A	7.01±0.04	99.00	97.15	91.73	89.02
B	7.08±0.01	98.73	97.18	94.92	90.96
C	6.97±0.04	99.57	95.98	90.82	84.79
D	6.85±0.02	99.42	96.06	89.64	85.26
E	6.70±0.04	98.51	94.48	90.30	86.27
F	6.35±0.07	98.58	94.80	91.65	89.61
G	6.43±0.05	97.98	95.80	89.58	87.40
H	6.84±0.02	91.23	86.55	83.77	79.39
I	6.56±0.05	93.75	90.24	79.88	69.82
J	6.85±0.02	91.97	87.01	81.31	72.85

BI*: Before incubation, cfu: colony forming unit.

In vitro assimilation of cholesterol

Significant correlations exist between elevated blood cholesterol and cases of coronary heart disease (Kern, 1991). Even a 1% decrease in cholesterol has been shown to lessen the incidence of cardiovascular illnesses by 2-3%. Coronary artery disease (CAD) is the leading cause of mortality worldwide (Manson *et al*., 1992). A significant risk factor for CAD is increased blood cholesterol, which can be treated with medication, dietary changes and behavioral adjustments. One strategy to lower blood cholesterol and lower the risk of ACD is dietary changes. The obtained data in Table 10 demonstrates the assimilation of cholesterol by the tested yeast strains. From the results, it could be seen that all tested cultures had the ability to reduce cholesterol concentration. However, our data revealed that tested strains assimilate cholesterol in a range varied from 18.42 to 44.74%. Moreover, it was evident that I strain assimilate more cholesterol than other tested strains, ranked 44.74%. In contrast, A strain possessed the lowest cholesterol assimilation (18.42%). The outcomes obtained supported the hypothesis that the only method by which yeast strains eliminated cholesterol from the growing media was by assimilation of cholesterol into yeast cells. According to several investigations, some probiotics can lower blood cholesterol levels in either people or animals (Usman and Hosono, 2000). However, multiple data suggest that yeasts can eliminate cholesterol from laboratory media supplemented with cholesterol micelles while they are growing (Ness *et al*., 1998). It is also important to note that, the examined yeast cultures never attained 100% cholesterol absorption. Since the lysing enzymes never achieved 100% cell wall lysis, this might be the cause.

Table 10. Cholesterol assimilation by yeast strains

Strains	Cholesterol (%)	Assimilation of cholesterol (%)
A	40.00	18.42
B	35.48	27.63
C	34.19	30.26
D	31.61	35.53
E	34.19	30.26
F	33.55	31.58
G	30.32	38.16
H	34.19	30.26
I	27.10	44.74
J	34.19	30.26

Conclusion

In this study, we examined yeasts that may have probiotic properties. They may offer protection from food-borne enteric infections and may be recommended to individuals receiving extended antibiotic therapy. Most of the yeast varieties we examined were capable of growing at body temperature and enduring high amounts of acidity, bile salts, sodium chloride, and enteric juice, among other harsh circumstances. For patients with hypercholesterolemia, their capacity to absorb cholesterol could be of great benefit. *Saccharomyces cerevisiae*, *Geotrichum candidum*, *Kluyveromyces lactis* and *Debaryomyces hansenii* are all beneficial probiotic agents that have the potential to be employed widely as food and feed additives in the future.

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خصائص الدعم الحيوي المحتملة لبعض سلالات الخميرة

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الملخص

بسبب تأثيرها المضاد للفطريات ، أصبحت الخمائر محبوبة أكثر فأكثر كمصادر طازجة لتعزيز سمات الطعام بما في ذلك النكهة واللون ومحتوى الفيتامينات وكذلك عوامل لتجنب تلف الطعام، وتهدف هذه الدراسة الى معرفة مدى احتمالية بعض سلالات الخميرة لبعض خصائص الدعم الحيوي (البروبيوتك)، وقد تم اجراء التجارب المعملية علي عشر سلالات تابعه لخمس انواع مختلفة هي : *Saccharomyces cerevisiae*, *Geotrichum candidum*, *Kluyveromyces lactis*, *Debaryomyces hansenii* and *Torulaspora delbrueckii*، وقد أوضحت النتائج ان ثمانية من هذه السلالات اظهرت قدرة على تحمل البقاء على قيد الحياة تحت ظروف مشابهه للمعدة بمعدل تراوح ما بين من 94 الى 99% عند درجة حموضه ما بين 1,5 : 2 pH وذلك باستثناء سلالتين هما *Debaryomyces hansenii* and *Torulaspora delbrueckii*، بينما كان لجميع السلالات القدرة على تحمل درجات الحرارة على نطاق واسع وكذا التركيزات العالية من كلوريد الصوديوم واملاح الصفراء عند تركيز 0.5 % وكذا النمو في بيئة مشابهه للأمعاء، بالإضافة الى ذلك جميع السلالات كانت لديها القدرة على تحليل الكوليسترول في نطاق تراوح ما بين 18-44% وذلك بعد التحضين لمدة 48 ساعة على درجة حرارة 37 درجة مئوية.