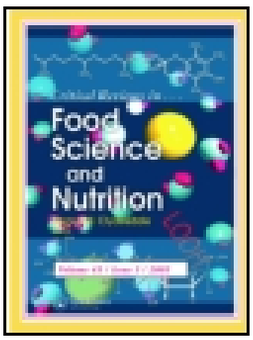

Taylor & Francis
Taylor & Francis Group

Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

Health-promoting properties of *Saccharomyces cerevisiae var. boulardii* as a probiotic; characteristics, isolation, and applications in dairy products

Fereshteh Ansari, Shohre Alian Samakkhah, Ali Bahadori, Seyedeh Maedeh Jafari, Mojtaba Ziaee, Mohammad Taghi Khodayari & Hadi Pourjafar

To cite this article: Fereshteh Ansari, Shohre Alian Samakkhah, Ali Bahadori, Seyedeh Maedeh Jafari, Mojtaba Ziaee, Mohammad Taghi Khodayari & Hadi Pourjafar (2021): Health-promoting properties of *Saccharomyces cerevisiae var. boulardii* as a probiotic; characteristics, isolation, and applications in dairy products, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2021.1949577

To link to this article: https://doi.org/10.1080/10408398.2021.1949577

Published online: 13 Jul 2021.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2021.1949577

Taylor & Francis
Taylor & Francis Group

REVIEW

Health-promoting properties of *Saccharomyces cerevisiae var. boulardii* as a probiotic; characteristics, isolation, and applications in dairy products

Fereshteh Ansari[a,b,c], Shohre Alian Samakkhah[d], Ali Bahadori[e], Seyedeh Maedeh Jafari[f], Mojtaba Ziaee[g], Mohammad Taghi Khodayari[h], and Hadi Pourjafar[i,j]

[a]Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran; [b]Research Center for Evidence-Based Medicine, Health Management and Safety Promotion Research Institute, Tabriz University of Medical Sciences, Tabriz, Iran; [c]Iranian EBM Centre: A Joanna Briggs Institute Affiliated Group; [d]Department of Food Hygiene and Quality Control, Faculty of Veterinary of Medicine, Amol University of Special Modern Technology, Amol, Iran; [e]Department of Medical Microbiology, Sarab Faculty of Medical Sciences, Sarab, Iran; [f]Department of Comparative Bioscience, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran; [g]Medicinal Plants Research Center, Maragheh University of Medical Sciences, Maragheh, Iran; [h]Department of Public Health, Maragheh University of Medical Sciences, Maragheh, Iran; [i]Alborz University of Medical Sciences, Dietary Supplements and Probiotic Research Center, Karaj, Iran; [j]Department of Food Sciences, Maragheh University of Medical Sciences, Maragheh, Iran

ABSTRACT

Saccharomyces cerevisiae var. *boulardii (S. boulardii)* has been isolated from lychee (*Litchi chinensis*), mangosteen fruit, kombucha, and dairy products like kefir. Dairy products containing *S. boulardii* have been revealed to possess potential probiotic activities owing to their ability to produce organic acids, essential enzymes, vitamins, and other important metabolites such as vanillic acid, phenyl ethyl alcohol, and erythromycin. *S. boulardii* has a wide spectrum of anti-carcinogenic, antibacterial antiviral, and antioxidant activity, and is known to reduce serum cholesterol levels. However, this yeast has mainly been prescribed for prophylaxis treatment of gastrointestinal infectious diseases, and stimulating the immune system in a number of commercially available products. The present comprehensive review article reviews the properties of *S. boulardii* related to their use in fermented dairy foods as a probiotic microorganism or starter culture. Technical aspects regarding the integration of this yeast into the dairy foods matrix its health advantages, therapeutic functions, microencapsulation, and viability in harsh conditions, and safety aspects are highlighted.

KEYWORDS

Dairy products; functional foods; probiotic; *Saccharomyces boulardii*; yeast

Introduction

Probiotics are live microorganisms that, when administered in a suitable amount, offer one or more specific health benefits on the host after consumption. The most significant health effects of probiotic microorganisms on host's health include their potential role in the reduction of different kinds of diarrheas, stimulation of the immunological system, antimutagenic and anticarcinogenic effects, and maintaining the healthiness of the urogenital tract (García et al. 2020; Hill et al. 2014; Khaneghah et al. 2020). To achieve the multiple health effects of probiotics, foods containing probiotic microorganisms ought to encompass at least 10^7 CFU/g probiotics and ingested at levels upper than 100 g/day to have supportive impacts on healthiness (Moslemi et al. 2016; Tang and Lu 2019). Some of the main difficulties in the use of probiotic microorganisms in food products; include the viability of these microorganisms in the food matrix and throughout passage in the gastrointestinal (GI) tract. Cold tension, exposure to bile, acid, osmotic, and oxidative stress may decrease the number of probiotic bacteria and lower their effective threshold (de Oliveira Ribeiro et al. 2020; Hill et al. 2014; Tripathi and Giri 2014).

Some of the most important probiotic microorganisms applied in food industries belong to the genus Bifidobacteria (e.g., *B. lactis*, *B. adolescentis*, *B. bifidum*, B. breve, *B. animalis*, *B. longum*), Lactobacilli (e.g., *L. rhamnosus*, *L. acidophilus*, *L. plantarum*, *L. casei*, *L. paracasei*), and some of Enterococcus species and yeasts (e.g., *Kluyveromyces marxianus*, and *Saccharomyces cerevisiae* var. boulardii) (Granato et al. 2010b; Nami et al. 2019; Vasiljevic and Shah 2008). It should be noted that every production of probiotic foods, must be in accordance with the guidelines of the FAO/WHO Working Group (See Figure 1).

In the last couple of years, the nonpathogenic serotypes of *Saccharomyces cerevisiae* var. boulardii (*S. boulardii*) recognized as probiotics have been prescribed by health professionals for treating various gut-related diseases. (Dendukuri et al. 2005; Moayyedi et al. 2010; Offei et al. 2019). The impacts of *S. boulardii* on bowel microflora and on enteric pathogens have been reported in various studies (Ansari et al. 2019; Ganguly, Chakraborty, and Sarkar 2019; Malviya et al. 2019; Peng et al. 2019). Controlled clinical trials have

CONTACT Hadi Pourjafar pourjafarhadi59@ut.ac.ir; pourjafarhadi59@gmail.com

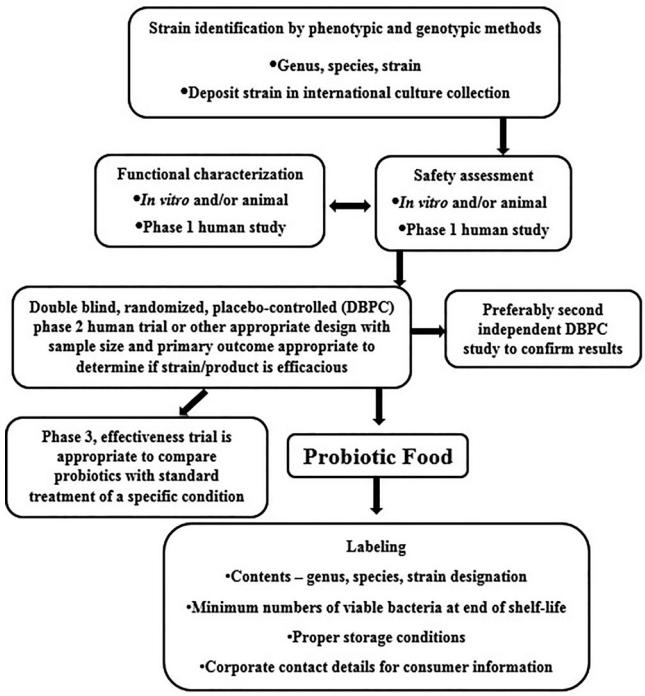

Figure 1. Guidelines for the evaluation of probiotics for food use, according to FAO/WHO Working Group. From strain identification by phenotypic and genotypic methods, safety and functional properties in animal and human studies until application in foods (Source: FAO/WHO 2002).

revealed that oral consumption of *S. boulardii* could cure or prevent GI diseases such as periodic *Clostridium difficile*-associated syndrome (Carstensen et al. 2018), Crohn's disease (Guslandi 2015), Traveler's diarrhea (Bisson et al. 2010), children acute diarrhea (Wang and Feng 2019), antibiotic-associated diarrhea (Ehrhardt et al. 2016), irritable bowel syndrome (Leventogiannis et al. 2019), and AIDS-associated diarrhea (Saint-Marc et al. 1995). According to reports, there is a close interaction between luminal flora and ileal surface enterocytes (Farache et al. 2013; Holzapfel et al. 1998; Johansson and Hansson 2016; Rozee et al. 1982).

S. boulardii has been isolated from lychee (*Litchi chinensis*), mangosteen fruit, kombucha, and from dairy products like kefir (Niamah 2017). *S. boulardii* has been applied as a probiotic (alone or in combination with other probiotic microorganisms) in dairy products manufacturing such as Kefir, kumis, cheese, yogurt, and ice-cream (Niamah 2017; Pandiyan and Kumaresan 2013). The mentioned yeast is added as an adjunct in combination with a number of other probiotic bacteria like Lactobacilli and Bifidobacteria, in these food products (Pandiyan and Kumaresan 2013; Pandiyan et al. 2012). *Saccharomyces boulardii* is a beneficial strain of yeasts for human and animal health, and a probiotic food to have helpful effects on health should contain at least 10^7 cfu/g *S. boulardii* and consumed at levels higher than 100 g/day (Tomičić et al. 2016). It is able to adhere efficiently to the gastric wall and intestinal mucosa, therefore it is highly viable in the human and animal GI tract (Lazo-Vélez et al. 2018). The current comprehensive review article, in addition to addressing the health benefits of *S. boulardii*, also summarizes its health aspects, and application in dairy products.

Figure 2. Scanning electron image of *S. boulardii* Biocodex in cell culture of the human epithelium. (Source: (McFarland 2017).

Microbiological properties of Saccharomyces boulardii

Cell morphology

Saccharomyces boulardii is an oval or spherical cell with 2–3 μm width and 2.5–10.5 μm length (Niamah 2017), and it has a thicker cell wall than other yeasts (Lazo-Vélez et al. 2018). This yeast reproduces sexually and non-sexually by cell budding and conjugation, respectively (Niamah 2017). Initially, *S. boulardii* was considered a distinct species of the hemiascomycete genus *Saccharomyces* (Edwards-Ingram et al. 2007). Edwards-Ingram et al. (2007) concluded that *S. cerevisiae* and *S. boulardii* belong to one species. Following taxonomic investigations on *S. boulardii* revealed that the yeast should be considered as a strain of *S. cerevisiae*. Consequently, it should be referred to as *Saccharomyces cerevisiae* var. boulardii (Tomičić et al. 2016). (See Figures 2 and 3)

Biochemical characteristics

Sugars are an important nutrient source of carbon for most probiotic microorganisms. Yeasts especially *S. cerevisiae* are the most desired organisms for ethanol production due to their diverse substrate specificity and ease of making ethanol under anaerobic conditions (See Figure 4). Moreover, they have high ethanol tolerance (Azhar et al. 2017; Du et al. 2012; Fakruddin et al. 2013) (See Table 1). Fakruddin, Hossain, and Ahmed (2017) published a paper on a strain of S. *cerevisiae* -IFST062013- isolated from the fruit. This strain was considered as a probiotic with high tolerance to GI secretions such as concentrate bile salt, and gastric acids. It also was able to produce antioxidants agents (glutathione, siderophore), vitamin B12, organic acids, killer toxin, and to produce a strong biofilm. It is also a cholesterol absorber with the ability of auto-aggregation and a hydrophobic cell surface. Although the isolated yeast induces intestinal enzyme production such as cellulose, amylase, lipase, and protease, it is unable to generate DNAase, galactosidase, and gelatinase. In fact, it is an antimicrobial agent against bacteria and fungi. Its lysate has more antimicrobial potential compared to the whole-cell and culture supernatant (Fakruddin, Hossain, and Ahmed 2017). The examination of

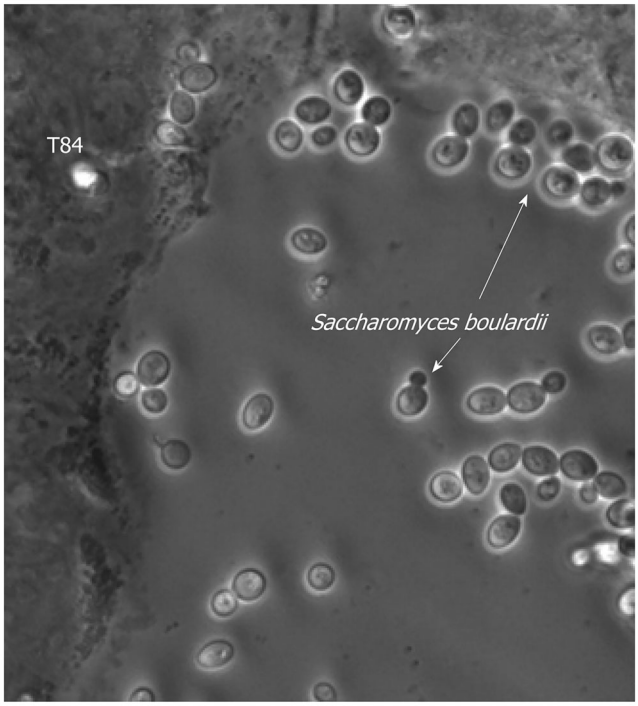

Figure 3. Transmission microscopy image displaying *S. boulardii* CNCM I-745 on a culture of human epithelial cells (T84 lineages). The arrows show budding yeast positioned either in the places between the cells or close the cell walls and the foundation of a protecting hurdle (Source: (Czerucka and Rampal 2019)).

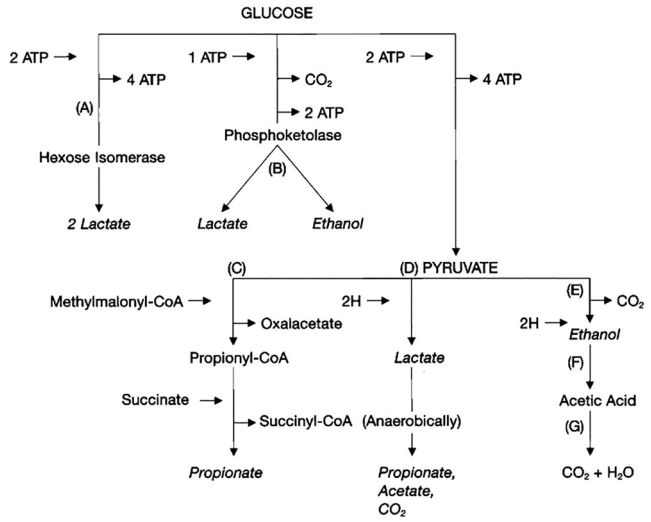

Figure 4. Comprehensive pathways for the production of some fermentation products from glucose via several probiotic microorganisms. (A) Homofermentative lactics; (B) Heterofermentative lactics; (C) and (D) Propionibacterium; (E) *Saccharomyces* spp.; (F) Acetobacter spp.; and (G) Acetobacter "over oxidizers." (Source: Jay, Loessner, and Golden 2008). In the complete process of glucose metabolism by *Saccharomyces* (E), glucose is first broken down into pyruvate. Then, due to the decomposition of pyruvate, ethanol and carbon dioxide are produced, then by the decomposition of ethanol, acetic acid is produced, and finally, by the decomposition of acetic acid, the final product of carbon dioxide and water is produced.

biochemical properties is one of the most important approaches for the diagnosis and identification of *S. boulardii* and other probiotic microorganisms (See Table 2).

Genetic characteristics

Due to the larger size of the yeast genome (in comparison to bacteria), 5S rRNA sequence explorations are used more than larger RNA fractions (Kreger-van Rij 2013). Although no significant study has been done on the structure of the *S. boulardii* genome-somewhat because of the fact that it is closely matching to that of *S. cerevisiae*- *S. cerevisiae* was the leading eukaryotic genome to be entirely sequenced. The achievement of the genome is a result of a global collaboration (Goffeau et al. 1996; Naumova et al. 2013). This sequence contains 13000000 base pairs, 6275 genes, 5885 protein-encoding genes (Drozdova et al. 2016; Goffeau et al. 1996). Roughly 140 genes are definitely for specifying rRNA, 275 functions as transfer RNA genes, and 40 genes are for small nuclear RNA molecules (Drozdova et al. 2016; Goffeau et al. 1996). It has been detected that there is a substantial amount of redundancy. Approximately 23% of the genome is matching to the hominid genome. Chromosomes have a single linear binary stranded DNA (Offei et al. 2019; Goffeau et al. 1996).

S. boulardii belongs to the *S. cerevisiae* strain, but it has some differences in its genome, including 1) chromosome IX trisomy, 2) some of its genes have different copy numbers. However, *S. boulardii* has strain-specific differences with *S. cerevisiae*, it has recently been considered as a member of the *S. cerevisiae* strain (Edwards-Ingram et al. 2007; Mousa, Wang, and Zhang 2019). *S. boulardii* has a distinguishing characteristic from *S. cerevisiae* that is the absence of Ty1, Ty3, Ty4 elements, and relevant LTR. In contrast, *S. boulardii* has Ty2 and Ty5 elements. All strains of *S. boulardii* lack hexose transporters HXT9, HXT11, and asparagine-utilization genes. Furthermore, identified *S. boulardii* compared to *S. cerevisiae* flocculin genes has different copy quantity of repeats and repeat periods. Different adhesion abilities are recognized to be responsible for this dissimilarity (Khatri et al. 2017). Another study demonstrated some other distinguishing characteristics of the *S. boulardii* genome in association with the number of genes copy in its subtelomeric zone and Ty elements (Edwards-Ingram et al. 2007).

Various kinds of literature have been published on genetic engineering as well as creating and specifying auxotroph mutants of *S. boulardii*. This auxotroph strain can be used as recombinant pharmaceutics to treat GI disorders (Al-Jailawi, Al-Shekdhaher, and Al-Zaiadi 2016; Hamedi et al. 2013; Liu et al. 2016). For example, the researchers have demonstrated that *S. boulardii* can produce and secrete human lysozyme, which could be applied to improve human and animal GI lumen health (Liu et al. 2016).

Sensitivity and resistance to ecological elements (intrinsic and extrinsic factors)

The selection of probiotics is based on important criteria such as their proliferation and survival in the intestinal lumen. Probiotic yeasts compared to probiotic bacteria have a higher surviving capacity in bile, gastric juice, digestive enzymes such as pepsin, and pancreatic enzymes (Chanthala and Appaiah 2014). Chanthala and Appaiah (2014) studied

Table 1. The high ethanol tolerance of *S. boulardii* (Source: Du et al. 2012).

Ethanol %	0	8	12	16	18	20	22	24
12 h	3+	3+	2+	1+	–	–	–	–
18h	3+	3+	3+	2+	1+	–	–	–
24h	3+	3+	3+	2+	2+	2+	–	–
48h	3+	3+	3+	3+	2+	2+	–	–
60h	3+	3+	3+	3+	3+	2+	–	–

3+: volume = full small tube; 2+: volume = 2/3 of small tube; 1+: volume = 1/3 of small tube; -: no gas

Table 2. The consequences of diagnostic tests applied for identification of *S. boulardii* (Source: Khidhr and Zubaidy 2014).

