

Probiotic potential of *Saccharomyces* strains isolated from *Litchi chinensis* (Lychee fruit)

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Abstract: Recently, probiotic yeasts have become an interesting topic of research all over the world. *Saccharomyces cerevisiae* is well proven probiotic yeast against several gastrointestinal diseases. Current study aimed to explore the probiotic potential and antibacterial properties of *Saccharomyces* strains isolated from fresh lychee fruits available in local markets of Karachi, Pakistan. Probiotic potential and antibacterial activity of locally isolated probiotic yeast strains (named as *S. cerevisiae* BEL 1 and *S. cerevisiae* BEL 9) was studied against gastrointestinal pathogens using standard *in vitro* screening methods. Comparative analysis was also carried out with commercially available *S. boulardii* probiotic preparations. Furthermore, for probiotic potential, all the studied yeast strains were exposed to various stress conditions inherent of gastrointestinal tract i.e., thermo tolerance, pH tolerance, bile salts survivability and osmo-tolerance. Isolated strains (BEL 1 and BEL 9) were able to tolerate at the temperatures (40°C and 45°C), moreover survived in the presence of gastric juices, extreme bile salt concentrations (range 0.5%-2%) and different osmotic stress conditions (1M and 1.5 M NaCl). Optimal growth was observed at 37°C. Similar growth pattern and viability of BEL 1 and 9 was found for most of the stress conditions, when compared with the commercially available strains of *S. boulardii*. Therefore, isolated yeast strains BEL 1 and 9 will be considered as a potential bio-therapeutic agent because of the promising probiotic potential.

Keywords: Probiotic yeast, diarrhea, *Saccharomyces cerevisiae*, p potential, *Litchi chinensis*

INTRODUCTION

Diarrhea is the most common manifestation of many gastrointestinal diseases. It is characterized by low fecal consistency, which is due to the imbalance between intestinal absorption and secretion of water and ions. Diarrhea is considered to be the most common health issue of developing and under developing countries and the most common cause of hospitalization in infants and children (Keusch *et al.*, 2006). Gastrointestinal disorders such as diarrhea (both specific and nonspecific) and colitis in most parts of Pakistan are very common (Ghayur and Iqbal, 1992). Conventionally used treatments for management of diarrhea is oral rehydration salts (ORS), that can replace lost fluids but it does not reduce the duration as well as intensity of diarrhea (Elmer, 2001, Kotowska *et al.*, 2005, Pattani *et al.*, 2013). Considering different strategies that can reduce the duration and intensity of diarrhea, probiotics are appeared to be the best alternative approach (Kelesidis and Pothoulakis, 2012).

Probiotics are applied to improve the patient's microbial balance, particularly the environment of the gastrointestinal tract and the vagina (Elmer, 2001, Kotowska *et al.*, 2005). Therefore, probiotic therapy is studied for its efficiency against a wide variety of gastrointestinal diseases such as inflammatory bowel disease, ulcerative colitis and pancreatitis. (Elmer, 2001, Kerry, Patra *et al.*, 2018, Kotowska *et al.*, 2005, Pattani *et*

al., 2013). Use of probiotic therapy for conditions related to allergic disorders such as atopic dermatitis and allergic rhinitis has also been reported (Plaza-Diaz, Ruiz-Ojeda *et al.* 2019). Probiotics usually exert their effect by inhibiting microbial toxins, pathogen attachment in intestine (Rowland *et al.*, 2010) and stimulation of immunoglobulin A (Hudson *et al.*, 2016, Stier and Bischoff, 2016).

Saccharomyces cerevisiae is non-pathogenic yeast that grows optimally at body temperature, and also tolerates acidic pH of stomach. A variant *Saccharomyces cerevisiae* var *boulardii* has proved its therapeutic effect for the treatment and prevention of certain gastric disorders (Sulik-Tyszka *et al.*, 2017). Certain other *in-vitro* and *in-vivo* studies have demonstrated anti-microbial, anti-inflammatory, enzymatic, metabolic and antitoxin activities of *saccharomyces* yeasts. *S. boulardii* has also been reported to stimulate immune response in intestinal mucosa by enhancing mucosal metabolism (Kelesidis and Pothoulakis, 2012, Czerucka *et al.*, 2007).