	Tests	Consequences
Application of carbon	Glucose	+
	Fructose	+
	Sucrose	+
	Galactose	+/−
	Raffinose	+
	Lactose	−
	Starch	+
Application of nitrogen	Peptone	+
	Aspargine	+
	Ammonium sulfate	+
	Nitrate	−
Acid production		+
Urea hydrolysis		−
Ester production		+
Cyclohexamide Resistance		−

the probiotic properties of yeasts (In vitro) and demonstrated that *S. boulardii* is highly resistant to digestive enzymes, and they declared that this probiotic yeast restricts the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. They also demonstrated that *S. boulardii* forms an inhibition zone in the culture medium against *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli*. The results showed that the reason for such antibacterial properties against these pathogens was the production of various organic acids such as capric acid, caprylic acid, and caproic acid by *S. boulardii*. (Chanthala and Appaiah 2014). Similarly, *S. boulardii* is able to tolerate ethanol with the maximum concentration resistance level of 20% (Du et al. 2012). *S. boulardii* is also naturally resistant to antibiotics (Czerucka, Piche, and Rampal 2007). After a brief introduction, we will discuss the intrinsic and extrinsic factors affecting the growth and activity of this yeast in the following items: (See Figure 5)

Temperature changes

S. boulardii is one of the few probiotics that have a great performance at human body temperature (37 °C). The optimal temperature for surviving most strains of *Saccharomyces* is from 22 to 30 °C. Other strains of *S. cerevisiae* prefer lower temperatures (Hossain et al. 2020; Paula et al. 2019). *S. boulardii* as a probiotic is available in capsules with two types, including heat-dried or lyophilized preparations. The heat-dried capsules lose their potency rapidly after opening, thus they are not able to survive at room temperature and must be kept in a refrigerator (4 °C). Lyophilized *S. boulardii* is stable at room temperature (25 °C) for one year (McFarland 2010). In Du et al. (2012) study, the heat stress test for *S. boulardii* at 28, 37, and 42 °C showed that (at

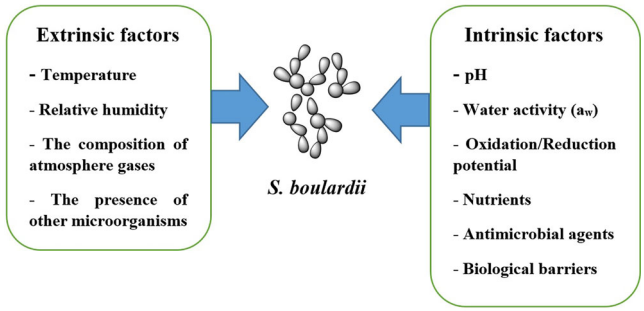

Figure 5. Intrinsic and extrinsic factors affecting the growth and activity of *S. boulardii*.

24 hours) 37 °C is the best temperature for the growth of this yeast, however, the growth rate decreases after 48 hours. The results demonstrated that a temperature between 55–56 °C is the thermal death point for *S. boulardii* (Du et al. 2012).

pH and acidity

S. boulardii is resistant to low pH and bile acid, however, other strains of *Saccharomyces* can't tolerate such harsh conditions (McFarland, 2010). It has been shown that *S. boulardii* can grow well at a pH 2 (Du et al. 2012). Another study on six strains of yeasts (including *S. cerevisiae* AAV2 (GenBank accession number KF551990) isolated from wine. *S. cerevisiae* 101. Non-Saccharomyces forms like *Pichia kudriavzevii* P1 (KC841145), *P. kudriavzevii* P2 (KC841146) and *P. kudriavzevii* P3 (KC841147) were isolated from coffee pulp effluent of pH 4.0–4.6. *Issatchenkia orientalis* CL1132 (KF551992) was isolated from fruit, and *S. boulardii* (as reference strain: *S. boulardii* NCDC 363 was procured from NCDC, Karnal, India) showed that acidic and alkaline tolerance of these strains (range from 1.5 up to 9) at 4, 24, and 48 hours of the incubation period are different from each other and non-*Saccharomyces* strains exhibited higher acidic and alkaline environment tolerance in comparison to *S. boulardii* (Chanthala and Appaiah 2014). The study of *S. boulardii* survivability in simulated gastric (pH 1.1) and intestinal (pH 6.8) conditions showed that the viability rate of yeasts in the freeze-dried formula is higher than liquid suspension form (Graff et al. 2008).

Water activity (a_w) and relative humidity (RH)

Water activity could affect the vitality and viability of *S. boulardii*. One study, investigating the viability of *S. boulardii* after freezing/thawing in different water activity (a_w) conditions showed that water activity (a_w) 0.98 (in comparion to 0.98, 0.97, 0.96, 0.95, 0.64, 0.93, 0.92, 0.91, and 0.90 water activity values) in media raised the survival to the freezing of *S. boulardii* cells stored at −20 °C for 2 months. Lower a_w conditions had a negative effect on yeast cell viability (Pardo, Galvagno, and Cerrutti 2009). In unpackaged foods, the amount of a_w can be affected by the relative humidity (RH) of the environment. In dry environments with low RH, the amount of a_w also decreases, and as a result, the survival rate of yeasts decreases.

Oxidation-reduction potential

Oxidation-reduction (O/R) potential or REDOX is measured in electron units of millivolts nominated as Eh. In Rattray, Schibeci, and Kidby (1975) study, in connection with the impact of Eh on lipid production by *Saccharomyces cerevisiae*, it has been revealed that anaerobically grown yeasts produce a minor total level, a greatly variable glyceride fraction, and reduced sterol and phospholipid components in comparison with aerobically grown yeasts. The lipid produced via anaerobically grown yeasts was described by a great content (50 per cent of total acid) of 8:0 to 14:0 acids and a small range of unsaturated fatty acid in the phospholipid fraction. In the case of aerobically grown yeasts, 80–90 per cent of the fatty acid component was related to glyceride, and the phospholipid was founded to be 16:1 and 18:1 acid. Dissimilar in the case of aerobically grown yeasts, anaerobically grown *Saccharomyces* was founded to have a sterol and lipid necessity (Rattray, Schibeci, and Kidby 1975). It seems that the growth and metabolic activities of *S. boulardii* are better and more in aerobic conditions, in other words in and around foods with positive Eh (Eh+).

Antimicrobial agents

Antibacterial activity of *S. boulardii* is due to the following mechanisms: (1) production of extracellular protease, discharge of enzymatic proteins, the stimulus of immunoglobulin A, removal of secreted toxins, sulfur dioxide, killer toxins, etc. (Fakruddin, Hossain, and Ahmed 2017) and (2) auto-aggregation ability and cell surface hydrophobicity (Fakruddin, Hossain, and Ahmed 2017; Syal and Vohra 2013).

S. boulardii increases the generation of enzymes through releasing polyamines that stimulate the amends of intestinal cells and the synthesis of the colonic mucosae (Tiencheu et al. 2016). *S. boulardii* proteases neutralize Toxin A and Toxin B of *Clostridium difficile*, which are primarily responsible for diarrhea and colitis (Castagliuolo et al. 1999). Auto-aggregation and cell surface hydrophobicity are very crucial characteristics of a probiotic that is involved in the adhesion of the probiotics to intestinal epithelial cells of patients. By occupying the binding sites on enterocytes by probiotics, other pathogenic bacteria are eliminated from the binding competition and excreted in the feces (Fakruddin, Hossain, and Ahmed 2017).

Antibacterial activity of *S. boulardii* reported against various enteric pathogens such as *Vibrio cholerae*, *Clostridium difficile*, *Salmonella*, *E. coli*, and *Shigella*. In a study, researchers showed that *S. boulardii* is significantly able to reduce the number of *Listeria monocytogenes*, *Staphylococcus aureus*, *Pseudomonas aerugenosa* (Syal and Vohra 2013). Similarly, another study reported that *S. boulardii* has antagonistic activity against enteric pathogens such as *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *E. coli*, and *Enterococcus faecalis* (Rajkowska, Kunicka-Styczyńska, and Rygala 2012). There are extensive reports on the antibacterial properties of *S. boulardii* against various types of pathogens, and only a few were mentioned here (more studies are mentioned in section "Health advantages of *Saccharomyces boulardii* as probiotic"). Besides the antibacterial properties, another thing that is important is that *S. boulardii* is not affected or inhibited by antibacterial drugs and does not change the normal microbiota of the GI tract. Hence, *S. boulardii* can be administered as a probiotic supplement to people with antibiotic-associated diarrhea (Tomičić et al. 2016).

Nutrient source of media for S. boulardii

Most yeasts grow well on several media including Sabouraud Dextrose Agar (SDA), Minimal Medium, YEPD (Yeast Extract Peptone Dextrose), and Rose Bengal Medium (Salimi and Mahzonieh 2015).YEPD contains 1% (w/v) yeast extract, 2% (w/v) peptone, and 2% (w/v) glucose or dextrose in distilled water and it is the best culture medium for yeast growth (Datta, Timson, and Annapure 2017). One of the important criteria for growth in yeasts is the presence of carbon, nitrogen, and inorganic ions in the composition of culture media. Glucose, sucrose, starch, fructose, maltose, etc. are the carbon source and peptone, and yeast extract powder, urea, $(NH4)_2SO4$, KNO3, etc. are the nitrogen source of culture media for yeasts. Furthermore, macro and microelements such as Phosphorus, Potassium, Sulfur, Zinc, Copper, Manganese, and so on are also critical for the growth of the yeast (Lei et al. 2016). It has been shown that the volume of a medium on the biomass of *S. boulardii* can affect it's growth. The optimum medium volume was 50 ml. Inoculum size may also have an effect on the growth of *S. boulardii*. When the inoculum size of *S. boulardii* was 8%, the maximum biomass occurred. The effect of adding molasses and animal serum to provide a cheap medium for *S. boulardii* culture has been investigated. Molasses contains sugar and the serum of animals' blood contains protein between 6.74–7.46 g/dl. The highest growth rate of yeast and production of most biomass was done in medium containing 10% molasses and 1% serum. (Salimi and Mahzonieh 2015).

Interactions between S. boulardii and other microorganisms

There are interactions between yeasts and bacteria. These interactions are due to competition for nutrients and the production of metabolites in media. The production of metabolites, for instance, lactic and acetic acids via Lactic acid bacteria (LAB) inhibits the extreme specific growth proportion and ethanol production of *S. cerevisiae*. on the other hand, it has been shown that the existence of yeast has a positive effect on the maximum specific growth rate of *L. sanfranciscensis* (Stadie et al. 2013).

Da Silva et al. (2015) displayed an actual biological control of probiotic strains of *S. boulardii* (CNCM-17) as they significantly decreased spore creation of *Aspergillus parasiticus* in peanuts. It was described that all probiotics were able to inhibit the sporulation of the molds, gaining better consequences with active cells. The existence of probiotics changed the color of the *A. parasiticus* colony but not the spore morphology. An important drop in the aflatoxin

production was found with the existence of yeast strains, but better results were obtained when *S. boulardii* and *L. delbrueckii* acted together. Probiotics remained viable in peanut grains even after 300 days of storage time (Silva et al. 2015).

In another study, Heling et al. (2017) analyzed the probable use of *S. boulardii* in the biological control of *Colletotrichum musae* in organic bananas. Bananas previously sanitized and treated with cells of *S. cerevisiae* or *S. boulardii* were inoculated with *C. musae* at three spots per fruit. Treatments with *S. cerevisiae* or *S. boulardii* reduced 48 and 35% of the incidence of anthracnose disease (anthracnose is caused via fungi in the genus *Colletotrichum*, a common group of plant pathogens that are responsible for diseases on many plant species. Infected plants develop dark, water soaked lesions on stems, leaves or fruit) at concentrations of 5.5 and 6.3 g L^{-1}, respectively. The authors concluded that these yeasts are potential agents for the biological control of this relevant pathogen (Heling et al. 2017).

Health advantages of Saccharomyces boulardii as probiotic

As a probiotic *S. boulardii* is able to improve the health position of the host through diverse mechanisms such as its beneficial effects on the immune system of the host and direct effects on pathogens. We will broadly discuss these effects in the following section.

Anti-carcinogenic effects

The potential effects of *S. boulardii* to prevent and treat neoplasm have been investigated in limited experimental studies with controversial results. For instance, the results obtained from a study on the effects of *S. boulardii* on the growth of dimethylhydrazine-induced preneoplastic lesions in the mice colon did not show any significant differences between the control and treatment group (Liboredo et al. 2013). Another study assessing the effects of the administration of oral cell wall extracts of *S. boulardii* on the colon cancer of rats treated with dimethylhydrazine concluded that the glucan part of the extract has an anti-cancer effect while the mannoprotein extract of the cell wall was related to more aberrant crypt foci/ACF/(are thought to be the earliest identifiable neoplastic lesions in the colon carcinogenetic model) in the colon (Fortin et al. 2018b).

On the other hand, the results of some studies are promising. Chen et al. (2009) showed that the EGF receptor is deactivated by exposure to *S. boulardii* which leads to inactivation of both the EGFR-Erk and EGFR-Akt pathways (Chen et al. 2009). They claimed that *S. boulardii* prevents EGF-induced proliferation, and reduces cell colony formation, and promotes apoptosis. They also founded that HER-2, HER-3, and IGF-1R were inactivated by *S. boulardii* and oral intake of *S. boulardii* reduced intestinal tumor growth and dysplasia in an animal model. They concluded that *S. boulardii* inhibited EGFR and other receptor tyrosine kinase signaling suggesting it as a novel therapeutic or prophylactic agent for intestinal neoplasia. It also has been shown that the anti-inflammatory effects of *S. boulardii* may be beneficial for oncologic patients to prevent adverse effect of radiotherapy and/or chemotherapy like mucositis (Picó-Monllor and Mingot-Ascencao 2019).

Immune system modulation

The beneficial effects of *S. boulardii* on the immune responses of the host against enteric pathogens have been thoroughly studied. It has been shown that *S. boulardii* stimulates the release of immunoglobulins and various cytokines and also has an impact on the maturation of immune cells. *S. boulardii* has also shown to be able to activate an unspecific immune system. The medical yeast also binds to the enteric pathogenic bacteria and neutralizes the toxins (Stier and Bischoff 2017). In a clinical trial on the children with acute gastroenteritis, a significant increase in serum immunoglobulin A and decreases in C-reactive protein levels were detected in the group receiving *S. boulardii* and in this group, the percentage of CD8 lymphocytes on day 7 was significantly higher compared to the control group (Lee, Kim, and Chi 2010). Another clinical trial studied the effects of a probiotic compound containing *S. boulardii* and *Bifidobacterium* in children with acute diarrhea. The results of the study demonstrated an improvement in clinical endpoints as well as an enhancement in the cellular immune function of the treatment group. CD3+, CD4+, and CD4+/CD8+ increased in the experimental group and Th1/Th2 ratio was significantly higher than the control group (Wang and Feng 2019). The effect of *S. boulardii* on immune responses in the healthy intestine was proven to be limited (Hudson et al. 2016).

The impacts of *S. boulardii* on the immune system function in other organs have not been well studied, however, there are promising results. For instance, it has been shown that the combination of *S. boulardii* and cetirizine hydrochloride has obvious clinical efficacy for the treatment of children with allergic rhinitis. Correction of interferon IFN-γ and IL-4 activation may be the leading mechanism of this observation (Fang et al. 2017).

The application of *S. boulardii* for the improvement of immune responses against parasitic infections has also been investigated. It was demonstrated that it can decrease *Schistosoma mansoni* worm burden in mice and increase both IgM and IgG against antigenic preparations from parasite developmental stages including soluble cercarial antigenic preparation, parasite soluble adult worm antigenic preparation, and soluble egg antigen (Bahgat et al. 2005).

Additionally, *S. boulardii* modulates the innate and adaptive immune responses by affecting the signaling cascades leading to attenuation of the inflammatory response induced by the infection. The mechanisms weaken the adhesion potential of enteric pathogens to the enteric epithelial layer and reduce the fluid loss of diarrhea (Stier and Bischoff 2017). *S. boulardii* downregulates several inflammatory and immune genes (Lee, Kim, and Chi 2010) and decreases the secretion of pro-inflammatory cytokines such as interleukin (IL)-1beta by intraepithelial lymphocytes and increase the

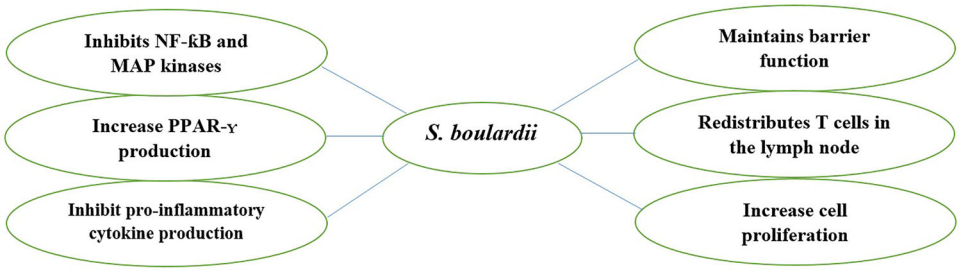

Figure 6. The chart shows the possible mechanism of action of *S. boulardii* in host immune modulation. *S. boulardii* increases the integrity of barrier function, rises epithelial cell proliferation, inhibits pro-inflammatory cytokine production including IL-8 via inhibition of the MAP kinases ErK 1/2 and p38 as well as the nF-κB, enhances anti-inflammatory PPAR-γ production and redistribution of T cells in mesenteric lymph nodes (Source: Im and Pothoulakis 2010).

level of anti-inflammatory cytokine-like IL-4 and IL-10. The anti-inflammatory effect of *S. boulardii* may be beneficial for the patient with inflammatory bowel disease (IBD). It has been demonstrated that *S. boulardii* decreases the frequency of CD40-, CD80-, and CD197 (CCR7; chemokine receptor-7)-expressing IBD mDC and reduces their secretion of tumor necrosis factor (TNF)-α and interleukin (IL)-6 and increases IL-8. It also inhibits T cell proliferation induced by IBD mDC and T(H)1 (TNF-α and interferon-γ) polarization induced by UC mDC and activates IL-8 and transforming growth factor-β-dependent mucosal healing (Fidan et al. 2009).

The modulatory effect of the yeast on immune responses is also very crucial in the case of antibiotics and chemotherapy drug administration. The antibiotic treatment enhances the phagocytic activity of dendritic cells leading to the destruction of intestinal microbiota and its homeostasis. It has been demonstrated that after antibiotic administration DCs are functionally activated (MHC class II antigen and CD 86 costimulatory molecule membrane expression are upregulated) and *S. boulardii* downregulates the membrane antigen expression of the cells. These mechanisms accelerate the restoration of the balance of intestinal microbiota and maintenance of intestinal homeostasis (Collignon, Sandre, and Barc 2010). Chemotherapy drugs that act via Toll-like receptors (TLRs) can worsen the mucosal injury by the production of cytokines. TLR2 and TLR4 activation lead to the release of NF-κB which initiates the inflammatory responses. *S. boulardii* is able to modulate the expressions of TLR2, TLR4, MyD88, NF-κB, ERK1/2, phospho-p38, phospho-JNK, TNF-α, IL-1β, and CXCL-1 in the jejunum and ileum cells following treatment with chemotherapy drugs (Justino et al. 2020).

The modulatory effect of *S. boulardii* on the immune system was an incentive to develop more efficient vaccines. *S. boulardii* has been examined in combination with *Bacillus toyonensis* in order to improve the efficacy of Bovine Herpesvirus 5 (BoHV-5) in sheep. It was shown that probiotics significantly increase seroconversions against BoHV-5, and neutralizing antibodies titers to BoHV-5. Splenocytes from the supplemented group had higher mRNA transcription levels of cytokines IL-10 and IL-17A (Roos et al. 2018). The effects of *S. boulardii* on the efficacy of DNA vaccines coding for the leptospiral protein fragments LigAni and LigBrep have also been examined. The results have shown a significant increase in the expression of the IL-10 in mice fed with *S. boulardii* (Silveira et al. 2017). (See Figure 6)

Antibacterial and antiviral properties

The effect of *S. boulardii* on intestinal microorganisms has been thoroughly investigated. Three main types of mechanisms have been suggested for antibacterial/antiviral properties of *S. boulardii* in the intestine. (1) a direct effect on enteropathogenic microorganisms or their virulence factors, (2) indirect effects by influencing the intestinal microbiota, and (3) effects on the host including trophic effects on the intestinal mucosa and immunomodulatory effects (Czerucka and Rampal 2019). The beneficial effects of a proper microbiota have been already discussed and in the previous section, we explained the immune-modulatory effects of *S. boulardii*. In the present section, the direct effects of the yeast on bacterial and viral will be considered.

Beneficial effects of S. *boulardii* against infections by various pathogenic bacteria such as *Vibrio cholera, C. Difficile, Bacillus Anthracis*, *Salmonella*, *Shigella*, *E. coli, Helicobacter pylori,* and viruses (Rotavirus) have been investigated (Czerucka et al. 2019; Czerucka and Rampal 2019; Das, Gupta, and Das 2016; Kelly et al. 2019; Namkin, Zardast, and Basirinejad 2016; Piatek et al. 2019). *S. boulardii* acts against the toxin of *vibrio Cholera* via binding the toxin to its cell wall. *S. boulardii* also produces a protein that inhibits the cAMP-induced secretion of chloride (Cl-) triggered by cholera toxin and leads to a significant reduction of the fluid and sodium secretion. These effects have been confirmed in models of intestinal epithelial cells and in vivo (Czerucka and Rampal 2019).

The mechanisms of actions of *S. boulardii* with respect to *C. difficile* infection have been well studied. The main suggested mechanisms are direct action on toxin A and B of *C. difficile* and their receptors. It has been demonstrated that a 54 KDa protease identified in the culture supernatant of *S. boulardii* degrade *C. difficile* toxins A and B and their receptors. Furthermore, *S. boulardii* inhibits pro-inflammatory pathways that are activated by toxins A and B. It has been shown that *S. boulardii* inhibits interleukin 8 (IL-8) synthesis as well as nuclear translocation of NF-κB and inhibits phosphorylation of ERK1/2 and JNK in epithelial cells (Czerucka and Rampal 2019).

Bacillus anthracis the agent of anthrax produces toxins containing three peptides; the protective antigen (PA), lethal

factor (LF), and the edematogenic factor (EF). The lethal toxin (LT) formed by the combination of PA with LF affects actin filaments in endothelial and epithelial cells and activates morphological changes leading to the opening of tight junctions. It has been shown that incubating cells with *S. boulardii* prior to exposure to LT conserves the structure of the actin fibers. The yeast acts directly on the LT subunits and also delays the intracellular pathways leading to morphological changes of the host cells (Czerucka and Rampal 2019).

S. boulardii is able to attenuate the symptoms caused by Enteropathogenic *E. coli* and Enterohaemorrhagic *E. coli* via different mechanisms. It inhibits the inflammatory pathways leading to the synthesis of IL8 and consequently decreases the cell permeability caused by the bacteria. Additionally, exposure to the yeast helps maintenance of tight junctions' structures in the infected cells. *S. boulardii* also acts directly on the expression of the virulence factors as well as lipopolysaccharide (LPS) of *E. coli* (Czerucka and Rampal 2019).

The beneficial effects of *S. boulardii* in the case of *Salmonella enterica* Typhimurium and *Shigella flexneri* have been investigated in various studies. It has been shown that *S. boulardii* reduces the severity and mortality rate caused by the infection (Czerucka and Rampal 2019; Khidhr and Zubaidy 2014; McFarland 2010). The maintenance of tight junctions and the anti-inflammatory effects of *S. boulardii* have been shown in both *Salmonella* and *Shigella* infections. Several mechanisms have been suggested for the treatment effects of the yeast in the case of *S. Typhimurium* (Czerucka and Rampal 2019; McFarland 2010). *S. boulardii* decreases invasion to enterocytes by reducing the activation of the Rac pathway (the pathway used for the invasion of bacteria). The yeast also modifies the motility of the bacteria and consequently reduces their invasiveness (two of the major mechanisms of *Salmonella* pathogenesis are motility ability and have an invading gene on a bacterial chromosome). Furthermore, *S. boulardii* adheres to the bacteria and decreases its propagation. The medical yeast also accelerates the excretion of bacteria in the stool (Czerucka and Rampal 2019).

Helicobacter pylori (H. pylori), the bacterium responsible for peptidic ulcer disease, has also been considered for the anti-bacterial effects of *S. boulardii*. The yeast prevents the binding of *H. pylori* on duodenal cells while bacterial probiotics do not show this kind of effect. In vivo studies have shown that *S. boulardii* decreases the *Helicobacter* load, and reduces expression levels of inflammatory cytokines and chemokines in the stomach. It also increases the production of anti-helicobacter-specific IgA and sIgA in the small intestine following infection (Czerucka and Rampal 2019). The anti-bacterial effects of *S. boulardii* have also been demonstrated against other pathogens such as *Listeria monocytogenes, Pseudomonas aeruginosa, and Staphylococcus aureus* (Rajkowska, Kunicka-Styczyńska, and Rygala 2012).

Rotavirus is the most common viral cause of gastroenteritis. *S. boulardii* has shown anti-viral effects in the case of Rotavirus infection by reducing oxidative stress in infected cells and consequently decreasing the chloride secretion induced by the virus (Czerucka and Rampal 2019).

Antioxidant activity

The antioxidant properties of *S. boulardii* have been widely investigated. *S. boulardii* isolated from the spontaneous fermentation of guajillo pepper, exhibited a cholesterol reduction capacity of 66.1% after 48 hours incubation. The antioxidant activity of *S. boulardii* according to the DPPHI (1, 1-diphenyl-2-picryl-hydrazyl free radical) method was 63% with Trolox equivalent (μM) of 174.54 (Lara-Hidalgo et al. 2019). According to another study aiming to analyze the effect of integrating *S. boulardii* for production of beers with increased healthy benefits the DPPH (DPPH/2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol) scavenging activity of the yeast was 2.3 milligrams of Trolox equivalents (TE) per liter of beer (mgTE/L). It was shown that *S. boulardii* is viable during beer processing and increases the antioxidant activity of the produced beer (Capece et al. 2018). *S. boulardii* isolated from fermented foods such as Brazilian indigenous fermented food, cocoa fermentation, and kefir showed DPPH scavenging of 40% (Menezes et al. 2019). Despite the evidence for antioxidant activity of *S. boulardii*, the result of a study on Cell-Free Supernatants (CFS) revealed that there is no significant difference between DPPH scavenging capacity of *S. boulardii* CFS and control medium (*without S. boulardii*) (De Marco et al. 2018).

It has been demonstrated that intact cells had higher antioxidant capacity than the extract, which mainly may be due to the high level of (1/3)-β-D-glucan and other β-glucans found in their cell wall in comparison with other cellular compounds (Chen et al. 2010). The insoluble glucan from *S. boulardii* is shown to have high antioxidant properties with increasing quinone reductase (QR) activity (Fortin et al. 2018a). Metabolites of *S. boulardii* such as a variety of metabolites e.g. vanillic acid, cinnamic acid, phenyl ethyl alcohol, erythromycin, and pyridoxine (vitamin B6) have antioxidant properties and are likely to contribute to the previously reported antioxidant effect of the yeast (Datta, Timson, and Annapure 2017).

In Suryavanshi et al. (2013) study, crude extract and diverse solvent extracts (dichloromethane, ethyl acetate, n-butanol) of *S. boulardii*'s growing were assessed for the antioxidant activity via superoxide radical scavenging (nitroblue tetrazolium; NBT) test, free radical scavenging DPPH test, and via assessing total flavonoid and phenolic contents (TFC and TPC). In their study, the antioxidative activity of *S. boulardii* extract was similarly assessed in a biological system that is A549 lung cancer cell line by means of dichlorofluorescein diacetate (DCF-DA) test. Comparative investigations on antiradical capability (NBT, DPPH) discovered that crude extract has the maximum activity than its other solvents via its greater TFC and TPC and minor IC50 values. In vitro assessments revealed that *S. boulardii* extracts efficiently reduce intracellular reactive oxygen types. Vanillin was recognized as one of the antioxidants, and it

was found that *S. boulardii* extract is an effective scavenger of free radicals which can be used as a possible novel resource of natural antioxidants (Suryavanshi et al. 2013).

Regarding the antioxidant activity of the yeast, it has been studied to be used for clinical indications. *S. boulardii* has been recommended as an adjunct for chemotherapy regimens in patients with neoplasms. It has been shown to have positive effects on cancerous cells and it also attenuates adverse effects of chemotherapy medications. Inhibition effects of *S. boulardii* on the proliferation of colorectal cancerous cell lines have been reported by Fortin et al. (2018a). It was claimed to be associated with the antioxidant properties of the yeast (Fortin et al. 2018a). Experiments on an animal model indicated that *S. boulardii* supports the antioxidant mechanisms of intestinal and hepatic tissues against clarithromycin- and methotrexate-induced oxidative stress (Duman et al. 2013). Decreasing the ratio between reduced (GSH) and oxidized (GSSG) glutathione is from the pathogenesis mechanisms of Rotavirus, in other words, there is a link between oxidative stress and RV-induced diarrhea. It was demonstrated that *S. boulardii* supernatant prevents oxidative stress in RV infected cells in an organ culture model using human intestinal biopsies (Buccigrossi et al. 2014).

Serum cholesterol reduction

S. boulardii as a probiotic yeast has the ability to enhance the lipidemic profile of the host. The results of the investigations on hypercholesterolemia induced by a cholesterol-enriched diet in animal models are promising. It has been demonstrated that administration of *S. boulardii* significantly reduces the total plasma level of cholesterol and increases fecal total cholesterol compared to the control group in hamsters (Briand et al. 2019; Girard, Pansart, and Verleye 2014). The Daily intake of the yeast has both preventive and curative effects on hypercholesterolemia (Girard, Pansart, and Verleye 2014). It also reduces serum triglyceride increased by a cholesterol-enriched diet (Girard, Pansart, and Verleye 2014). Similar results have been obtained from the rat animal model which shows beneficial effects of *S. boulardii* administration on levels of triglycerides, cholesterol, free blood sugar, and liver enzymes (Ghorbani-Choboghlo et al. 2019).

The administration of *S. boulardii* for hypercholesterolemia in human subjects has also been examined. According to the obtained results, the probiotic decreases Remnant lipoprotein particles without any significant effects on other lipidemic indexes such as Fasting concentrations of total cholesterol, low-density lipoprotein-cholesterol [LDL-C], high-density lipoprotein-cholesterol [HDL-C], triglycerides, lipoprotein particles (very-low-density lipoprotein-particle [VLDL-P], total LDL-P, LDLIII-P, LDLIV-P, total HDLP, and HDL 2b-P (Ryan et al. 2015). It should be noticed that the administration of *S. boulardii* for human hypercholesterolemia needs further evidence on human subjects and potential mechanisms involved.

Increase nutritional value

Adding *S. boulardii* in food processing will improve the nutritional value of the final product. It has been shown that the yeast produces beneficial products such as aminobutyric gamma acid (a non-protein amino acid) and B-vitamins including thiamin, riboflavin, biotin, and pyridoxine. Consumption of the products containing *S. boulardii* decreases the concentration of antinutrients like phytates and improves the bioavailability of essential minerals and B-vitamins. It also adds nutritional value by helping the biofortification of folates and other relevant B-vitamins (*S. boulardii* synthesizes folates and eliminates phytates and other antinutrients (Lazo-Vélez et al. 2018).

S. boulardii can increase the antioxidant capacity of a product by stimulating the synthesis of active phytochemicals such as isoflavones. The antioxidant properties of the yeast have been widely explained in the previous section (Lazo-Vélez et al. 2018). *S. boulardii* also enhances the digestibility of starch and proteins by decreasing the phytic acid, polyphenols, and trypsin inhibitor activity. It has been shown that fermentation with *S. boulardii* improves protein, crude fiber, neutral detergent fiber, acid detergent fiber, amino acid, and fatty acid levels (Lazo-Vélez et al. 2018). It can be concluded that this probiotic can be applied for human food and animal feed to add nutritional value, the bioavailability of essential nutritional elements, and improvement of digestibility (Lazo-Vélez et al. 2018).

Antibiotic resistance

S. boulardii is obviously resistant to antibiotics and consequently is not affected via antibiotics and sulfamide. It is regarded as an active means for the inhibition and treatment of diarrhea which was induced by the imbalance of the intestinal microbiota in patients with long-term antibiotic treatment, however, many other probiotic microorganisms (bacterial probiotics) do not have this capability (Qamar et al. 2001).

The therapeutic function of saccharomyces boulardii as a probiotic

Probiotics are described as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balances" (FAO/WHO 2002). *S. boulardii* has been used as a probiotic in some pharmaceutical products, and fermented dairy foods (such as yogurt, kefir, buttermilk, and cheese). (Lourens-Hattingh and Viljoen 2001; Pandiyan and Kumaresan 2013), vegetable juice (Diplock et al. 1998), and ice-cream (Clydesdale 1997; Pandiyan et al. 2012). *S. boulardii* as a probiotic, when ingested in proper amounts (at least 10^7 cfu/g or ml), exert beneficial effects on host health. These effects include immune modulation, prevention of diarrhea and inflammatory bowel disease, prevention of enteric infections, improvement of intestinal flora (Czerucka, Piche, and Rampal 2007). *S. boulardii* is able to produce water-soluble vitamins such as thiamin, riboflavin, biotin, pyridoxine, folic acid, and antimicrobial compounds such as bacteriocins (Guo et al. 2014; McFarland 2010). Some selected publications on recommendations for clinical employ of *S. boulardii* are shown in Table 3.

Table 3. Some selected publications on recommendations for clinical employ of *S. boulardii*.

Employ pro disease	Employing dose daily dose cfu/d (mg/d)	Treatment duration	References
Clostridium difficile			
	Not reported-(2–250 mg capsules bid)	30 d	(Surawicz et al. 1989)
	Not reported-(500 mg)	30 d	(Kimmey et al. 1990)
	Not reported-(500–1000 mg)	15 d	(Buts, Corthier, and Delmee 1993)
	3×10^{10} (1000 mg)	28 d	(McFarland et al. 1994)
	1×10^{10} (1000 mg)	28 d	(Elmer et al. 1999)
	2×10^{10} (1000 mg)	28 d	(Surawicz et al. 2000)
	1×10^{10} (500 mg)	12 d	(Bravo et al. 2008)
	Not reported-1 g or 250 mg	28 d	(McFarland 2009)
	5×10^{9} (500 mg)	7 d + 12 wk	(Pozzoni et al. 2012)
	3×10^{10} (not reported) animal model	7 d	(Koon et al. 2016)
	1.8×10^{10} (250 mg)	7 d + 6 wk	(Ehrhardt et al. 2016)
	5×10^{9} (not reported)	7 d	(Carstensen et al. 2018)
Antibiotic-associated diarrhea			
	4×10^{10} (200 mg)	7 d	(Adam et al. 1977)
	Not reported-4 capsule/day	6 d	(Monteiro et al. 1981)
	2×10^{10} (1000 mg)	14 d	(Surawicz et al. 1989)
	2×10^{10} (1000 mg)	3 d	(McFarland et al. 1995)
	4.5×10^{9} (226 mg)	14 d	(Lewis, Potts, and Barry 1998)
	5×10^{9} (not reported)	14 d	(Cremonini et al. 2002)
	2×10^{10} (1000 mg)	14 d	(Duman et al. 2005)
	1×10^{10} (500 mg)	Not reported	(Can et al. 2006)
	2×10^{10} (1000 mg)	14 d	(Cindoruk et al. 2007)
	1×10^{10} (500 mg)	12 d	(Bravo et al. 2008)
	Not reported- (500 mg)	14 d	(McFarland 2009)
	5×10^{9} (500 mg)	7 d + 12 wk	(Pozzoni et al. 2012)
	1×10^{10} (not reported)	14 d	(Shan et al. 2013)
	Not reported- (500 mg)	Not reported	(Casem 2013)
	1.8×10^{10} (250 mg)	7d + 6 wk	(Ehrhardt et al. 2016)
Acute diarrheas			
	2×10^{10} (not reported)	4 d	(Cetina-Sauri and Sierra Basto 1994)
	5×10^{9} (not reported)	3 d	(Urganci et al. 2001)
	1.5×10^{10} (750 mg)	10 d	(Mansour-Ghanaei et al. 2003)
	Not reported- (250 mg)	5 d	(Kurugöl and Koturoğlu 2005)
	Not reported- (250 mg)	5 d	(Billoo et al. 2006)
	Not reported- (250 mg)	7 d	(Ozkan et al. 2007)
	Not reported- (500 mg)	5 d	(Htwe et al. 2008)
	Not reported- (500 mg)	10 d	(Dinleyici et al. 2009)
	Not reported- (200 mg)	6 d	(Le, Makhoul, and Duhamel 2010)
	Not reported- (500 mg)	10 d	(Dinleyici et al. 2011)
	Not reported- (500 mg)	5 d	(Riaz et al. 2012)
	Not reported- (282.5 mg)	5–10 d	(Erdoğan et al. 2012)
	Not reported- (500 mg)	5 d	(Dinleyici et al. 2015)
	Not reported- (500 mg)	5 d	(Das, Gupta, and Das 2016)
	Not reported- (250 mg)	5 d	(Sharif et al. 2016)
	Not reported- (500 mg)	7 d	(Wang and Feng 2019)
Traveler's diarrhea			
	2×10^{9} (250 mg)	5 d before travel and mean 21 d travel	(Kollaritsch et al. 1989)
	5×10^{9} (500 mg)	5 d before travel and mean 21 d travel	(Kollaritsch et al. 1989)
	2×10^{9} (250 mg)	5 d before travel and mean 21 d travel	(Kollaritsch et al. 1993)
	2×10^{10} (1000 mg)	5 d before travel and mean 21 d travel	(Kollaritsch et al. 1993)
	1×10^{10} (600 mg)	5 d	(Bruns and Raedsch 1995)
	Not reported- (150–450 mg)	11 d	(Kirchhelle, Frühwein, and Tobüren 1996)
	2×10^{8} (4 mg) animal model	14 d	(Bisson et al. 2010)
Enteral feeding-related diarrhea			
	1×10^{10} (not reported)	11–21 d	(Tempe et al. 1983)
	4×10^{10} (2000 mg)	8–28 d	(Schlotterer et al. 1987)
	4×10^{10} (2000 mg)	21 d	(Bleichner et al. 1997)
	Not reported- (500 mg)	6 d + 2–10 d after treatment	(Schneider et al. 2005)
	Not reported- (500 mg)	21 d	(Whelan 2007)
	Not reported- (1000–2000 mg)	Not reported	(Jack et al. 2010)
Helicobacter pylori			
	5×10^{9} (not reported)	14 d	(Cremonini et al. 2002)
	2×10^{10} (1000 mg)	14 d	(Duman et al. 2005)
	2×10^{10} (1000 mg)	14 d	(Cindoruk et al. 2007)

(*continued*)

Table 3. Continued.