The current study was designed with the objective to explore the probiotic potential of the local *Saccharomyces* strains. The study aimed to isolate, purify and identify the *Saccharomyces* strains from fresh lychee fruits available in local markets of Karachi. Furthermore, *in vitro* assessment of the probiotic potential and antibacterial activity of the isolated strains was also evaluated and comparison studies with commercially available probiotic

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preparation of *Saccharomyces cerevisiae* var *boulardii* were also carried out.

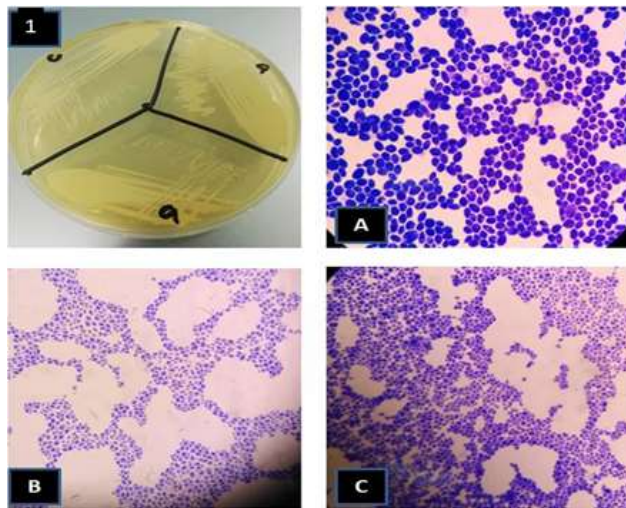
MATERIALS AND METHODS

Sample collection and isolation of yeast strains

Fresh lychee fruits (*Litchi chinensis*) were purchased from local market of Karachi, Pakistan during the month of May 2018. All the samples were transported to laboratory and stored at 4°C for further processing. Fruits with no apparent defect in their skin were used. Commercial preparation of *Saccharomyces boulardii* (Actiflor) was used as control strain. Isolation was performed by using the protocols of Alberghina and Fakriddin (Alberghina *et al.*, 2012, Fakriddin *et al.*, 2017). Strains that showed yeast like colonial and microscopic characteristics were selected for further evaluations.

Germ tube production test

Strains that showed yeast like morphology were subjected to germ tube test, according to the protocol of Banerjee (Banerjee *et al.*, 2017). Germ tube is a rapid test for the presumptive identification of pathogenic yeast strains and negative germ tube test indicates non virulence. In this assay a single isolated colony of isolated strains were inoculated into serum and incubated for 24hr at 37°C. After incubation the slides were prepared and staining was done by using crystal violet dye and observed for germ tube formation.



A= *Saccharomyces cerevisiae* var *boulardii*, B= Isolated Yeast Strain (BEL 1) & C= Isolated Yeast Strain (BEL 9)

Fig. 1: Colony morphology (1) & Microscopic investigation of the isolated and commercial probiotic yeast strains at 100x magnification.

Molecular identification of isolated strains

For specie identification, molecular characterization was performed. DNA extraction and PCR amplification was done through the method described by Murray *et al.*, 2016

(Murray *et al.*, 2016). Primers ITS 1, used as forward primer, (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 as reverse primer, (5' TCCTCCGCTTATTGATATGC 3') to amplify the region of 5.8S rRNA gene and 2 non coding internally transcribed regions namely ITS 1 and ITS 2. BLAST analysis of the amplified regions was also performed to identify the species.

In-vitro screening of probiotic potential

Gastrointestinal (GI) Stress Tolerance Studies

For the determination of stress tolerance, yeast cultures were inoculated onto YEPD broth and inoculated flasks were incubated at 37°C for 24hr. After incubation period, number of cells was adjusted by using fresh YEPD broth to 10⁶ CFU/mL for further analysis. Growth patterns and cell viability were done by measuring optical density (OD) at 600 nm and plating on YEPD agar respectively.

All experiments of GI stress tolerance studies were performed in triplicate and results given are the average values of the triplicates.

i. Thermo tolerance

Thermo tolerance of yeast strains was determined according to Fietto *et al.*, 2004 (Fietto *et al.*, 2004) with slight modifications. Briefly, cells were subjected to heat shock stress by shifting the incubation temperature from 30°C to 37°C, 40°C and 45°C in YEPD liquid media. After 24hr the OD was measured and cell viability was determined.

ii. Tolerance to Gastric enzymes

Sensitivity of the isolated strains in simulated gastric enzymes was also determined by using the protocol of Fietto *et al.*, 2004 (Fietto *et al.*, 2004). In this assay aqueous solution of 3g/L pepsin (pH 2.0) and 1mg/L of pancreatin (pH 8.0) were used. Absorbance and cell viability was determined at the interval of 30 min for 6 hr.

iii. Bile salt Tolerance

Bile salt tolerance was determined by the method of Narayanan *et al.*, 2012 (Narayanan, 2012) with slight modifications. Adjusted cells were inoculated in YEPD broth supplemented with different concentrations of bile salts (0.5%, 1%, 1.5% & 2%), and incubated at 37°C with 200 rpm for 24 hours. A control without bile salt was also prepared. Cellular growth and viability was determined.

iv. Osmo-tolerance

Isolated and commercial yeast strains were also exposed to osmotic stress created by 1M and 1.5M concentration of sodium chloride (NaCl) (Narayanan *et al.*, 2012). Cellular growth and viability was monitored after 24 hr.

Cell Viability Measurement

Viability count of the isolated and commercial yeast strains after exposure to different gastrointestinal stress

conditions was measured. Cell viability was determined by plating the cell suspension on YEPD agar and incubation at 37°C for 24 hours. Number of colony forming units were counted and categorized as:

- + = 1-100 colonies
- ++ = 101-200 colonies
- +++ = 201-300 colonies
- ++++ = > 300 colonies
- = 0 or No colony

Antibacterial Activity

Antibacterial activity of isolated and commercial strain against GI pathogen was evaluated by using the protocol of Zokaeifar *et al.*, 2012 (Patton *et al.*, 2006, Zokaeifar *et al.*, 2012) with slight modifications. Cultures of GI pathogens which include *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus* and *Salmonella typhi* in suspension, were adjusted at 0.5 McFarland standards by using spectrophotometer. Cultures of GI pathogens were obtained from Microbial bank, microbial Bioresource and biotechnology laboratory, DRIBBS, DUHS. Antibacterial activity was checked in supernatant and percent inhibition was determined using the following formula:

$$\text{Percent (\%) inhibition} = 1 - \frac{\text{OD of test well}}{\text{OD of Control Well}} \times 100$$

STATISTICAL ANALYSIS

Descriptive statistics were done using MS excel (Version 2010). Data is shown as mean \pm standard error (SE).

RESULTS

Yeast strain characterization

The yeast strains isolated from locally purchased lychee fruit were identified as *S. cerevisiae* based on their microscopic and morphological characteristics (fig. 1). Germ tube test of the isolates was performed and found out to be negative (fig. 2). Further identification of the strains was done by amplification of the internally transcribed spacer (ITS) regions of the isolates. The sequence obtained from the two isolated strains after amplification of their ITS regions was run on BLAST and their identities were confirmed as *S. cerevisiae*. The control strain was identified as *S. boulardii*. Based on this identification, the local strains were named as *S. cerevisiae* BEL 1 and *S. cerevisiae* BEL 9 (BEL 1 and BEL 9) and during this study they will be referred as same.

Probiotic Potential Screening

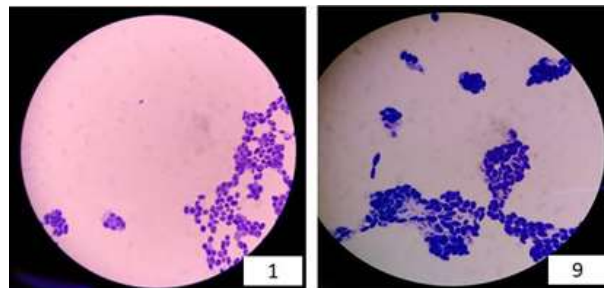
Thermo tolerance

Thermo tolerance pattern of the isolated and commercial probiotic yeasts strains are shown in fig. 3 and table 1. Locally isolated and commercial strains showed similar growth patterns at different temperatures (30°C, 37°C, 40°C and 45°C). Moreover, viability up to 40°C was also observed, with comparable viability at variable

temperatures (30°C, 37°C, 40°C and 45°C). However, suppressed growth observed at 45°C, and few viable cells were observed.