Employ pro disease	Employing dose daily dose cfu/d (mg/d)	Treatment duration	References
	3×10^{10} (not reported)	14 d	(Song et al. 2010)
	Not reported- (500 mg)	14 d	(Chu et al. 2012)
	6×10^{6} (not reported)	14 d	(Kyriakos et al. 2013)
	1×10^{10} (not reported)	14 d	(Zojaji et al. 2013)
	1×10^{10} (not reported)	14 d	(Zhao et al. 2014)
	Not reported- (500 mg)	14 d	(Bin et al. 2015)
	Not reported- (250 mg)	56 d	(Namkin, Zardast, and Basirinejad 2016)
	Not reported- (750 mg)	14 d	(Cardenas et al. 2020)
	Not reported- (250 mg)	28 d	(Seddik et al. 2019)
	Not reported- (750 mg)	14 d	(Cárdenas et al. 2020)
Giardiasis			
	1×10^{10} (600 mg)	10 d with 28 d follow-up time	(Besirbellioglu et al. 2006)
	1×10^{8} (not reported)	15 d	(Ribeiro et al. 2018)
Blastocystis hominis			
	Not reported- (500 mg)	10 d	(Dinleyici et al. 2011)
Crohn's disease			
	1.5×10^{10} (750 mg)	49 d	(Plein and Hotz 1993)
	2×10^{10} (1000 mg)	180 d	(Guslandi et al. 2000)
	Not reported- (750 mg)	90 d	(Joossens et al. 2005)
	4×10^{8} (500 mg) $\times 3$	30 d and 90 d	(Garcia Vilela et al. 2008)
	Not reported- (1000 mg)	364 d	(Bourreille et al. 2013)
	Not reported- (500 mg)	180 d	(Guslandi 2015)
AIDS-related diarrhea			
	6×10^{10} (3000 mg)	7 d	(Saint-Marc et al. 1995)
Irritable bowel syndrome			
	9×10^{9} (not reported)	28 d	(Maupas, Champemont, and Delforge 1983)
	Not reported- (0–10 mg) animal model	28 d	(Dalmasso et al. 2006)
	2×10^{11} (not reported)	28 d	(Choi et al. 2011)
	Not reported- (500 mg)	30 d	(Kabir et al. 2011)
	Not reported- (200 mg)	30 d	(Bafutto et al. 2013)
	Not reported- (750 mg)	42 d	(Abbas et al. 2014)
	1.5×10^{9} (not reported)	30 d	(Leventogiannis et al. 2019)

Summary of several health benefits of *S. boulardii* including anti-mutagenic and anti-carcinogenic effects, immune system stimulation, anti-infection properties, serum cholesterol reduction, alleviation of lactose intolerance symptoms, and nutritional enhancements has been presented in the previous section. To provide health benefits related to probiotic microorganisms, food industries apply the recommended consumption level of 10^7 CFU/g of probiotic microorganisms, and probiotic food must be consumed at levels higher than 100 g/day (Beena Divya et al. 2012; Granato et al. 2010a; Mohammadi and Mortazavian 2011).

S. boulardii has an unusually high optimal growth temperature of 37 °C. It is a unique probiotic, known to survive gastric acidity and it is not adversely affected or inhibited by antibiotics or does not alter or adversely affect the normal microbiota in the bowel (McFarland and Bernasconi 1993; Van der Aa Kühle, Skovgaard, and Jespersen 2005). It has been proven that *S. boulardii* has antagonistic activity against various bacterial pathogens. It reduces the number of intracellular bacteria but does not modify the number of cell-associated bacteria (Czerucka, Piche, and Rampal 2007).

S. boulardii has been utilized worldwide as a probiotic supplement to support GI health. It benefits the GI tract by increasing intestinal populations of healthy Bifidobacteria and Lactobacilli while decreasing numbers of disease-causing microorganisms (Kabbani et al. 2017; Wang and Feng 2019).

Application of Saccharomyces boulardii in dairy foods and fermented milks as probiotic

Background of the utilization of S. boulardii in dairy products as probiotic

The fast-paced lifestyle accompanied by the unwanted side effects of antibiotic therapies has directed the public to select precautionary rather than curative methods toward diseases. Food industries, particularly dairy industries, have been rapid to understand the massive market potential formed via the several positive healthiness advantages of these probiotic microorganisms. The development of innovative food products turns out to be gradually challenging, as it has to satisfy the consumer's expectancy for products that are simultaneously relishing and healthy.

Dairy food serves as the ideal system for the delivery of probiotic bacteria to the human GI tract due to the provision of a favorable environment that promotes product growth and enhances the viability of these microorganisms (Lourens-Hattingh and Viljoen 2001). Probiotic strains need to hold their functional healthiness properties, containing the capability to survive, transport over the stomach and small intestine, and colonize the human GI zone. A foodstuff can be said to be functional if it comprises a component (which may or may not be a nutrient) that impacts one or a restricted number of functions in the body in a targeted way so as to have positive effects on health (Diplock

et al. 1998; Mirzaei, Pourjafar, and Rad 2011; Pourjafar et al. 2016). Functional food has a physiologic or psychological effect beyond the traditional nutritional impact (Clydesdale 1997; Peng et al. 2020). Probiotics are gradually combined into foodstuffs as dietary adjuncts to aid to keep a strong microbial GI balance with probable resulting profits for human health (Czerucka, Piche, and Rampal 2007). The origin of such beneficial microorganisms as Lactobacillus and Bifidobacteria are mainly fermented dairy foods. So far, the use of yeasts for human consumption has been limited, but in recent years, however, special attention has been paid to the use of yeasts such as Saccharomyces to produce fermented products, including dairy fermented products.(Fleet and Mian 1987; Kuda, Takahashi, and Kimura 2016). In this regard, several studies have been performed on the use of Saccharomyces yeast in the production of fermented dairy foods, and this indicates the potential use of yeasts for incorporation into dairy products as probiotic agents (Arevalo-Villena et al. 2017; Jakobsen and Narvhus 1996).

The usage of probiotic microorganisms gives back to a time earlier than microbes were discovered. Fermented milk foodstuffs were visualized in Egyptian hieroglyphs, and fermented yak milk has conventionally been applied via Tibetan nomads to preserve milk throughout their long treks (Guo et al. 2014). The apparent health influence of consumption amounts of fermented milk products was noticed via researchers in the 1800s, nevertheless, the reason for these health effects remained undiscovered. Louis Pasteur recognized the bacteria and yeasts responsible for the process of fermentation but did not link these microbes to any apparent health effects (Barnett 2000). Elie Metchnikoff (in 1905), who had worked with Pasteur (in the 1860s), was accredited with making the relationship of longevity among Bulgarians to the Lactobacilli used to ferment the yogurt and the presence of these Lactobacilli in the colon (Metchnikoff 1907). Isolated Bifidobacterium from an infant and claimed it could displace pathogenic bacteria in the GI lumen (McFarland 2015).

S. boulardii was discovered by Henri Boulard in 1923. Henri Boulard was a visitor throughout a cholera outbreak who observed that some people who did not develop cholera were consuming an unusual tea (Schlotterer et al. 1987). This tea was made by the outer skin from tropical fruit (lychee and mangosteen) which were cooked down to make tea. He succeeded in isolating the effective agent which was a distinctive strain of yeast he called "*Saccharomyces boulardii*" (McFarland 2010).

Technological aspects of incorporating S. boulardii in dairy products as probiotic

The application of yeast species as a probiotic appears promising specifically in usual milk and simple yogurts since no alcohol or gas was formed. The creation of extreme volumes of alcohol and gas formation, nevertheless, are the main limitations in applying the yeast species into flavored and fruit yogurts (Ansari et al. 2019; Lourens-Hattingh and Viljoen 2001). *S. boulardii* can be well combined into dairy-based food products to progress functional and therapeutic nutrients (Lazo-Vélez et al. 2018; Pandiyan and Kumaresan 2013). Yeasts hardly ever grow in milk deposited at refrigeration temperature due to the fact that they are out-grown via psychotropic bacteria. Nevertheless, in sterilized milk in the absence of competition (addition of yeast after the sterilization process and its growth in the absence of any other microorganisms), *S. boulardii* is capable of growth to populations of 10^8 −109 CFU/ml (Lazo-Vélez et al. 2018; Niamah 2017).

Cheese. *S. boulardii* is often existing in soft mold-ripened cheeses, semi-hard, and hard cheeses like Cheddar cheese. It is thought that the growth of *S. boulardii* in such products is due to its ability to use protein, lipid, and lactic acid compounds in the food environment (Homayouni et al. 2020a; Roostita and Fleet 1996; Zamora-Vega et al. 2013).

Yogurt. Lourens-Hattingh and Viljoen (2001) collected *S. boulardii* ATCC 74012 from YNB (Yeast Nitrogen base, oxoid, Basingstoke) broth via centrifugation technique and mixing with sterile water in order to contain more than 10^6 CFU yeast cells/ml. The yogurt samples such as plain yogurt, fruit cocktail yogurt, and UHT treated yogurt were prepared by inoculating 2.5 per cent (weight cells per volume product) of the yeast culture (Lourens-Hattingh and Viljoen 2001).

Lourens-Hattingh and Viljoen (2001) indicated that the yeast numbers in plain and UHT yogurts remained virtually the same (∼7.6 logs10 CFU/ml) over a storage period of 29 days at 5 °C (Lourens-Hattingh and Viljoen 2001). In fruit yogurt, the cell numbers of *S. boulardii* enhanced from 7.7 logs 10 CFU/ml to 8.1 logs 10 CFU/ml over the storage at 5 °C for 29 days. This rise of cell number in fruit yogurt is related to fermented sugars, sucrose, and fructose derived from the added fruit. The number of *S. boulardii* cells was significantly higher in fruit-based yogurt, mainly because of the presence of proportions of sucrose and fructose derived from the fruit. Regardless of the failure of using lactose, the yeast species utilized accessible organic acids, glucose, and galactose derived from bacterial metabolism of lactose existing in the dairy products (Lourens-Hattingh and Viljoen 2001; Niamah 2017; Pandiyan and Kumaresan 2013).

Additionally, yeast is the chief source of B complex vitamins, which need LAB for growing. A rise in the *S. boulardii* concentration completely affects the amount of lactic acid production. It has been shown that the yogurt starter with added *S. boulardii* had higher proteolytic activity compared with starter cultures lacking yeast. *S. boulardii* has a complex proteolytic system, which generates biopeptides (Marteau 2001; Pandiyan et al. 2012).Water soluble nitrogen (WSN) proportion is higher in yogurt made via starter culture of *S. boulardii* (2%) in comparison with other treatments due to the high proteolytic bustle of *S. boulardii*. Saccharomyces had phospholipase enzyme which breaks down for phospholipids and releases free fatty acids. The water holding capacity (WHC) of a protein gel is a significant parameter in making yogurt. During the production of yogurt, WHC drops and the whey is separated to form the

gel structure of yogurt (Niamah 2017; Niamah, Sahi, and Al-Sharifi 2017). *S. boulardii* can enhance the total solid of yogurt and might cause an improved density of protein gel in the yogurt texture. the significant fact is that the existence of yeast encouraged the viability of LAB. Integration of probiotic yeasts shows a synergistic impact by improving the growth and cell viability of LAB in dairy fermentation. The incorporation of *S. boulardii* into different yogurts appeared valuable in improving the proteolytic activity of yogurts throughout fermentation (Niamah 2017).

UHT treated milk. In Lourens-Hattingh and Viljoen (2001) study, *S. boulardii* treated UHT milk was prepared via inoculating 2.5% of the yeast culture. In the prepared UHT milk, the number of *S. boulardii* increased slightly from 8.15 logs 10 CFU/ml to 8.5 logs 10 CFU/ml over the storage at 5 °C for 29 days. The pH of the *S. boulardii* treated UHT milk was 6.55 on day one and remained 6.59 until 29 days of storage at 5 °C. By reason of UHT treatment, a slight percentage of hydrolyzed lactose was utilized via the yeast cells in the UHT-treated milk. Likewise, *S. boulardii* growth did produce trivial volumes of lactic acid (1.04%) and alcohol (0.5%) suggesting carbohydrate metabolism (Lourens-Hattingh and Viljoen 2001). Furthermore, Lourens-Hattingh and Viljoen (2001) concluded that the application of yeast species such as *S. boulardii* as a probiotic microorganism seems promising in milk since no gas or alcohol was formed (Lourens-Hattingh and Viljoen 2001).

The regular existence of yeasts in dairy foods and related products indicates their capacity to metabolize milk ingredients. *S. boulardii* cannot ferment lactose so they grow in milk as a secondary flora after bacterial growth. LAB can ferment the lactose of milk to galactose and glucose during hydrolysis. The majority of glucose is fermented to lactic acid. Lactic acid produces a high acid milieu, nevertheless, the capability of some yeasts to use lactic acid (as a carbon source) can generate a discriminating environment for the growth of yeasts and for the development of less acid-tolerant LAB (Giang et al. 2012; Roostita and Fleet 1996).

Yeast-acidophilus milk is produced by skim milk and inoculation with 2% probiotic *S. boulardii* yeast culture (10^6 CFU/g) after fermentation at 37 °C for 24 hours. The addition of probiotic yeast in acidophilus milk enhances its demand as a probiotic drink since it has beneficial properties such as the quick recovery of diarrhea as well as tuberculosis (Gaon et al. 2003; Nagashima et al. 2013). *S. boulardii* is known to produce several protein enzymes that break down milk protein into peptides that are used as a nitrogen source over starter bacteria growth in milk and during yogurt production. This, in turn, leads to higher starter growth and acidity-produced rate in the milk (Niamah 2017).

Ice cream. Pandiyan et al. (2012) studied the development of synbiotic (probiotic + prebiotic) ice cream combining *L. acidophilus* and *S. boulardii*. In their investigation, the ice cream mixture was prepared by adding prebiotic ingredients including viz., honey, oligofructose, and inulin at the rate of 3%. In each treatment, mixed ingredients were homogenized and then heated to 80 °C for 30 sec. The mixed samples were stored at 5 °C overnight. After aging, they were heat-treated to 80 °C for 30 seconds and cooled to 37 °C. Probiotic cultures such as *L. acidophilus* and *S. boulardii* (alone and in combination) were inoculated into an ice cream mix at the rate of 4% and incubated at 37 °C until the pH of 5.5 was reached. *L. acidophilus* maintained in the sterile skim milk was inoculated in the ice cream mix. *S. boulardii* culture which was activated in YPD broth then inoculated into the sterile skim milk at 37 °C for 24 h, inoculated in the ice cream mix. Both cultures were inoculated at the rate of 4% into ice cream mix and incubated at 37 °C until the pH of 5.5 was reached. After freezing, the ice cream was stored at −18 °C to −23 °C. Ice cream samples supplemented with or without prebiotics, were satisfactory and gave a good total impression without any off-flavor or losing the therapeutic level of 10^6 cell counts/ml. *S. boulardii* growth was better in combinations than alone in the ice cream samples (Pandiyan et al. 2012).

The ice cream samples either with *L. acidophilus* or *S. boulardii*, or in combinations, consumed by human volunteers could significantly improve gut health by increasing the probiotic population thereby reducing the coliforms in the feces. Since ice cream is a delicious product consumed by all age groups; it can be used as a medium for the growth and transfer of probiotic bacteria and yeast as well as prebiotic substances to maintain the normal flora and thereby restorative of the gut microbes in combating the gut-associated illness (Homayouni et al. 2018; Pandiyan et al. 2012; Tabrizi et al. 2019).

Viability of saccharomyces boulardii in harsh conditions (e.g. fermented products and GI tract)

Dairy food products serve as the perfect system for the delivery of probiotic microorganisms into the human GI tract, due to the provision of a favorable milieu that promotes the growth and survivability of these microorganisms.

To be an active probiotic, it needs to stay alive through the passageway to the target organ (most normally the colon). Probiotic microorganisms need to survive at 37 °C (body temperature), be resistant to bile acids and stomach acids and tolerate the competitive situation of the intestinal lumen. Some strains of *Saccharomyces* have been revealed to have these capabilities. Even though the optimum temperature for most strains of *Saccharomyces* range from 22–30 °C, *S. boulardii* lives superlative at 37 °C, giving it an exceptional benefit of being one of the rare yeasts that do best at body temperatures (Fietto et al. 2004). *S. boulardii* is recognized to endure gastric acidity and it is not inhibited or affected via antibiotics, also can't change the regular microflora in the GI. Upon consumption, *S. boulardii* able to attain a high concentration in the colon (Pardo, Galvagno, and Cerrutti 2009).

Inside the intestinal tract, *S. boulardii* may interface with toxins of pathogenic microorganisms and their binding to the enterocytes, maintain cellular physiology, interact with

regular microbiota, and contribute to regenerating short-chain fatty acid levels. This probiotic similarly can do as an immune regulator, both inside the GI tract and systemically (Graff et al. 2008).

Lourens-Hattingh and Viljoen (2001) investigated the survivability of *S. boulardii* in dairy foods including UHT yogurt, bio-yogurt, and UHT milk during 4-week storage time (kept at 4 °C)It was detected that *S. boulardii* had the aptitude to grow in bioyogurt and reach extreme number beyond 10^7 CFU g^{-1} (from 7.7 log to 8.6 log). The number of yeast populations was significantly greater in the fruit-based yogurt, chiefly because of the existence of fructose and sucrose derived from the fruit. In spite of the inability of *S. boulardii* to use lactose, it used accessible glucose, galactose, and organic acids derived from bacterial metabolism of the milk lactose existing in the dairy foods (Araújo et al. 2012).

Some studies have shown that the colon concentration is dose-dependent in human volunteers. When *S. boulardii* was given to healthy volunteers at doses normally used therapeutically (1–2 × 10^{10}/day), colonic levels were 2 × 10^8/g in the stool (Buts 1999; Klein et al. 1993). Some clinical trials using probiotics have studied the level of microorganisms existing in the terminal site (colonic lumen in their study subjects). In one trial of patients with repeated *C. difficile* infection (CDI) given *S. boulardii* (2 × 10^{10}/day) for 28 days, patients who had a successive CDI recurrence were detected to have considerably lesser numbers of *S. boulardii* (2 × 10^4/g stool) compared with those without recurrence (1 × 10^6/g stool) (Czerucka, Piche, and Rampal 2007).