Gastric Environment

Test and control strains showed comparable growth pattern under conditions simulating the gastric environment as shown in fig. 4 and 5. Cell count of all yeast strains was comparable.



1= Isolated Yeast Strain (BEL 1), 9 = Isolated Yeast Strain (BEL 9).

Fig. 2: Germ tube results of the isolated yeast strains stained with crystal violet and observed at 100x magnification.

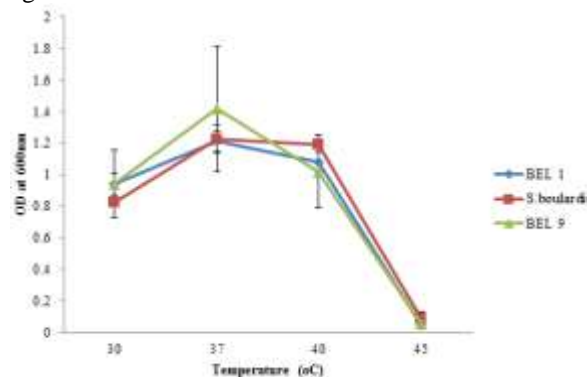


Fig. 3: Effect of temperature on growth of isolated and commercial yeast strains.

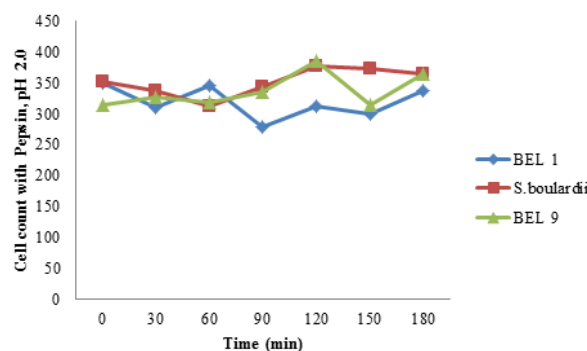


Fig. 4: Growth pattern of isolated and commercial yeast strains under gastric conditions (Pepsin, pH 2.0)

Bile salt stress tolerance

Test and control strains were able to survive in presence of different concentrations of bile salts and showed

similar growth pattern. Viability count of all three strains was found to be similar at different concentrations of bile salt, except for *BEL 9* which showed slightly less growth in 1.5% concentration of bile salts (fig. 6, table 1).

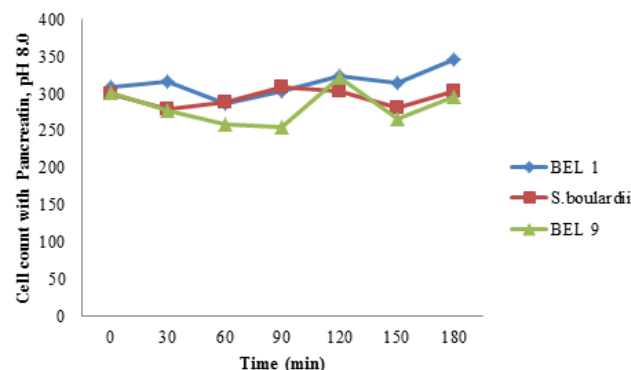
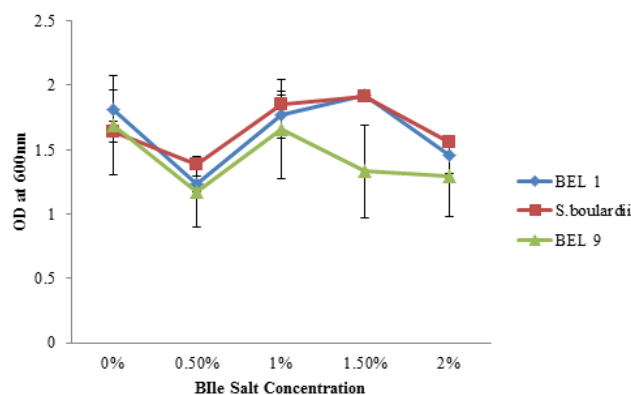


Fig. 5: Growth pattern of isolated and commercial yeast strains under pancreatic conditions (Pancreatin, pH 8.0).



*0% indicates control without bile salt

Fig. 6: Effect of Bile salt stress on growth of isolated and commercial strains at 37°C.

Osmo-tolerance

Test and control strains, when compared with control, showed growth and viability under osmotic stress condition induced by 1M NaCl. Viability count was comparable in all three strains. Higher NaCl concentration (1.5M) inhibited the growth and viability (fig. 7, table 1).

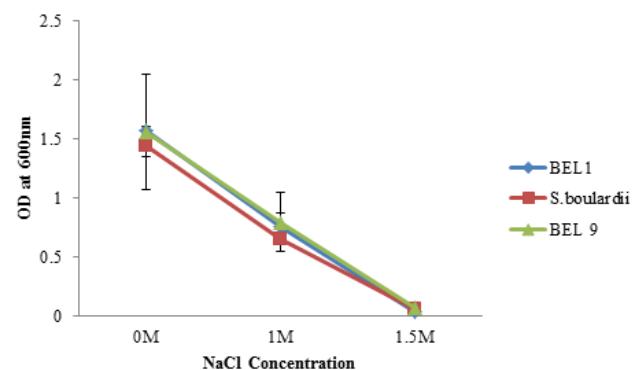
Antibacterial activity

Test and control strains, showed significant antibacterial activity against common GI pathogenic strains. The locally isolated strains showed significant antibacterial activity than the commercial strain. Among the two local isolates, *BEL 9* performed better than *BEL 1* (table 2).

DISCUSSION

Global market of Probiotics, live microbial cells with different beneficiary characteristics, has been flourishing because of the growing interest in self-care and integrative medicines (Reid, 2015). *S. boulardii* is

clinically and experimentally proven yeast strain that confers probiotic effect to humans and can be used as a bio therapeutic agent against a number of gastrointestinal disorders (Czerucka *et al.*, 2007, Kelesidis and Pothoulakis, 2012).



*0M NaCl indicates control

Fig. 7: Effect of sodium chloride (NaCl) concentration on growth of isolated and commercial strains at 37°C.

In the presented research, potential probiotic yeast strains *BEL 1* and *BEL 9* have been isolated from lychee fruit, identified and characterized as *Saccharomyces cerevisiae* on the basis of comparative morphological, biochemical, physiological and molecular characteristics. Isolated strains showed promising probiotic activities that were found to be comparable with the commercial *S. boulardii* control probiotic strain used. Current study to the best of our knowledge is first attempt to isolate and characterize the probiotic potential of locally isolated yeasts against commercial *S. boulardii* in Pakistan. Several studies in different regions of the world reported the isolation of probiotic yeast (from genus *Saccharomyces*) from different samples including fruits and have *in vitro* screened their probiotic activities through various physiological and biochemical parameters (Fakruddin *et al.*, 2017, Khanda and Zainab, 2014, Qvirist *et al.*, 2016).

In probiotic screening microorganisms are exposed to various stress conditions that are present in these gastrointestinal tract and their ability to survive in these stressful conditions is tested (Klaenhammer and Kullen, 1999). Current study tested the probiotic potential of the isolated strains by evaluating their tolerance against various mammalian body stresses including temperature, gastric juices, bile salt and osmolarity.

Thermo tolerance is an important characteristic of probiotic organism, and special focus has been placed on the capacity of yeast cells to grow at mammalian body temperature (37°C) (Fietto *et al.*, 2004). The isolated strains *BEL 1* and *BEL 9* showed growth between 30°C to 45°C, while optimum growth was observed at 37°C. The pH of human gastrointestinal tract ranges from being acidic to alkaline (Psomas *et al.*, 2001). Within stomach

Table 1: Viability count of the isolated and commercial yeast strains after exposure to different gastrointestinal stress conditions

Viability Count			
Stress Conditions	<i>S.bouardii</i>	<i>S. cerevisiae BEL 1</i>	<i>S. cerevisiae BEL 9</i>
Osmo-tolerance			
Control	++++	++++	++++
1M	++++	++++	++++
1.5M	+	+	+
Thermotolerance			
30°C	++++	++++	++++
37°C	++++	++++	++++
40°C	+++	+++	+++
45°C	+	+	+
Bile Salt Tolerance			
Control	++++	++++	++++
0.50%	++++	++++	++++
1%	++++	++++	++++
1.50%	++++	++++	++++
2%	++++	++++	++++

Key: + = 1-100 colonies, ++ = 101-200 colonies, +++ = 201-300 colonies, ++++ = > 300 colonies, - = 0 or No colony

Table 2: Antibacterial activity of the isolated and commercial yeast strains against clinically isolated gastrointestinal pathogens.