Presence of sodium chloride

The adaptation and response of yeasts to several tension conditions including osmotic pressure, oxidative tension, ethanol, extreme pHs, and heat shock have been well recognized (Chae et al. 2013). As Marshall and Odame-Darkwah found, the existence of sodium chloride (NaCl) decreases the total fermentation rate and increases the final product. Furthermore, a similar investigation discovered that higher amounts of NaCl prevented the viability of some yeast strains existing in the wheat dough (Marshall and Odame-Darkwah 1995). Nevitt et al. (2004) concluded that enhancing amounts of NaCl can have triggered hyperosmotic pressure which could have changed the transcription and expression of genes due to reduced or entirely arrested cell growth (Nevitt et al. 2004).

In the latest years, scientists have changed their view from liquid to solid and semisolid culture situations. Previous investigations on liquid cultures have shown the inhibitory role of mineral salts like NaCl on the fermentation rate of sugars by yeasts (Wei, Tanner, and Malaney 1982). Prominent amounts of inorganic electrolytes in a suitable liquid growth medium have been found to affect some parameters of yeast activity including:

i. Cell growth and proliferation: (a) as the amount of salt present in the culture medium increases, the survival rate of probiotic yeast decreases (b) the biomass of the culture (specifically, the full weight of yeast cells per unit volume of liquid growth medium) decreases following salt rises (c) the length of the lag phase (specifically, the incubation period between inoculation of the culture and detectable beginning of cell growth) elongates following salt concentration rises.
ii. Use of the main carbon and energy source is decreased
iii. Alteration in the concentration of metabolic products: (a) There is a reduction in the production of ethanol as salt content rises and (b) there is a surge in the concentration of other fermentation products (for instance acetaldehyde, glycerol, etc.) following salt content rises (Tanner, Wei, and Woodward 1981; Tempest, Meers, and Brown 1970).

Low temperatures, and lyophilization effects

The use of *S. boulardii* for the production of commercial probiotic bio yogurt has been demonstrated by maintaining a high cell count during storage time, and the use of this yeast is recommended for the production of other similar probiotic products.(Lourens-Hattingh and Viljoen 2001).

It has been shown that ice cream's colloidal design, in cooperation with its low-temperature storing situation, makes it a very promising carrier for the stabilization and in vivo delivery of bioactive compounds and advantageous microorganisms (Homayouni et al. 2020b; Soukoulis, Fisk, and Bohn 2014). With the aim of reaching the useful impacts of probiotics, these useful microorganisms must be lively, active, and abundant in the product up to the expiration date. Nevertheless, temperature variation referred to as "cold shock", throughout freezing and melting can cause a decrease or even comprehensive loss of metabolic activity (Niamah, Al-Manhel, and Al-Sahlany 2018). Microencapsulation is one of the important methods for increasing the survival rate of probiotics in harsh conditions (Heydari et al. 2011; Homayouni et al. 2020b; Pourjafar et al. 2018; Pourjafar et al. 2020). According to numerous findings, microencapsulation is a valuable alternative to rise the viability rate of probiotic microorganisms in ice cream and other fermented frozen dairy desserts (Homayouni et al. 2008; Huq et al. 2013; Norouzi et al. 2019). (**Also see section: Microencapsulation of *Saccharomyces boulardii***)

Ahmadi et al. (2014) have concluded that the live numbers of free probiotic microorganisms reduced from ∼9.55 to ∼7.3 Log CFU/g after 60 days of frozen storage while the microencapsulated cells only reduced under 1 Log cycle. Encapsulation by means of alginate microbeads protected the probiotic cells against damages in the freezing phase and throughout frozen storage (Ahmadi et al. 2014). Lyophilized products are stable at room temperature (∼ 25 °C), and they have the benefit of transportability, and retain high survivability counts over prolonged periods (Graff et al. 2008), whereas heat-dried preparations must be refrigerated and may not be stable at room temperature (McFarland 2010). A study of *S. boulardii* products found that lyophilized products survived yeast cells better than other heated products (Schwenzer 1998). Lyophilized *S. boulardii* is obviously different from dietary probiotic foods that contain different

strains of probiotic microorganisms and are applied either in animals to improve zootechnical produces or in healthy humans to support host physiology in the absence of any pathological background. It has been shown that *S. boulardii* can be regarded as an instance of a probiotic medicine (Czerucka, Piche, and Rampal 2007; Hennequin et al. 2000).

pH profile

Probiotic microorganisms that are consumed orally ought to initially survive throughout the transport system and then persist in the GI lumen to offer their useful effects for the host. *S. boulardii* can survive in the human GI tract due to its resistance to high temperature and low pH (Czerucka, Piche, and Rampal 2007; Douradinha et al. 2014). *S. boulardii* can compete with diarrhea-causing pathogens for growth in the gut, making it a promising candidate for treating and preventing diarrhea (Micklefield 2014). Adequate viable probiotic microorganisms (∼ 7 log cell/g or ml) are required to play a useful role on the consumer (FAO/WHO 2002). GI lumen resistance is also an imperative property for probiotics when gut microbiome balance is the target, however, only alive microorganisms can confer additional benefits (Paula et al. 2019). The survival of *S. boulardii* is challenged after oral administration and a ratio between 1 to 3% of the live yeast administered orally is recovered in feces. This weak ratio could result from the destruction of the yeast during its transit within the GI tract, which may be due to pH variations (Guslandi et al. 2000). *S. boulardii* is more tolerant of acidic pH variation than other *S. cerevisiae* strains and can survive at pH values as low as 2.0 (Moradi et al. 2018).

A comparative investigation between *S. boulardii* and *S. cerevisiae* disclosed that the *S. boulardii's* survivability remained down to pH 2 although the *S. cerevisiae*'s reduced considerably at pH 6, 3, and 2. Nevertheless, none of the yeast strains survived at pH 1 (Edwards-Ingram et al. 2007). Enhancing the percentage of alive yeast inside the intestine would potentiate the therapeutical profit of *S. boulardii* administration. The initial phase toward this goal is better information of *S. boulardii* sensitivity to environmental circumstances in the GI lumen (Bai et al. 2004).

S. boulardii is now commercialized as a freeze-dried powder that upholds the stability of the probiotic. Nonetheless, the freeze-drying method is expensive and leads to a loss of 20 to 30% of viability compared to the initial aqueous suspension of the yeast whose viability is about 100% (Guslandi et al. 2000).

Microencapsulation of saccharomyces boulardii

The survivability of probiotics in food products is very reliant on the species and strain used, metabolic interaction with lactic acid starters, fermentation conditions, pH of products, presence of oxygen and storage temperature, and presence of protective compounds such as protein and fat droplets. A probiotic organism should possess several attributes such as adhesive ability, acid, and H_2O_2 production ability, bile tolerance, and significant antibacterial activity, and immunomodulatory activity (Barzegari et al. 2014; Ghasemnezhad et al. 2017; Shah 2000; Vinderola, Mocchiutti, and Reinheimer 2002) and must be nonpathogenic (probiotic microorganisms must be GRAS/generally recognized as safe: means they must to be non-pathogen, and have health promoting properties). *S. boulardii* and some *S. cerevisiae* strains are studied or commercialized as probiotics (Holzapfel et al. 2001; Szajewska and Mrukowicz 2005). It is important to mention that for a microorganism to be considered probiotic, it must survive passage through the stomach and maintain its viability and metabolic activity in the intestine (Ansari et al. 2017b; Mirzaei, Pourjafar, and Rad 2011; Van der Aa Kühle, Skovgaard, and Jespersen 2005). Different techniques are available for improving the survival of *S. boulardii,* and microencapsulation is one of the best and most outstanding methods. This technique can be effective in both product storage as well as GI conditions (de la Cruz-Gavia et al. 2018; Ghorbani-Choboghlo et al. 2015). Microencapsulation is a process by which bioactive substances/cells are coated with protective materials. A way for encapsulation and maintenance of probiotic yeast as *S. boulardii* in polymeric or biopolymeric fibers has been technologically advanced. Biopolymers are natural polymers (alginate, chitosan, resistant starch) that are plentifully accessible and extractable from natural sources and these biopolymers offer a wide range of unique applications (Ghorbani-Choboghlo et al. 2015; Sultana et al. 2017).

Relationship between saccharomyces boulardii and other probiotics

S. boulardii has been applied in combination with other probiotic microorganisms and also administered with or without dairy foods (Pandiyan and Kumaresan 2013). *S. boulardii* has numerous profits compared to other live microorganisms proposed as drug delivery vehicles. As a eukaryotic microorganism, it is able to express complex glycosylated antigens. *S. boulardii* can possibly express a much broader collection of compounds (such as vanillic acid, phenyl ethyl alcohol, and erythromycin) than probiotic bacteria. Also, *S. boulardii* is resistant to lower pH and higher temperatures compared with conventional laboratory strains of *S. cerevisiae* (Edwards-Ingram et al. 2007; Fietto et al. 2004), which could translate to an increased ability of *S. boulardii* to survive through the intestine (Hudson et al. 2014).

The advantage of probiotic yeast over probiotic bacteria is possibly due to the normal resistance of yeast to antibiotics, which maintains their survivability and probiotic characteristics (Czerucka, Piche, and Rampal 2007). Several investigations applying cell/or animal models have shown that *S. boulardii* may exert a positive influence against several enteric pathogens such as *C. difficile*, *Vibrio cholera*, *Salmonella*, *Shigella*, and *E. coli*. *S. boulardii* seemed to act via two chief mechanisms: (i) production of factors that neutralized bacterial toxins and (ii) modulation of the host cell signaling pathway implicated in proinflammatory response during bacterial infection (McFarland 1996).

S. boulardii also produces a phosphatase that can dephosphorylate endotoxins for example LPS from *E. coli* O55B5 and can partly neutralize its cytotoxic effects (Czerucka, Piche, and Rampal 2007). *S. boulardii* is found in the human gastrointestinal tract as a probiotic and has been used in combination with antibiotics to treat some infectious diarrhea. for instance, this probiotic yeast secretes several metabolites, some of which inactivate *C. difficile* toxins or its phosphatase enzyme (EC 3.1.3.X) neutralizes endotoxin caused by gram-negative bacteria. Studies have shown that the metabolites of *S. boulardii* can inhibit the activity of 26 species of food-borne pathogenic bacteria (Niamah, Al-Manhel, and Al-Sahlany 2018).

Yeasts mainly grow in milk and dairy products as secondary flora, ie after the growth and activity of starter bacteria. LAB can ferment about 35% of the lactose in milk through hydrolysis to galactose and glucose. Solitary glucose is altered into lactic acid, whereas the galactose moiety is released mostly via *Streptococcus thermophilus* into the extracellular milieu (Goodenough and Kleyn 1976). Giudici, Masini, and Caggia (2003) showed that the high amount of galactose (approximately 1%) was the key reason for the growing of non-fermenting and galactose positive yeasts (*Hansenula* and *S. cerevisiae*) in yogurt (Giudici, Masini, and Caggia 2003). Additionally, the low pH of yogurt (and other similar products) and the ability of yeasts to utilize organic acids create a selective environment for yeast growth (Ansari, Pourjafar, Esmailpour 2017a; Suriyarachchi and Fleet 1981). Milk products that contain yeast in their starter culture are acidophilus-yeast milk (Lang and Lang 1975). *S. boulardii* is the only yeast probiotic that has been confirmed effective in double-blind investigations, and it outperformed other known probiotics, such as Lactobacillus and Bifidobacterium, regarding immunomodulation (Ansari, Pourjafar, Esmailpour 2017a; Martins et al. 2009).

Methods of isolation, identification, and enumeration of Saccharomyces boulardii

S. cerevisiae var. *boulardii* belongs to the cluster of eukaryotic cells and therefore, differs from bacterial probiotics that are prokaryotes. Conventionally, acidified cultures (acidification of the culture medium with 10% tartaric acid until the pH of the medium reaches about 3.5. Bacteria are not able to grow in this pH range) have been applied to count yeasts in foods. Such cultures are currently known as inferior to antibiotic supplemented media that have been formulated to overturn bacterial colony growth, improve resuscitation of damaged yeasts, and reduce precipitation of food particles (Endo and Gueimonde 2016).

Yeasts had better be counted via a surface plate method rather than with a pour plate. This method offers the highest contact of the cells to atmospheric oxygen and avoids heat stress from molten agar (Da Silva et al. 2018; Speck 1984). Also, the "most probable number" (MPN) method may be suitable for certain foodstuffs. The membrane filter method is appropriate for beverages, and for some solid foodstuffs. Sterile 0.1% peptone water is the most proper diluent when culturing samples for *Saccharomyces* species. Solutes, for instance, glucose, sucrose, or glycerol should be added to the diluent when counting *Saccharomyces* species in foods, for example, fruit juice and sirups concentrate (Da Silva et al. 2018; Speck 1984). Some important media for yeasts culture are Dichloran Rose Bengal Chloramphenicol (DRBC) Agar, Dichloran 18% Glycerol (DG 18) Agar, Plate Count Agar (PCA) supplemented with Chloramphenicol, Acetic Acid Dichloran Yeast Extract (ADYS), Dichloran Rose Bengal Yeast Extract Sucrose Agar (DRYES), Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), and Sabouraud Dextrose Agar (SDA) (Speck 1984).

In recent years alternative procedures to the traditional plate count have been technologically advanced. Rapid techniques that evaluate levels of both viable and nonviable yeasts in foodstuffs are required for analytical consulting firms, research laboratories, and food industries. The investigation is being prepared on rapid approaches that have the potential for detection and identification of *S. cerevisiae* var boulardii in foods. The important possibly methods include: (a) microscopic examination by means of selective stains or fluorescent dyes; (b) optically-based techniques, for instance, flow-cytometry; (c) apply of impedimetric methods based on conductance; (d) recognition of a metabolic or cellular constituent produced via yeast that is lacking in foodstuff; (e) immunological detection of yeasts based on antibodies that are specific for yeasts; (f) molecular detection of DNA or RNA fractions that are specific to a given yeast genus and species (Da Silva et al. 2018; Fung 2002; Speck 1984).

Selective culture media

If an enumeration of particular groups of species of yeasts is desired, selective agar can be applied. Plate on either tryptone glucose yeast extract (TGY) agar or MEA are incubated at 25 °C for 3 days. If bacteria are existing in the foodstuffs, it is better to supplement agar with 100 ppm oxytetracycline or chloramphenicol. If molds are existing, DRBC agar is the best choice (Da Silva et al. 2018; Reethu Narayanan and Ch 2012; Sardonini and DiBiasio 1987; Speck 1984).

Trigueros et al. (2016) have done a study entitled "medium optimization and kinetics modeling for the fermentation of hydrolyzed cheese whey permeate as a substrate for *S. cerevisiae* var. *boulardii*". In their study, *S. cerevisiae* var. *boulardii* cells were motivated in a 250 ml yeast extract peptone dextrose (YEPD) culture medium with the following composition: 20 g/L glucose, 10 g/L meat peptone, and 5 g/L yeast extract. In maintenance culture, *S. boulardii* cells were inoculated in the YEPD motivation medium and moved to a shaker incubator with 100 rpm tension and a controlled temperature of 30 °C for 24 h (Trigueros et al. 2016).

Niamah (2017) investigated microbial properties of yogurt with supplemented *S. boulardii*. In this study, yeast culture was motivated in potato dextrose broth at 30 °C for 24 h. In microbiological tests, chloramphenicol glucose yeast extract agar medium was used for culture and counting of *S. boulardii* and then the plates were incubated at 30 °C for 48–72 h. (Niamah 2017).

Khidhr and Zubaidy (2014) studied isolation and identification of *S. cerevisiae* var. *boulardii* from fresh and dried fruits including apricot, fig, lychees, mangosteen, and grape. 1 g of fruit sample, blended with sterilized 9 milliliters peptone water 0.1% then a serial of dilution was made until 10^{-5}, 1 ml of the latter dilution was cultured via pour plate technique using SDA medium (pH 5.5) (Khidhr and Zubaidy 2014).

In Liu et al. (2016) study, the *S. boulardii* strain was grown on YP medium (20 g/L peptones, 10 g/L yeast extract) having 20 g/L glucose and supplemented with the corresponding antibiotics at 30 °C (Liu et al. 2016). Also, Wu et al. (2008) cultured *S. boulardii* in Sabouraud dextrose (SD) broth (100 mg/ml) overnight at 37 °C (Wu et al. 2008).

Du et al. (2012) cultured *S. boulardii* in YEPD liquid medium (at natural pH enclosing 2% (w/v) peptone, glucose 2% (w/v), 1% (w/v) yeast extract, and agar 2%) at 30 °C for 24 h for activation. Also, they cultured *S. boulardii* in YEPD plates at 30 °C or 37 °C for observing morphological alterations. To induce sporulation, the grown strain in YEPD was transmitted to sporulation agar (sodium acetate 5 g/L, tryptone 2.5 g/L, NaCl 0.62 g/L, agar 20 g/L, pH natural) and incubated at 25 °C for 2 weeks (Du et al. 2012).

Microscopic examination/selective stains/fluorescent dyes

Staining techniques have been applied to fast detect viable yeasts chiefly in semi-solid foods and beverages (Speck 1984). Yeasts have been stained with methylene blue for the reason that alive cells will decrease the dye to the colorless form whereas dead cells will take up the blue color. Meanwhile, some alive cells may stain blue, fluorescent dyes and optical brighteners such as analine blue, viablue, acridine orange, and fluorescine-diacetete have been assessed (Da Silva et al. 2018; Speck 1984). Fluorescent dyes have been vigorously investigated with the direct epifluorescent filter technique (DEFT) for the detection of yeasts in foodstuffs. DEFT offered a satisfactory correlation with plate counts for yeasts counted from foodstuffs. Differentiating between viable and nonviable cells has not permanently been probable with DEFT and has consequently been joined with immunological and gel microdroplets methods to develop detection of yeasts (Da Silva et al. 2018; Speck 1984). Morphological form and microscopic analysis of the colonies can be done after staining them with a simple stain (methylene blue). Generally, it is important to select an identification pattern, trail the procedures carefully, and apply an excellent microscope to view slides (Da Silva et al. 2018; Speck 1984).

Hudson et al. (2016) studied *S. boulardii* in all imaging, in vivo, and in vitro. *S. cerevisiae* W303 and BY4741 are well-described laboratory haploid strains applied in EM imaging. They have proved that the cell wall of *S. boulardii* is thicker than in *S. cerevisiae* strains. *S. boulardii* and *S. cerevisiae* were cryopreserved and imaged by transmission electron microscopy (TEM). Scale bars indicate 500 nm and 50 nm. The thickness of the full cell wall for every strain was intended by an average of 23 cells per strain (Hudson et al. 2016). (See Figure 7)

Chiron, Tompkins, and Burguière (2018) surveyed some probiotic bacteria and *S. boulardii* via epifluorescence microscopy. Microscopy interpretations of probiotic products stained by antibodies were comprehended to imagine antibody staining place and fluorescence intensity. Phase contrast and epifluorescence photographs were taken by means of an inverted microscope. Far-red fluorescence was evaluated by Cy5-4040A filter, and green fluorescence was evaluated by green fluorescent protein (GFP) narrow filter (Chiron, Tompkins, and Burguière 2018).

Biochemical assays

A simple enumeration of yeasts may not be adequate to help the identification and it may be required to identify the genus and even the species of them. Biochemical methods that can be applied to recognize yeasts to genus and species levels have been described.