Yeast Strains	<i>Salmonella typhi</i> (% inhibition)	<i>Pseudomonas aeruginosa</i> (% inhibition)	<i>Escherichia coli</i> (% inhibition)	<i>Bacillus cereus</i> (% inhibition)
<i>S.bouardii</i>	53.17	68.95	84.24	75.54
<i>S. cerevisiae BEL 1</i>	67.85	76.76	86.72	80.78
<i>S. cerevisiae BEL 9</i>	91.82	80.77	96	96.15

the environment is highly acidic due to the secretion of hydrochloric acid; however, the presence of food changes the pH from 0.9 to 3.0 (Erkkila and Petaja, 2000). Additionally, pH gradually increases in the small intestine from pH 6 in duodenum to about pH 7.4 in the terminal ileum. Therefore, one of the very first hindrances that microorganisms face after ingestion is the strong acidic environment of the stomach and their ability to tolerate this environment determine their potential as successful probiotics (Psomas *et al.*, 2001, Erkkila and Petaja, 2000). We have tested the abilities of yeast strain to endure the pH of acidic pepsin and alkaline pancreatin environment. Isolated strains' growth pattern was comparable with the commercial available control *saccharomyces* strain. Results obtained were comparable with other studies that have shown acid resistance of various *saccharomyces* strains (Fietto *et al.*, 2004, Khanda and Zainab, 2014, Pennacchia *et al.*, 2008).

Besides gastric juices tolerance, determination of the bile salt tolerance of microorganisms is a critical step in the screening of intestinal probiotics. Bile salts are synthesized in liver from cholesterol as main components of bile and are released in to small intestine from gall bladder during digestion to perform their physiological

functions in the gastrointestinal tract, which is to facilitate the digestion of lipids coming from diet (Miskovitz, 1999). Additionally, bile salts possess strong antimicrobial property since they are able to disrupt the lipid bilayer of the cell membrane and can trigger the DNA damage (Kandell and Bernstein, 1991). Therefore, probiotic microorganisms must have an intrinsic resistance to counteract the toxic effects of bile salts, in order to be able to survive the gastrointestinal transit and to transiently colonize the gut (Ruiz *et al.*, 2013). Although concentration of bile salts varies in the GI tract ranging from 0.2 to 2% (w/v) (Miskovitz, 1999), the minimum concentration reported in the literature for screening of a resistant probiotic strain is 0.3% (w/v) (Gilliland *et al.*, 1984). In this study, all strains were screened against various concentrations of bile salts ranging from 0.5 to 2%; with 2% represent the extreme concentration of bile salts obtained during first hour of digestion. Isolated strains showed strong tolerance against bile salts (with growth and viability up to 2%.) The results were comparable with previous studies that have shown yeast growth in higher concentration of bile salts (Fakruddin *et al.*, 2017, Khanda and Zainab, 2014, Syal and Vohra, 2013).

High osmolarity is another extreme condition that microbes face during gastric passage (Fietto *et al.*, 2004). The isolates showed tolerance to high NaCl and tolerate the osmolarity similar to the commercial probiotic strain.

Antibacterial activity against the human pathogens is one of the most desirable properties of probiotic organisms. The isolates showed good antibacterial activity against entero-pathogen *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Bacillus cereus*. Results were in accordance with the previous studies that reported antibacterial activity of *S. boulardii* against number of human pathogens (Rajkowska and Kunicka-Styczyńska, 2012, van der Aa Kühle *et al.*, 2005).

CONCLUSION

In conclusion, the isolated yeast strains (*BEL 1* and *BEL 9*) show promising probiotic activity *in vitro*, which are comparable to the commercially available *S. boulardii* products. The isolates have a potential to be used therapeutically, however, before any therapeutic application, further research is required to evaluate the biosafety profile of the potential probiotic yeasts. Furthermore, *in vivo* studies have also been needed to establish the identified potential.