Du et al. (2012) studied the properties and cultural circumstances of *S. boulardii*. In their study the composition of sugar fermentation medium was 1% (w/v) peptone, 0.3% (w/v) NaCl, 0.2% (w/v) Na2HPO4•12H2O, 5% (w/v) glucose, 0.5% (w/v) beef extract, and 0.2% (v/v) bromocresol purple (as an indicator) (Du et al. 2012). They added soluble starch, maltose, sucrose, and lactose to the sugar fermentation medium instead of glucose. Also, they used two basic media. Nitrogen integration medium includes: 0.05% (w/v) MgSO4•7H2O, 0.1% (w/v) KH2PO4, 0.1% (w/v) yeast extract, 2% (w/v) glucose, and 2% (w/v) agar; Carbon integration medium include: 0.1% (w/v) KH2PO4, 0.05% (w/v) MgSO4•7H2O, 0.5% (w/v) (NH4)2SO4, 0.02% (w/v) yeast extract, and 2% agar. In sugar fermentation, the motivated strain was inoculated in 50 ml YEPD broth at 30 °C/12 h. Then, 1% of seed culture was injected into a 10 ml tube with a sugar fermentation medium at 30 °C. The production of CO_2 offers *S. boulardii*'s fermentative activity. In nitrogen and carbon integration, nitrogen integration was tried via inoculating the yeast from a YEPD plate to plates using Urea, (NH4) 2SO4, or KNO3 as the nitrogen source; carbon integration was tried via inoculating the yeast from a YEPD plate to plates with maltose, galactose, or lactose as the carbon source. In the case of ethanol tolerance, the triggered strain was inoculated in 50 ml of YEPD broth at 30 °C/24 h. Then, 1% of seed culture was inoculated to a 10 ml tube with a YEPD medium having diverse ethanol concentrations (0%-24% [v/v]) at 30 °C. The volume of CO_2 generated offers *S. boulardii*'s fermentative activity. In acid tolerance culture, the yeast motivated at 30 °C/24h was inoculated in 10 ml YEPD broth adjusted to dissimilar pH (1–6) with 10% NaOH and 6 mol/L HCl. The cultures were incubated at 160r/min at 30 °C. 1 ml sample was diluted 10 times with sterile distilled water after 12 and 24 h. The growth of yeast was measured via the optical density (at 600 nm = OD600) (Du et al. 2012).

Khidhr and Zubaidy (2014) studied the identification of isolated *S. boulardii* (via pour plate method using SDA medium) by use of biochemical tests for instance; nitrogen source utilization, carbon source fermentation, acid creation

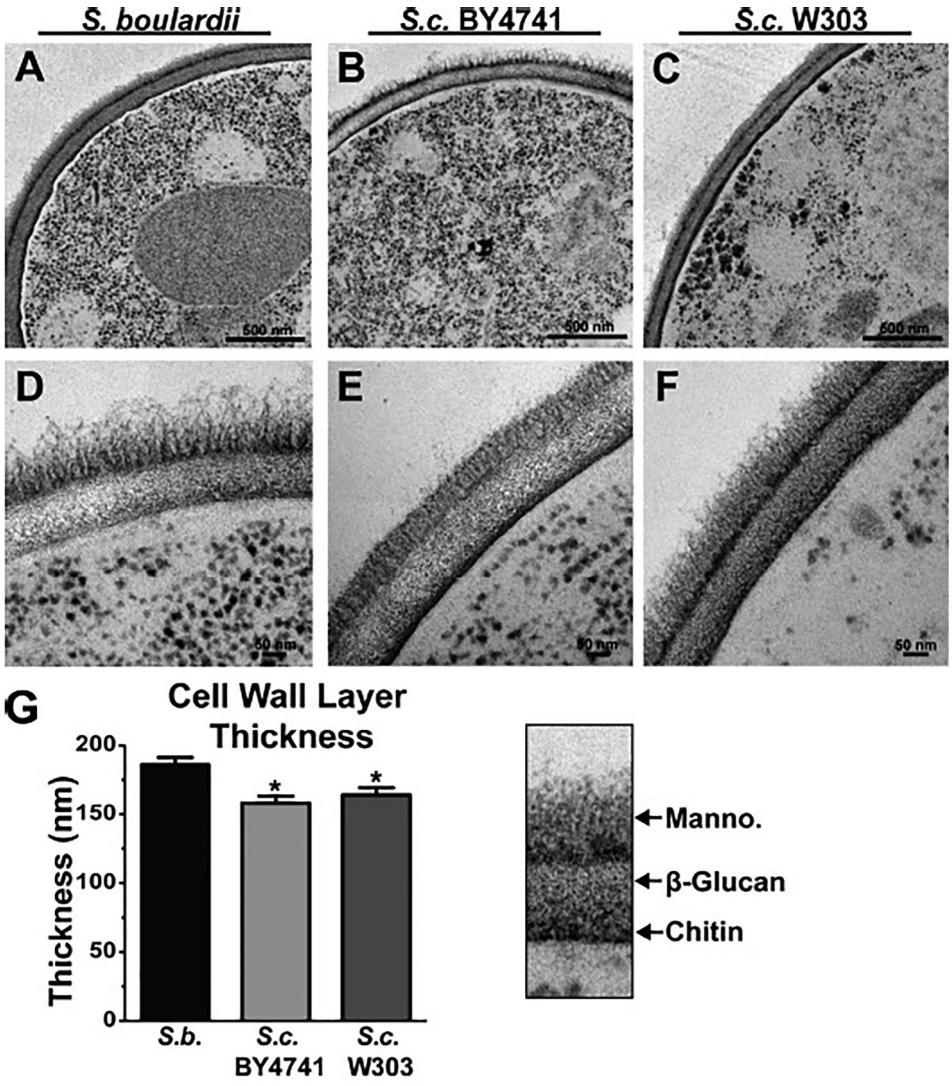

Figure 7. "The cell wall of *S. boulardii* is thicker than in *S. cerevisiae* strains. *S. boulardii* (A, D) and S. cerevisiae BY4741 (B, E) and W303 (C, F) were cryopreserved and imaged via transmission electron microscopy. Scale bars denote 500 nm (A–C) and 50 nm (D–F). (G) Quantification of total cell wall thickness for each strain was calculated taking the average of 23 cells per strain. Error bars depict the standard error of the mean (SEM), * $p < 0.05$ relative to *S. boulardii*, Kruskal-Wallis with Dunn's multiple comparison test" (Source: (Hudson et al. 2016).

from fermented sugars, ester creation, and urea hydrolysis (Khidhr and Zubaidy 2014).

Flow-cytometry

Traditional microbiology methods are the gold standard for probiotic microorganism's enumeration. Nevertheless, these procedures are restricted by factors of time, incapability, and specificity to identify viable but non-culturable (VBNC), and non-viable microorganisms. Flow cytometry is a technique for the particular quantification of viable and non-viable probiotic microorganisms (Chiron, Tompkins, and Burguière 2018). This technique is an optically-based method that has been applied to identify and enumerate yeasts in semi-solid foods and beverages. It has been applied to measure cell concentration, protein, enzymatic activity, DNA, and analogous parameters. The light source of the flow cytometer measures the power of light scatter that is translated into data on external morphology, cell shape and size, cell density and survivability, and other information (e.g. total DNA, DNA gene expression, surface receptors, intracellular proteins, transient signal). However, the cost of the apparatus and the prerequisite for skilled operators are disadvantages for possible users (Pettipher 1991).

Pettipher (1991) studied the initial assessment of flow cytometry for the detection of yeasts in soft drinks. *S. cerevisiae* was detected at a number of 50 cells/mL in lemonade, on the other hand in tomato juice 14000 cells/mL were desired. Once food samples had >10 cells/g and were pre-incubated at 25 °C for 22 h, the process was comparable to the plate count. Flow cytometry has been jointed with RNA probes, immunofluorescence, and 2 D-image analysis. Detection of *S. cerevisiae* by means of flow cytometry with a fluorescent rRNA probe has been testified (Pettipher 1991).

Hudson et al. (2016) studied the properties of *S. boulardii* in the healthy mucosal immune system. In their study, for detection of *S. boulardii* antibody, stained cells were fixed with 2% paraformaldehyde and read on a BD LSRII flow-cytometer (Hudson et al. 2016).

Chiron, Tompkins, and Burguière (2018) studied flow cytometry as useful equipment for precise quantification and survivability valuation of microorganisms in multistrain probiotic products. A flow cytometry technique conjoining specific antibodies and survivability valuation with SYTO 24 and propidium iodide was used to enumerate these strains (one of the probiotic strains was *S. cerevisiae* var. *boulardii*, and culture condition was: YEPD media, aerobiosis 30 °C, 24–48 h) in three commercial products. Examines were directed on two flow cytometry devices and compared using selective media. Results showed that flow cytometry offers higher cell counts than the classical microbiology method (Chiron, Tompkins, and Burguière 2018).

Various studies have been shown that flow cytometry offers complete and precise quantification of viable and non-viable strains of probiotics in a very short time (<2 h) in comparison with traditional methods (>48 h), conveying effective apparatuses for investigation and quality control (Chiron, Tompkins, and Burguière 2018; Díaz et al. 2010; Ruyssen et al. 2007; Wilkinson 2018).

Impedimetric methods (conductance)

Activities of bacteria's metabolites cause a confrontation to the stream of an electrical current, thus, enhancing conductance. Nonetheless, the contrary takes place for yeasts and an indirect conductance requisite to measure (Carvalho et al. 2003; Jayan, Pu, and Sun 2019; Silley and Forsythe 1996). Indirect conductance can identify as few as 10 yeast cells/mL of juices in 2–3 days (Deak and Beuchat 1993). The measurements of conductance compared satisfactorily with plate counts on acidified PDA and DRBC agar. Also, 1 cell/10 mL can be identified via indirect conductance in carbonated beverages with an initial incubation in tryptone glucose yeast extract (TGYE) broth; nevertheless, the requirement for an incubation era (48–72 h) does not permit fast analysis (Deak and Beuchat 1995).

Metabolic or cellular constituent

Every cellular component or metabolite that is produced by yeasts and is not existing in animal or plant tissues is a possible candidate for fast technique development. Cellular components applied to detect yeasts include mannan. It should also be noted that depending on the substrate present in the yeast culture medium, the type of metabolites produced will also vary, and depending on the yeast genus, species, and variety some of these metabolites are specific for every yeast (Da Silva et al. 2018; Speck 1984).

Liu et al. (2016) studied the metabolic engineering of probiotic *S. boulardii*. They analyzed galactosidase activity and produced metabolites during the fermentation process. In galactosidase activity analysis, one unit of enzyme activity is defined as the quantity of enzyme that catalyzes 1 mol of the substrate (per min at 30 °C). The bicinchoninic acid (BCA) technique was applied to determine the concentration of protein of the yeast extract. In the analysis of metabolites during fermentation, the xylose fermentation mix was prepared via inoculating a pre-culture grown overnight (5 ml of YP medium + 20 g/L glucose) into 50 ml YPX40 medium (YP + 40 g/L of xylose). The OD600 was measured via a spectrophotometer, and extracellular metabolite was measured and detected via HPLC (Liu et al. 2016).

Ryan et al. (2011) studied the produced metabolite profile of *S. boulardii* after the fermentation process on rice bran. They measured and detected metabolites by Gas Chromatography-Mass Spectrometry (Ryan et al. 2011).

Immunological detection

Immunoassays are founded on the binding of an antibody that was created to a species of yeast to an antigen from food, and the following visualization of that reaction via joining the antibody to an enzyme that will yield a colored complex upon reaction with the proper substrate. Unlike molds especially toxigenic ones, very little investigations have been done on the use of ELISA to detect yeasts in foods, but it can still be considered one of the ways to detect *S. boulardii* and other yeasts (Da Silva et al. 2018; De Ruiter, Notermans, and Rombouts 1993; Speck 1984; Wang and Yang 2017).

Molecular methods

Molecular methods for the detection of yeasts are based on the identification of unique DNA or RNA profiles. Approaches that have displayed the most potential for identifying yeasts in foodstuffs are those based on polymerase chain reaction (PCR), comprising random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP), electrophoretic karyotyping, pulsed-field gel electrophoresis (PFGE), or a mixture of these techniques (Da Silva et al. 2018; De Llanos et al. 2006; Postollec et al. 2011; Speck 1984).

Deak and Beuchat (1995) initially reviewed the usage of PFGE to identify yeasts for taxonomic along with industrial usages such as wine production. This investigation has been completed by PCR, RFLP, and RAPD for the identification of yeasts (Deak and Beuchat 1995). McCullough et al. (1998) studied the species identification and virulence attributes of *S. boulardii*. They used restriction fragment length polymorphisms (RFLPs) of the PCR-amplified intergenic transcribed spacer regions (including the 5.8S ribosomal DNA) (McCullough et al. 1998). Recent studies show applying molecular methods for the detection of *S. boulardii* (Douradinha et al. 2014; Fietto et al. 2004; Mitterdorfer et al. 2002; Ragavan and Das 2017; Trotta et al. 2012; Van der Aa Kühle, Skovgaard, and Jespersen 2005). However, with more investigations, PCR may be a suitable instrument for detecting and identifying *S. boulardii* and other yeasts in foodstuffs.

Safety of saccharomyces boulardii

In some investigations on immunocompromised people, *S. boulardii* has been associated with localized infection or fungemia, which can be lethal (Santino et al. 2014). Generally, *S. boulardii* is safe for use in healthy populaces and fungemia with *S. boulardii* has not been described, to the top of

the current evidence in immunocompetent patients (Kelesidis and Pothoulakis 2012). Berni et al. (2011) published a review article focused on the existing evidence for efficacy and safety of *S. boulardii* in diverse diseases in pediatric and adult patients with the purpose of offering practical guidance for gastroenterology clinical practice. This review showed that HIV-1-infected patients given therapy by *S. boulardii* don't encounter any health problems related to fungemia (Berni et al. 2011). A surveying investigation on 32000 oncohematological patients indicated no existence of fungal sepsis with *S. boulardii* application (Sulik-Tyszka et al. 2018).

Conclusions

Widespread public notice in natural foods has invigorated industrial investigation for the production of some healthy metabolites and organic acids by probiotic microorganisms. *S. boulardii* as a probiotic is a valuable strain of yeast for human and animal health. The suggested level of 10^6 CFU/g or ml to 10^7 CFU/g or ml of product is needed to offer health profits related to probiotics. The viability of *S. boulardii* to ecological situations depends on the fermentation conditions, pH, water activity, oxidation/reduction potential, presence of nutrients, presence of antimicrobial agents, biological barrier, temperature, relative humidity, and the combination of gases (O_2, Co_2, N_2, …) in the atmosphere. Dairy foods serve as the perfect system for the delivery of *S. boulardii* to the GI lumen, because of the provision of a favorable milieu that encourages the growth and improves the survivability of this probiotic.

In probiotic foods, *S. boulardii* is regularly combined with Lactobacilli and Bifidobacteria, and there is a wide tendency for its commercial application. In summary, the application of *S. boulardii* as a probiotic, particularly in different dairy foods, can assist consumers to overcome several clinical health disorders and henceforth decrease wide therapeutic costs. Despite extensive studies and proving the safety of *S. boulardii*, it is necessary to use any probiotic product more carefully in people with specific patients and with the advice of a physician.

Disclosure statement

The authors have no competing interests to disclose.

Abbreviations

ACF	Aberrant Crypt Foci
ADYS	Acetic Acid Dichloran Yeast Extract
BCA	Bicinchoninic Acid
CFS	Cell-Free Supernatants
CFU	Colony Forming Unit
DEFT	Epifluorescent Filter Technique
DG 18	Dichloran 18% Glycerol
DRBC	Dichloran Rose Bengal Chloramphenicol
DRYES	Dichloran Rose Bengal Yeast Extract Sucrose Agar
EF	Edematogenic Factor
FAO	Food and Agriculture Organization
GFP	Green Fluorescent Protein
GI	Gastrointestinal
IBD	Inflammatory Bowel Disease
LAB	Lactic Acid Bacteria
LF	Lethal Factor
LPS	Lipopolysaccharide
LT	Lethal Toxin
MEA	Malt Extract Agar
MPN	Most Probable Number
O/R	Oxidation/Reduction
PA	Protective Antigen
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PFGE	Pulsed-Field Gel Electrophoresis
QR	Quinone Reductase
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SDA	Sabouraud Dextrose Agar
TE	Trolox Equivalents
TGY	Tryptone Glucose Yeast Extract (TGYE)
TLRs	Toll-like receptors
UHT	Ultra High Temperature
VBNC	Viable but Non-Culturable
WHC	Water Holding Capacity
WHO	World Health Organization
YEPD	Yeast Extract Peptone Dextrose

References

Abbas, Z., J. Yakoob, W. Jafri, Z. Ahmad, Z. Azam, M. W. Usman, S. Shamim, and M. Islam. 2014. Cytokine and clinical response to Saccharomyces boulardii therapy in diarrhea-dominant irritable bowel syndrome: randomized trial. *European Journal of Gastroenterology & Hepatology* 26:630–9. doi: 10.1097/MEG.0000000000000094.

Adam, J., C. Barret, A. Barret-Bellet, E. Benedetti, A. Calendini, P. Darchen, J. Galibert, P. Guerci, G. Guiot, and M. Haechler. 1977. Controlled double-blind clinical trials of Ultra-Levure: Multicentre study by 25 physicians in 388 cases. *Medicine and Chirurgie Digestives* 84:2072–8.

Ahmadi, A., E. Milani, A. Madadlou, S. A. Mortazavi, R. R. Mokarram, and D. Salarbashi. 2014. Synbiotic yogurt-ice cream produced via incorporation of microencapsulated lactobacillus acidophilus (la-5) and fructooligosaccharide. *Journal of Food Science and Technology* 51:1568–74. doi: 10.1007/s13197-012-0679-y.

Al-Jailawi, M. H., A. Al-Shekdhaher, and R Al-Zaiadi. 2016. Genetic improvement of Saccharomyces boulardii R7 and generate suitable strains for synthesis and expression of recombinant products. *British Biotechnology Journal* 11 (2):1–9. doi: 10.9734/BBJ/2016/22927.

Ansari, F., H. Pourjafar, and S. Esmailpour. 2017a. Study on citric acid production and antibacterial activity of kombucha green tea beverage during production and storage. *Annual Research & Review in Biology* 16 (3):1–8. doi: 10.9734/ARRB/2017/35664.

Ansari, F., H. Pourjafar, V. Jodat, J. Sahebi, and A. Ataei. 2017b. Effect of Eudragit S100 nanoparticles and alginate chitosan encapsulation on the viability of Lactobacillus acidophilus and Lactobacillus rhamnosus. *AMB Express* 7:144. doi: 10.1186/s13568-017-0442-x.

Ansari, F., H. Pourjafar, A. Kangari, and A. Homayouni. 2019. Evaluation of the glucuronic acid production and antibacterial properties of Kombucha Black Tea. *Current Pharmaceutical Biotechnology* 20:985–90. doi: 10.2174/1389201020666190717100958.

Araújo, E. A., A. C. dos Santos Pires, M. S. Pinto, G. Jan, and A. Carvalho. 2012. Chapter 6: Probiotics in dairy fermented products. In *Probiotics*, ed. E. Rigobelo, 129–48. London: IntechOpen doi: 10.5772/51939..

Arevalo-Villena, M., A. Briones-Perez, M. Corbo, M. Sinigaglia, and A. Bevilacqua. 2017. Biotechnological application of yeasts in food science: Starter cultures, probiotics and enzyme production. *Journal of Applied Microbiology* 123:1360–72. doi: 10.1111/jam.13548.

Azhar, S. H. M., R. Abdulla, S. A. Jambo, H. Marbawi, J. A. Gansau, A. A. M. Faik, and K. Rodrigues. 2017. Yeasts in sustainable bioethanol production: A review. *Biochemistry and Biophysics Reports* 10:52–61. doi: 10.1016/j.bbrep.2017.03.003.

Bafutto, M., J. R. d Almeida, N. V. Leite, M. B. G. Costa, E. C. d Oliveira, and J. Resende-Filho. 2013. Treatment of diarrhea-predominant irritable bowel syndrome with mesalazine and/or Saccharomyces boulardii. *Arquivos de Gastroenterologia* 50:304–9. doi: 10.1590/S0004-28032013000400012.

Bahgat, M., A. Maghraby, O. D.-F. Ab, and A. Elshafei. 2005. Immunization of mice with crude extract of Saccharomyces boulardii yeast induces cross-reactive immune responses with antigenic preparations from different developmental stages of the Schistosoma mansoni and reduces the parasite worm burden. *Journal of the Egyptian Society of Parasitology* 35:563–80.

Bai, F., L. Chen, Z. Zhang, W. Anderson, and M. Moo-Young. 2004. Continuous ethanol production and evaluation of yeast cell lysis and viability loss under very high gravity medium conditions. *Journal of Biotechnology* 110:287–93. doi: 10.1016/j.jbiotec.2004.01.017.

Barnett, J. A. 2000. A history of research on yeasts 2: Louis Pasteur and his contemporaries, 1850–1880. *Yeast* 16 (8):755–71. doi: 10.1002/1097-0061(20000615)16:8<755::AID-YEA587>3.0.CO;2-4.

Barzegari, A., S. Eslami, E. Ghabeli, and Y. Omidi. 2014. Imposition of encapsulated non-indigenous probiotics into intestine may disturb human core microbiome. *Frontiers in Microbiology* 5:393. doi: 10.3389/fmicb.2014.00393.

Beena Divya, J., K. Kulangara Varsha, K. Madhavan Nampoothiri, B. Ismail, and A. Pandey. 2012. Probiotic fermented foods for health benefits. *Engineering in Life Sciences* 12:377–90. doi: 10.1002/elsc.201100179.

Berni, R. C., S. Cucchiara, R. Cuomo, F. Pace, and F. Papale. 2011. Saccharomyces boulardii: a summary of the evidence for gastroenterology clinical practice in adults and children. *European Review for Medical and Pharmacological Sciences* 15:809–22.

Besirbellioglu, B. A., A. Ulcay, M. Can, H. Erdem, M. Tanyuksel, I. Y. Avci, E. Araz, and A. Pahsa. 2006. Saccharomyces boulardii and infection due to Giardia lamblia. *Scandinavian Journal of Infectious Diseases* 38:479–81. doi: 10.1080/00365540600561769.

Billoo, A. G., M. Memon, S. Khaskheli, G. Murtaza, K. Iqbal, M. S. Shekhani, and A. Q. Siddiqi. 2006. Role of a probiotic (Saccharomyces boulardii) in management and prevention of diarrhoea. *World Journal of Gastroenterology* 12:4557–60. doi: 10.3748/wjg.v12.i28.4557.

Bin, Z., X. Ya-Zheng, D. Zhao-Hui, C. Bo, J. Li-Rong, and Y. Vandenplas. 2015. The efficacy of Saccharomyces boulardii CNCM I-745 in addition to standard Helicobacter pylori eradication treatment in children. *Pediatric Gastroenterology, Hepatology & Nutrition* 18:17–22. doi: 10.5223/pghn.2015.18.1.17.

Bisson, J.-F., S. Hidalgo, P. Rozan, and M. Messaoudi. 2010. Preventive effects of different probiotic formulations on travelers' diarrhea model in wistar rats: Preventive effects of probiotics on TD. *Digestive Diseases and Sciences* 55:911–9. doi: 10.1007/s10620-009-0822-4.

Bleichner, G., H. Blehaut, H. Mentec, and D. Moyse. 1997. Saccharomyces boulardii prevents diarrhea in critically ill tube-fed patients. *Intensive Care Medicine* 23:517–23. doi: 10.1007/s001340050367.

Bourreille, A., G. Cadiot, G. Le Dreau, D. Laharie, L. Beaugerie, J.-L. Dupas, P. Marteau, P. Rampal, D. Moyse, A. Saleh, et al. 2013. Saccharomyces boulardii does not prevent relapse of Crohn's disease. *Clinical Gastroenterology and Hepatology* 11:982–7. doi: 10.1016/j.cgh.2013.02.021.

Bravo, M. V., D. Bunout, L. Leiva, M. P. de la Maza, G. Barrera, J. de la Maza, and S. Hirsch. 2008. Effect of probiotic Saccharomyces boulardii on prevention of antibiotic-associated diarrhea in adult outpatients with amoxicillin treatment. *Revista Médica de Chile* 136:981–8.

Briand, F., T. Sulpice, P. Giammarinaro, and X. Roux. 2019. Saccharomyces boulardii CNCM I-745 changes lipidemic profile and gut microbiota in a hamster hypercholesterolemic model. *Beneficial Microbes* 10:555–67. doi: 10.3920/BM2018.0134.

Bruns, R., and R. Raedsch. 1995. Therapy of traveller's diarrhea. *Medizinische Welt* 46:591–6.

Buccigrossi, V., G. Laudiero, C. Russo, E. Miele, M. Sofia, M. Monini, F. Ruggeri, and A. Guarino. 2014. Chloride Secretion Induced by Rotavirus Is Oxidative Stress-Dependent and Inhibited by Saccharomyces boulardii in Human Enterocytes. *PloS One* 9:e99830. doi: 10.1371/journal.pone.0099830.

Buts, J. P. 1999. Mechanisms of action of biotherapeutic agents. In *Biotherapeutic agents and infectious diseases*, G. W. Elmer, L. V. McFarland, C. M. Surawicz, 27–46. Totowa, NJ: Springer.

Buts, J. P., Corthier, G. Delmee. and M. 1993. Saccharomyces boulardii for Clostridium difficile-associated enteropathies in infants. *Journal of Pediatric Gastroenterology and Nutrition* 16:419–25. doi: 10.1097/00005176-199305000-00013.

Can, M., I. Y. Avci, C. M. Beker, and A. Pahsa. 2006. Prophylactic Saccharomyces boulardii in the prevention of antibiotic-associated diarrhea: A prospective study. *Medical Science Monitor* 12:PI19–PI22.

Capece, A., R. Romaniello, A. Pietrafesa, G. Siesto, R. Pietrafesa, M. Zambuto, and P. Romano. 2018. Use of Saccharomyces cerevisiae var. boulardii in co-fermentations with S. cerevisiae for the production of craft beers with potential healthy value-added. *International Journal of Food Microbiology* 284:22–30. doi: 10.1016/j.ijfoodmicro.2018.06.028.

Cárdenas, P. A., D. Garcés, B. Prado-Vivar, N. Flores, M. Fornasini, H. Cohen, I. Salvador, O. Cargua, and M. E. Baldeón. 2020. Effect of Saccharomyces boulardii CNCM I-745 as complementary treatment of Helicobacter pylori infection on gut microbiome. *European Journal of Clinical Microbiology & Infectious Diseases* 39:1365–72. doi: 10.1007/s10096-020-03854-3.

Carstensen, J. W., M. Chehri, K. Schønning, S. C. Rasmussen, J. Anhøj, N. S. Godtfredsen, C. Ø. Andersen, and A. M. Petersen. 2018. Use of prophylactic Saccharomyces boulardii to prevent Clostridium difficile infection in hospitalized patients: a controlled prospective intervention study. *European Journal of Clinical Microbiology & Infectious Diseases* 37:1431–9. doi: 10.1007/s10096-018-3267-x.

Carvalho, A. S., J. Silva, P. Ho, P. Teixeira, F. X. Malcata, and P. Gibbs. 2003. Impedimetric method for estimating the residual activity of freeze-dried Lactobacillus delbrueckii ssp. bulgaricus. *International Dairy Journal* 13:463–8. doi: 10.1016/S0958-6946(03)00049-9.

Casem, R. A. O. 2013. Saccharomyces boulardii in the prevention of antibioticassociated diarrhea in children: a randomized controlled trial. *Pediatric Infectious Disease Society of the Philippines Journal* 14:70–6.

Castagliuolo, I., M. F. Riegler, L. Valenick, J. T. LaMont, and C. Pothoulakis. 1999. *Saccharomyces boulardii* protease inhibits the effects of *Clostridium difficile* toxins A and B in human colonic mucosa. *Infection and Immunity* 67:302–7.

Cetina-Sauri, G., and G. Sierra Basto. 1994. Evaluation of Saccharomyces boulardii for the treatment of acute diarrhea in pediatric patients. *Annales de Pediatrie* 41 (6):397–400.

Chae, Y. K., S. H. Kim, J. E. Ellinger, and J. L. Markley. 2013. Dosage effects of salt and pH stresses on Saccharomyces cerevisiae as monitored via metabolites by using two dimensional NMR spectroscopy. *Bulletin of the Korean Chemical Society* 34:3602–8. doi: 10.5012/bkcs.2013.34.12.3602.

Chanthala, L., and A. Appaiah. 2014. In vitro probiotic characterization of yeasts of food and environmental origin. *International Journal of Probiotics and Prebiotics* 9:87–92.

Chen, X., J. Fruehauf, J. D. Goldsmith, H. Xu, K. K. Katchar, H. W. Koon, D. Zhao, E. G. Kokkotou, C. Pothoulakis, and C. P. Kelly. 2009. Saccharomyces boulardii inhibits EGF receptor signaling and intestinal tumor growth in Apc(min) mice. *Gastroenterology* 137:914–23. doi: 10.1053/j.gastro.2009.05.050.

Chen, L. S., Y. Ma, J.-L. Maubois, L-j Chen, Q.-H. Liu, and J.-P. Guo. 2010. Identifcation of yeasts from raw milk and selection for some

specific antioxidant properties. *International Journal of Dairy Technology* 63:47–54. doi: 10.1111/j.1471-0307.2009.00548.x.

Chiron, C., T. A. Tompkins, and P. J. Burguière. 2018. Flow cytometry: a versatile technology for specific quantification and viability assessment of micro-organisms in multistrain probiotic products. *Journal of Applied Microbiology* 124:572–84. doi: 10.1111/jam.13666.

Choi, C. H., S. Y. Jo, H. J. Park, S. K. Chang, J.-S. Byeon, and S.-J. Myung. 2011. A randomized, double-blind, placebo-controlled multicenter trial of Saccharomyces boulardii in irritable bowel syndrome: Effect on quality of life. *Journal of Clinical Gastroenterology* 45:679–83. doi: 10.1097/MCG.0b013e318204593e.

Chu, Y., H. Zhu, Y. Zhou, L. Lv, and J. Huo. 2012. Intervention study on Saccharomyces boulardii with proton pump inhibitor (PPI)-based triple therapy for Helicobacter pylori related peptic ulcer. *African Journal of Pharmacy and Pharmacology* 6:2900–4. doi: 10.5897/AJPP12.400.

Cindoruk, M., G. Erkan, T. Karakan, A. Dursun, and S. Unal. 2007. Efficacy and safety of Saccharomyces boulardii in the 14-day triple anti-Helicobacter pylori therapy: A prospective randomized placebo-controlled double-blind study. *Helicobacter* 12:309–16. doi: 10.1111/j.1523-5378.2007.00516.x.

Clydesdale, F. M. 1997. A proposal for the establishment of scientific criteria for health claims for functional foods. *Nutrition Reviews* 55: 413–22. doi: 10.1111/j.1753-4887.1997.tb01588.x.

Collignon, A., C. Sandre, and M.-C. Barc. 2010. Saccharomyces boulardii modulates dendritic cell properties and intestinal microbiota disruption after antibiotic treatment. *Gastroentérologie Clinique et Biologique* 34:S71–S78. doi: 10.1016/S0399-8320(10)70024-5.

Cremonini, F., S. Di Caro, M. Covino, A. Armuzzi, M. Gabrielli, L. Santarelli, E. C. Nista, G. Cammarota, G. Gasbarrini, and A. Gasbarrini. 2002. Effect of different probiotic preparations on anti-Helicobacter pylori therapy-related side effects: A parallel group, triple blind, placebo-controlled study. *The American Journal of Gastroenterology* 97:2744–9. doi: 10.1111/j.1572-0241.2002.07063.x.

Czerucka, D., L. Ibáñez,, R. Pontier-Bres, F. Larbret, A. Rekima, V. Verhasselt, C. Blin-Wakkach. 2019. Saccharomyces boulardii strain CNCM I-745 modifies the mononuclear phagocytes response in the small intestine of mice following salmonella typhimurium infection. *Frontiers in Immunology* 10:643. doi: 10.3389/fimmu.2019.00643.

Czerucka, D., T. Piche, and P. Rampal. 2007. Review article: yeast as probiotics - Saccharomyces boulardii. *Alimentary Pharmacology & Therapeutics* 26:767–78. doi: 10.1111/j.1365-2036.2007.03442.x.

Czerucka, D., and P. Rampal. 2019. Diversity of Saccharomyces boulardii CNCM I-745 mechanisms of action against intestinal infections. *World Journal of Gastroenterology* 25:2188–203. doi: 10.3748/wjg.v25.i18.2188.

Da Silva, N., M. H. Taniwaki, V. C. Junqueira, N. Silveira, M. M. Okazaki, and R. A. R. Gomes. 2018. *Microbiological examination methods of food and water: A laboratory manual*. Boca RAton, FL: CRC Press..

Dalmasso, G., F. Cottrez, V. Imbert, P. Lagadec, J.-F. Peyron, P. Rampal, D. Czerucka, H. Groux, A. Foussat, and V. Brun. 2006. Saccharomyces boulardii inhibits inflammatory bowel disease by trapping T cells in mesenteric lymph nodes. *Gastroenterology* 131: 1812–25. doi: 10.1053/j.gastro.2006.10.001.

Das, S., P. K. Gupta, and R. R. Das. 2016. Efficacy and safety of Saccharomyces boulardii in acute rotavirus diarrhea: double blind randomized controlled trial from a developing country. *Journal of Tropical Pediatrics* 62:464–70. doi: 10.1093/tropej/fmw032.

Datta, S., D. J. Timson, and U. S. Annapure. 2017. Antioxidant properties and global metabolite screening of the probiotic yeast Saccharomyces cerevisiae var. boulardii. *Journal of the Science of Food and Agriculture* 97:3039–49. doi: 10.1002/jsfa.8147.

de la Cruz-Gavia, A., C. Pérez-Alonso, C. Barrera-Díaz, J. Alvarez-Ramírez, H. Carrillo-Navas, and A. Guadarrama-Lezama. 2018. Survival of Saccharomyces cerevisiae microencapsulated with complex coacervate after freezing process. *Food Hydrocolloids* 82:45–52. doi: 10.1016/j.foodhyd.2018.03.045.

De Llanos, R., A. Querol, J. Pemán, M. Gobernado, and M. T. Fernández-Espinar. 2006. Food and probiotic strains from the Saccharomyces cerevisiae species as a possible origin of human systemic infections. *International Journal of Food Microbiology* 110: 286–90. doi: 10.1016/j.ijfoodmicro.2006.04.023.

De Marco, S., M. Sichetti, D. Muradyan, M. Piccioni, G. Traina, R. Pagiotti, and D. Pietrella. 2018. Probiotic cell-free supernatants exhibited anti-inflammatory and antioxidant activity on human gut epithelial cells and macrophages stimulated with LPS. *Evidence-Based Complementary and Alternative Medicine* 2018: 1756308. doi: 10.1155/2018/1756308.

de Oliveira Ribeiro, A. P., F. dos Santos Gomes, K. M. O. dos Santos, V. M. da Matta, D. de Grandi CastroFreitas de Sá, M. C. P. de AraujoSantiago, C. Conte, S. D. de Oliveira Costa, L. de Oliveira Ribeiro, R. L. de Oliveira Godoy, et al. 2020. Development of a probiotic non-fermented blend beverage with juçara fruit: Effect of the matrix on probiotic viability and survival to the gastrointestinal tract. *LWT* 118:108756. doi: 10.1016/j.lwt.2019.108756.

De Ruiter, G. A., S. W. Notermans, and F. M. J. T. Rombouts. 1993. New methods in food mycology. Trends *in Food Science & Technology* 4:91–7. doi: 10.1016/0924-2244(93)90089-S.

Deak, T., and L. Beuchat. 1993. Evaluation of the indirect conductance method for the detection of yeasts in laboratory media and apple juice. Food *Microbiology* 10:255–62. doi: 10.1006/fmic.1993.1029.

Deak, T., and L. Beuchat. 1995. Modified indirect conductimetric technique for detecting low populations of yeasts in beverage concentrates and carbonated beverages. Food *Microbiology* 12:165–72. doi: 10.1016/S0740-0020(95)80093-X.

Dendukuri, N., V. Costa, M. McGregor, and J. M. Brophy. 2005. Probiotic therapy for the prevention and treatment of Clostridium difficile-associated diarrhea: A systematic review. *Canadian Medical Association Journal* 173:167–70. doi: 10.1503/cmaj.050350.

Díaz, M., M. Herrero, L. A. García, and C. Quirós. 2010. Application of flow cytometry to industrial microbial bioprocesses. *Biochemical Engineering Journal* 48:385–407. doi: 10.1016/j.bej.2009.07.013.

Dinleyici, E. C., M. Eren, N. Dogan, S. Reyhanioglu, Z. A. Yargic, and Y. Vandenplas. 2011. Clinical efficacy of Saccharomyces boulardii or metronidazole in symptomatic children with Blastocystis hominis infection. *Parasitology Research* 108:541–5. doi: 10.1007/s00436-010-2095-4.

Dinleyici, E. C., M. Eren, Z. A. Yargic, N. Dogan, and Y. Vandenplas. 2009. Clinical efficacy of Saccharomyces boulardii and metronidazole compared to metronidazole alone in children with acute bloody diarrhea caused by amebiasis: a prospective, randomized, open label study. *The American Journal of Tropical Medicine and Hygiene* 80: 953–5. doi: 10.4269/ajtmh.2009.80.953.

Dinleyici, E. C., A. Kara, N. Dalgic, Z. Kurugol, V. Arica, O. Metin, E. Temur, O. Turel, S. Guven, O. Yasa, et al. 2015. Saccharomyces boulardii CNCM I-745 reduces the duration of diarrhoea, length of emergency care and hospital stay in children with acute diarrhoea. *Beneficial Microbes* 6:415–21. doi: 10.3920/BM2014.0086.

Diplock, A., J.-L. Charuleux, G. Crozier-Willi, F. Kok, C. Rice-Evans, M. Roberfroid, W. Stahl, and J. Vina-Ribes. 1998. Functional food science and defence against reactive oxidative species. *British Journal of Nutrition* 80:S77–S112. doi: 10.1079/BJN19980106.