REFERENCES

Alberghina, L, Mavelli, G, Drovandi, G, Palumbo, P, Pessina, S, Tripodi, F, Coccetti P and Vanoni M (2012). Cell growth and cell cycle in *Saccharomyces cerevisiae*: Basic regulatory design and protein-protein interaction network. *Biotechnol. Adv.*, **30**(1): 52-72.

Banerjee, P, Sulaiman, I. M, Schneider, G, Ray U and Jagadeesan B (2017). Novel microbial diagnostic methods for clinical, environmental and food samples. *BioMed Res. Int.*, 2017.

Czerucka D, Piche T and Rampal P (2007). Review article: Yeast as probiotics -- *Saccharomyces boulardii*. *Aliment Pharmacol Ther*, **26**(6): 767-78.

Elmer, G. W. (2001). Probiotics: "living drugs". *Am. J. Health-Syst. Ph.*, **58**: 1101-1109.

Erkkila S and Petaja E (2000). Screening of commercial meat starter cultures at low pH and in the presence of bile salts for potential probiotic use. *Meat. Sci.*, **55**(3): 297-300.

Fakruddin M, Hossain MN and Ahmed MM (2017). Antimicrobial and antioxidant activities of *Saccharomyces cerevisiae* IFST062013, a potential probiotic. *BMC Complement. Altern. Med.*, **17**(1): 64.

Fietto JL, Araujo RS, Valadao FN, Fietto LG, Brandao RL, Neves MJ, Gomes FC, Nicoli JR and Castro IM (2004). Molecular and physiological comparisons between *Saccharomyces cerevisiae* and *Saccharomyces boulardii*. *Can J. Microbiol*, **50**(8): 615-21.

Ghayur MA and Iqbal MQ (1992). *Census and Socio-demographic Survey of Mustafabad: A Squatter Settlement of Karachi*, Population Studies Centre, University of Karachi.

Gilliland SE, Staley TE and Bush LJ (1984). Importance of bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct. *J. Dairy. Sci.*, **67**(12): 3045-51.

Hudson LE, McDermott CD, Stewart TP, Hudson WH, Rios D, Fasken, MB, Corbett AH and Lamb TJ (2016). Characterization of the Probiotic Yeast *Saccharomyces boulardii* in the Healthy Mucosal Immune System. *PLoS One*, **11**(4): e0153351.

Kandell RL and Bernstein C (1991). Bile salt/acid induction of DNA damage in bacterial and mammalian cells: implications for colon cancer.

Kelesidis T and Pothoulakis C (2012). Efficacy and safety of the probiotic *Saccharomyces boulardii* for the prevention and therapy of gastrointestinal disorders. *Ther. Adv. Gastroenter.*, **5**(2): 111-125.

Kerry RG, JK Patra, S Gouda, Y Park, HS Shin and G Das (2018). Benefaction of probiotics for human health: A review. *J. Food Drug Anal.*, **26**(3): 927-939.

Keusch GT, Fontaine O, Bhargava A, Boschi-Pinto C, Bhutta ZA, Gotuzzo E, Rivera J, Chow J, Shahid-Salles SA and Laxminarayan R (2006). Diarrheal diseases. Disease control priorities in developing countries, 2nd Ed., pp.371-388.

Khanda OK and Zainab MALZ (2014). Isolation and Identification of *Saccharomyces cerevisiae* var *boulardii* and its Uses as a Probiotic (*in vitro*). *Rafidain J. Sci.* **25**(5): 1-11.

Klaenhammer TR and Kullen MJ (1999). Selection and design of probiotics. *Int. J. Food Microbiol.*, **50**(1-2): 45-57.

Kotowska M, Albrecht P and Szajewska H (2005). *Saccharomyces boulardii* in the prevention of antibiotic associated diarrhoea in children: A randomized double blind placebo controlled trial. *Aliment. Pharmacol. Ther.*, **21**(5): 583-590.

Miskovitz P (1999). Sleisenger & Fordtran's Gastrointestinal and Liver Disease Pathophysiology/ Diagnosis/ Management. Mosby.

Murray JM, Watson AT and Carr AM (2016). Extraction of chromosomal DNA from *Schizosaccharomyces pombe*. *Cold Spring Harb. Protoc.*, **2016**: pdb. prot 090985.

Narayanan R (2012). Evaluation of probiotic potential of stress tolerant *Saccharomyces cerevisiae* and development of economically viable media for maximum growth. *J. Food Process. Technol.*, **3**(178): 2.

Narayanan R, Reddy K and Jyothi C (2012). Evaluation of probiotic potential of stress tolerant *Saccharomyces cerevisiae* and development of economically viable media for maximum growth. *J. Food Process. Technol.*, **3**(178): 2.

- Pattani R, Palda VA, Hwang SW and Shah PS (2013). Probiotics for the prevention of antibiotic-associated diarrhea and *Clostridium difficile* infection among hospitalized patients: Systematic review and meta-analysis. *Open Med.*, **7**(2): 56.
- Patton T, Barrett J, Brennan J and Moran N (2006). Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. *J. Microbiol. Methods.* **64**(1): 84-95.
- Pennacchia C, Blaiotta G, Pepe O and Villani F (2008). Isolation of *Saccharomyces cerevisiae* strains from different food matrices and their preliminary selection for a potential use as probiotics. *J. Appl. Microbiol.*, **105**(6): 1919-28.
- Plaza-Diaz, J, FJ Ruiz-Ojeda, M Gil-Campos and A Gil (2019). Mechanisms of action of probiotics. *Adv. Nutr.* **10**(suppl 1): S49-S66.
- Psomas E, Andrighetto C, Litopoulou-Tzanetaki E, Lombardi A and Tzanetakis N (2001). Some probiotic properties of yeast isolates from infant faeces and Feta cheese. *Int. J. Food Microbiol.*, **69**(1-2): 125-33.
- Qvirist LA, De Filippo C, Strati F, Stefanini I, Sordo M, Andlid T, Felis GE, Mattarelli P and Cavalieri D (2016). Isolation, Identification and Characterization of Yeasts from Fermented Goat Milk of the Yaghnob Valley in Tajikistan. *Front. Microbiol.*, **7**: 1690.
- Rajkowska K and Kunicka-Styczyńska A (2012). Probiotic activity of *Saccharomyces cerevisiae* var. *boulardii* against human pathogens. *Food Technol. Biotech.*, **50**(2): 230-236.
- Reid G (2015). The growth potential for dairy probiotics. *Int. Dairy J.*, **49**: 16-22.
- Rowland IR, Capurso L, Collins K, Cummings J, Delzenne N, Goulet O, Guarner F, Marteau P and Meier R (2010). Current level of consensus on probiotic science-Report of an expert meeting-London, 23 November 2009: Report of an expert meeting-London, 23 November 2009. Taylor & Francis.
- Ruiz L, Margolles A and Sanchez B (2013). Bile resistance mechanisms in *Lactobacillus* and *Bifidobacterium*. *Front. Microbiol.*, **4**: 396.
- Stier H and Bischoff SC (2016). Influence of *Saccharomyces boulardii* CNCM I-745 on the gut-associated immune system. *Clin. Exp. Gastroenterol.*, **9**: 269-279.
- Sulik-Tyszka B, Snarski E, Niedźwiedzka M, Augustyniak M, Myhre TN, Kacprzyk A, Swoboda-Kopeć E, Roszkowska M, Dwilewicz-Trojaczek J and Jędrzejczak WW (2017). Experience with *Saccharomyces boulardii* probiotic in Oncohaematological patients. *Probiotics Antimicrob. Protiens.*, pp.1-6.
- Syal P and Vohra A (2013). Probiotic potential of yeasts isolated from traditional Indian fermented foods. *Int. J. Microbiol. Res.*, **5**(2): 390.
- Van Der Aa Kuhle A, Skovgaard K and Jespersen L (2005). *In vitro* screening of probiotic properties of *Saccharomyces cerevisiae* var. *boulardii* and food-borne *Saccharomyces cerevisiae* strains. *Int. J. Food Microbiol.*, **101**(1): 29-39.
- Zokaeifar H, Balcazar JL, Kamarudin MS, Sijam K, Arshad A and Saad CR (2012). Selection and identification of non-pathogenic bacteria isolated from fermented pickles with antagonistic properties against two shrimp pathogens. *J. Antibiot. (Tokyo)*, **65**(6): 289-94.