Douradinha, B., V. C. Reis, M. B. Rogers, F. A. Torres, J. D. Evans, and E. T. Marques Jr. 2014. Novel insights in genetic transformation of the probiotic yeast Saccharomyces boulardii. *Bioengineered* 5 (1): 21–9. doi: 10.4161/bioe.26271.

Drozdova, P. B., O. V. Tarasov, A. G. Matveenko, E. A. Radchenko, J. V. Sopova, D. E. Polev, S. G. Inge-Vechtomov, and P. V. Dobrynin. 2016. Genome sequencing and comparative analysis of Saccharomyces cerevisiae strains of the Peterhof genetic collection. *PloS One* 5:e0154722. 11 doi: 10.1371/journal.pone.0154722.

Du, L. P., R. X. Hao, D. G. Xiao, L. L. Guo, and W. D. Gai. 2012. Research on the characteristics and culture conditions of Saccharomyces boulardii. *Advanced Materials Research* 343–344: 594–8. doi: 10.4028/www.scientific.net/AMR.343-344.594.

Duman, D. G., S. Bor, O. Ozütemiz, T. Sahin, D. Oğuz, F. Iştan, T. Vural, M. Sandkci, F. Işksal, I. Simşek, et al. 2005. Efficacy and safety of Saccharomyces boulardii in prevention of antibiotic-associated diarrhoea due to Helicobacterpylori eradication. *European*

Journal of Gastroenterology & Hepatology 17:1357–61. doi: 10.1097/00042737-200512000-00015.

Duman, D. G., Z. N. Ö. Kumral, F. Ercan, M. Deniz, G. Can, and B. Cağlayan Yeğen. 2013. Saccharomyces boulardii ameliorates clarithromycin- and methotrexate-induced intestinal and hepatic injury in rats. *The British Journal of Nutrition* (3)110:493–9. doi: 10.1017/S000711451200517X.

Edwards-Ingram, L., P. Gitsham, N. Burton, G. Warhurst, I. Clarke, D. Hoyle, S. G. Oliver, and L. Stateva. 2007. Genotypic and physiological characterization of Saccharomyces boulardii, the probiotic strain of Saccharomyces cerevisiae. *Applied and Environmental Microbiology* 73:2458–67. doi: 10.1128/AEM.02201-06.

Ehrhardt, S., N. Guo, R. Hinz, S. Schoppen, J. May, M. Reiser, M. P. Schroeder, S. Schmiedel, M. Keuchel, E. C. Reisinger, et al. 2016. Saccharomyces boulardii to prevent antibiotic-associated diarrhea: a randomized, double-masked, placebo-controlled trial. *Open Forum Infectious Diseases* 3 (1):ofw011. doi: 10.1093/ofid/ofw011.

Elmer, G., L. McFarland, C. Surawicz, L. Danko, and R. Greenberg. 1999. Behaviour of Saccharomyces boulardii in recurrent Clostridium difficile disease patients. *Alimentary Pharmacology & Therapeutics* 13:1663–8. doi: 10.1046/j.1365-2036.1999.00666.x.

Endo, A., and M. Gueimonde. 2016. *Isolation, identification and characterisation of potential new probiotics. Advances in probiotic technology*. Abingdon: Taylor and Francis Group.

Erdoğan, Ö., B. Tanyeri, E. Torun, E. Gönüllü, H. Arslan, U. Erenberk, and F. Öktem. 2012. The comparition of the efficacy of two different probiotics in rotavirus gastroenteritis in children. *Journal of Tropical Medicine* 2012:787240. doi: 10.1155/2012/787240.

Fakruddin, M., M. N. Hossain, and M. M. Ahmed. 2017. Antimicrobial and antioxidant activities of Saccharomyces cerevisiae IFST062013, a potential probiotic. *BMC Complementary and Alternative Medicine* 17 (1):64. doi: 10.1186/s12906-017-1591-9.

Fakruddin, M., M. A. Islam, M. M. Ahmed, and N. J. Chowdhury. 2013. Process optimization of bioethanol production by stress tolerant yeasts isolated from agro-industrial waste. *International Journal of Renewable and Sustainable Energy* 2 (4):133–9. doi: 10.11648/j.ijrse.20130204.11.

Fang, G., Z. Li, C. Su, and G. Hu. 2017. Clinical observation of saccharomyces boulardii combined with cetirizine hydrochloride in children allergic rhinitis. *Journal of Clinical Otorhinolaryngology, Head, and Neck Surgery* 31:1649–52.

FAO/WHO. 2002. Guidelines for the evaluation of probiotics in food. https://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf.

Farache, J., I. Koren, I. Milo, I. Gurevich, K.-W. Kim, E. Zigmond, G. C. Furtado, S. A. Lira, and G. Shakhar. 2013. Luminal bacteria recruit CD103+ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation. *Immunity* 38:581–95. doi: 10.1016/j.immuni.2013.01.009.

Fidan, I., A. Kalkanci, E. Yesilyurt, B. Yalcin, B. Erdal, S. Kustimur, and T. Imir. 2009. Effects of Saccharomyces boulardii on cytokine secretion from intraepithelial lymphocytes infected by Escherichia coli and Candida albicans. *Mycoses* 52:29–34. doi: 10.1111/j.1439-0507.2008.01545.x.

Fietto, J. L., R. S. Araújo, F. N. Valadão, L. G. Fietto, R. L. Brandão, M. J. Neves, F. C. Gomes, J. R. Nicoli, and I. M. Castro. 2004. Molecular and physiological comparisons between Saccharomyces cerevisiae and Saccharomyces boulardii. *Canadian Journal of Microbiology* 50:615–21. doi: 10.1139/w04-050.

Fleet, G. H., and M. J. Mian. 1987. The occurrence and growth of yeasts in dairy products. *International Journal of Food Microbiology* 4:145–55. doi: 10.1016/0168-1605(87)90021-3.

Fortin, O., B. Aguilar-Uscanga, K. D. Vu, S. Salmieri, and M. Lacroix. 2018a. Cancer chemopreventive, antiproliferative, and superoxide anion scavenging properties of Kluyveromyces marxianus and Saccharomyces cerevisiae var. boulardii cell wall components. *Nutrition and Cancer* 70:83–96. doi: 10.1080/01635581.2018.1380204.

Fortin, O., B. R. Aguilar-Uscanga, K. D. Vu, S. Salmieri, and M. Lacroix. 2018b. Effect of Saccharomyces boulardii cell wall extracts on colon cancer prevention in male F344 rats treated with 1,2-Dimethylhydrazine. *Nutrition and Cancer* 70:632–42. doi: 10.1080/01635581.2018.1460672.

Fung, D. Y. F. 2002. Predictions for rapid methods and automation in food microbiology. *Journal of AOAC International* 85:1000–2. doi: 10.1093/jaoac/85.4.1000.

Ganguly, R., R. Chakraborty, and K. Sarkar. 2019. Inulin Induced Co-Aggregation of Saccharomyces boulardii with Potential Pathogenic Bacteria. *International Journal of Probiotics & Prebiotics* 14 (1): 18–23.

Gaon, D., H. Garcia, L. Winter, N. Rodríguez, R. Quintas, S. N. Gonzalez, and G. Oliver. 2003. Effect of Lactobacillus strains and Saccharomyces boulardii on persistent diarrhea in children. *Medicina (B Aires)* 63:293–8.

Garcia Vilela, E., M. De Lourdes De Abreu Ferrari, H. Oswaldo Da Gama Torres, A. Guerra Pinto, A. Carolina Carneiro Aguirre, F. Paiva Martins, E. Marcos Andrade Goulart, and A. Sales Da Cunha. 2008. Influence of Saccharomyces boulardii on the intestinal permeability of patients with Crohn's disease in remission. *Scandinavian Journal of Gastroenterology* 43:842–8. doi: 10.1080/00365520801943354.

García, G., M. E. Agosto, L. Cavaglieri, and C. Dogi. 2020. Effect of fermented whey with a probiotic bacterium on gut immune system. *Journal of Dairy Research* 87:134–7. doi: 10.1017/S0022029919000980.

Ghasemnezhad, R., V. Razavilar, H. Pourjafar, K. Khosravi-Darani, and K. Ala. 2017. The viability of free and encapsulated Lactobacillus casei and Bifidobacterium animalis in chocolate milk, and evaluation of its pH changes and sensory properties during storage. *Annual Research & Review in Biology* 21 (3):1–8. doi: 10.9734/ARRB/2017/37885.

Ghorbani-Choboghlo, H., D. Nikaein, A. Khosravi, R. Rahmani, and Z. Farahnejad. 2019. Effect of microencapsulation on Saccharomyces cerevisiae var. boulardii viability in the gastrointestinal tract and level of some blood biochemical factors in wistar rats. *Iranian Journal of Microbiology* 11:160–5. doi: 10.18502/ijm.v11i2.1082.

Ghorbani-Choboghlo, H., T. Zahraei-Salehi, J. Ashrafi-Helan, R. Yahyaraeyat, H. Pourjafar, D. Nikaein, A. Balal, and A.-R. Khosravi. 2015. Microencapsulation of Saccharomyces cerevisiae and its evaluation to protect in simulated gastric conditions. *Iranian Journal of Microbiology* 7:338.

Giang, H. H., T. Q. Viet, B. Ogle, and J. E. Lindberg. 2012. Growth performance, digestibility, gut environment and health status in weaned piglets fed a diet supplemented with a complex of lactic acid bacteria alone or in combination with Bacillus subtilis and Saccharomyces boulardii. *Livestock Science* 143:132–41. doi: 10.1016/j.livsci.2011.09.003.

Girard, P., Y. Pansart, and M. Verleye. 2014. Anti-Hypercholesterolemic Effect of Saccharomyces boulardii in the Hamster. *Pharmacology* 94:239–44. doi: 10.1159/000365009.

Giudici, P., G. Masini, and C. Caggia. 2003. The role of galactose fermenting yeast in plain yogurt spoilageet. *Annali di Microbiologia ed Enzimologia* 46 (1):11–9.

Goffeau, A., B. G. Barrell, H. Bussey, R. W. Davis, B. Dujon, H. Feldmann, F. Galibert, J. D. Hoheisel, C. Jacq, M. Johnston, et al. 1996. Life with 6000 genes. *Science* 274:546–67. doi: 10.1126/science.274.5287.546.

Goodenough, E., and D. Kleyn. 1976. Qualitative and quantitative changes in carbohydrates during the manufacture of Yogurt1. *Journal of Dairy Science* 59:45–7. doi: 10.3168/jds.S0022-0302(76)84153-7.

Graff, S., J.-C. Chaumeil, P. Boy, R. Lai-Kuen, and C. Charrueau. 2008. Influence of pH conditions on the viability of Saccharomyces boulardii yeast. *The Journal of General and Applied Microbiology* 54: 221–7. doi: 10.2323/jgam.54.221.

Granato, D., G. F. Branco, A. G. Cruz, J. d A. F. Faria, and N. P. Shah. 2010a. Probiotic dairy products as functional foods. *Comprehensive Reviews in Food Science and Food Safety* 9:455–70. doi: 10.1111/j.1541-4337.2010.00120.x.

Granato, D., G. F. Branco, F. Nazzaro, A. G. Cruz, and J. A. Faria. 2010b. Functional foods and nondairy probiotic food development:

Trends, concepts, and products. *Comprehensive Reviews in Food Science and Food Safety* 9:292–302. doi: 10.1111/j.1541-4337.2010.00110.x.

Guo, X., R. Long, M. Kreuzer, L. Ding, Z. Shang, Y. Zhang, Y. Yang, and G. Cui. 2014. Importance of functional ingredients in yak milk-derived food on health of Tibetan nomads living under high-altitude stress: a review. *Critical Reviews in Food Science and Nutrition* 54: 292–302. doi: 10.1080/10408398.2011.584134.

Guslandi, M. 2015. Role of probiotics in Crohn's disease and in Pouchitis. *Journal of Clinical Gastroenterology* 49:S46–S49. doi: 10.1097/MCG.0000000000000351.

Guslandi, M., G. Mezzi, M. Sorghi, and P. A. Testoni. 2000. Saccharomyces boulardii in maintenance treatment of Crohn's disease. *Digestive Diseases and Sciences* 45:1462–4. doi: 10.1023/A:1005588911207.

Hamedi, H., A. Misaghi, M. H. Modarressi, T. Z. Salehi, D. Khorasanizadeh, and V. Khalaj. 2013. Generation of a uracil auxotroph strain of the probiotic yeast Saccharomyces boulardii as a host for the recombinant protein production. *Avicenna Journal of Medical Biotechnology* 5 (1):29–34.

Heling, A. L., O. J. Kuhn, J. R. Stangarlin, N. P. Henkemeier, S. Coltro-Roncato, and E. D. V. Gonçalves. 2017. Controle biológico de antracnose em pós colheita de banana "Maçã" com Saccharomyces spp. *Summa Phytopathologica* 43:49–51. doi: 10.1590/0100-5405/2105.

Hennequin, C., C. Kauffmann-Lacroix, A. Jobert, J. Viard, C. Ricour, J. Jacquemin, and P. Berche. 2000. Possible role of catheters in Saccharomyces boulardii fungemia. *European Journal of Clinical Microbiology & Infectious Diseases* 19 (1):16–20. doi: 10.1007/s100960050003.

Heydari, S., A. M. Mortazavian, M. R. Ehsani, M. A. Mohammadifar, and H. Ezzatpanah. 2011. Biochemical, microbiological and sensory characteristics of probiotic yogurt containing various prebiotic compounds. *Italian Journal of Food Science* 23 (2):153–64.

Hill, C., F. Guarner, G. Reid, G. R. Gibson, D. J. Merenstein, B. Pot, L. Morelli, R. B. Canani, H. J. Flint, S. Salminen, et al. 2014. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology* 11:506–14. doi: 10.1038/nrgastro.2014.66.

Holzapfel, W. H., P. Haberer, R. Geisen, J. Björkroth, and U. Schillinger. 2001. Taxonomy and important features of probiotic microorganisms in food and nutrition. *The American Journal of Clinical Nutrition* 73:365S–73s. doi: 10.1093/ajcn/73.2.365s.

Holzapfel, W. H., P. Haberer, J. Snel, U. Schillinger, and J. H. Huis in't Veld. 1998. Overview of gut flora and probiotics. *International Journal of Food Microbiology* 41:85–101. doi: 10.1016/S0168-1605(98)00044-0.

Homayouni, A., F. Ansari, A. Azizi, H. Pourjafar, and M. Madadi. 2020a. Cheese as a potential food carrier to deliver probiotic microorganisms into the human gut: a review. *Current Nutrition & Food Science* 16:15–28. doi: 10.2174/1573401314666180817101526.

Homayouni, A., A. Azizi, M. Ehsani, M. Yarmand, and S. Razavi. 2008. Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. *Food Chemistry* 111:50–5. doi: 10.1016/j.foodchem.2008.03.036.

Homayouni, A., M. Javadi, F. Ansari, H. Pourjafar, M. Jafarzadeh, and A. Barzegar. 2018. Advanced methods in ice cream analysis: A review. *Food Analytical Methods* 11:3224–34. doi: 10.1007/s12161-018-1292-0.

Homayouni, A., R. R. Mokarram, S. Norouzi, A. Dehnad, A. Barkhordari, H. Homayouni, and H. Pourjafar. 2020b. Soy ice cream as a carrier for efficient delivering of Lactobacillus casei. *Nutrition & Food Science* 51 (1):61–70. doi: 10.1108/NFS-11-2019-0349.

Hossain, M. N., S. Afrin, S. Humayun, M. M. Ahmed, and B. K. Saha. 2020. Identification and Growth Characterization of a Novel Strain of Saccharomyces boulardii Isolated From Soya Paste. *Frontiers in Nutrition* 7:27. doi: 10.3389/fnut.2020.00027.

Htwe, K., K. S. Yee, M. Tin, and Y. Vandenplas. 2008. Effect of Saccharomyces boulardii in the treatment of acute watery diarrhea in Myanmar children: a randomized controlled study. *The American Journal of Tropical Medicine and Hygiene* 78:214–6. doi: 10.4269/ajtmh.2008.78.214.

Hudson, L. E., M. B. Fasken, C. D. McDermott, S. M. McBride, E. G. Kuiper, D. B. Guiliano, A. H. Corbett, and T. J. Lamb. 2014. Functional heterologous protein expression by genetically engineered probiotic yeast Saccharomyces boulardii. *Plos One* 9 (11):e112660. doi: 10.1371/journal.pone.0112660.

Hudson, L. E., C. D. McDermott, T. P. Stewart, W. H. Hudson, D. Rios, M. B. Fasken, A. H. Corbett, and T. J. Lamb. 2016. Characterization of the probiotic yeast Saccharomyces boulardii in the healthy mucosal immune system. *PloS One* 11:e0153351. doi: 10.1371/journal.pone.0153351.

Huq, T., A. Khan, R. A. Khan, B. Riedl, and M. Lacroix. 2013. Encapsulation of probiotic bacteria in biopolymeric system. *Critical Reviews in Food Science and Nutrition* 53:909–16. doi: 10.1080/10408398.2011.573152.

Im, E., and C. Pothoulakis. 2010. Recent advances in Saccharomyces boulardii research. Gastroentérologie *Clinique* et *Biologique* 34: S62–S70. doi: 10.1016/S0399-8320(10)70023-3.

Jack, L., F. Coyer, M. Courtney, and B. Venkatesh. 2010. Probiotics and diarrhoea management in enterally tube fed critically ill patients-what is the evidence? *Intensive & Critical Care Nursing* 26: 314–26. doi: 10.1016/j.iccn.2010.07.001.

Jakobsen, M., and J. Narvhus. 1996. Yeasts and their possible beneficial and negative effects on the quality of dairy products. *International Dairy Journal* 6:755–68. doi: 10.1016/0958-6946(95)00071-2.

Jay, J. M., M. J. Loessner, and D. A. Golden. 2008. *Modern food microbiology*. New York: Springer Science & Business Media.

Jayan, H., H. Pu, and D. W. Sun. 2019. Recent development in rapid detection techniques for microorganism activities in food matrices using bio-recognition: A review. *Trends in Food Science & Technology* 95:233–46. doi: 10.1016/j.tifs.2019.11.007.

Johansson, M. E., and G. C. Hansson. 2016. Immunological aspects of intestinal mucus and mucins. *Nature Reviews Immunology* 16: 639–49. doi: 10.1038/nri.2016.88.

Joossens, S., P. Suenaert, M. Noman, S. Vermeire, and P. Rutgeerts. 2005. Saccharomyces boulardii in Crohn's disease: effect on anti-Saccharomyces cerevisiae antibodies and intestinal permeability. *Inflammatory Bowel Disease* 11:863–4. doi: 10.1097/01.mib.0000183543.72225.08.

Justino, P. F. C., A. X. Franco, R. Pontier-Bres, C. E. S. Monteiro, A. L. R. Barbosa, M. H. L. P. Souza, D. Czerucka, and P. M. G. Soares. 2020. Modulation of 5-fluorouracil activation of toll-like/MyD88/NF-κB/MAPK pathway by Saccharomyces boulardii CNCM I-745 probiotic. *Cytokine* 125:154791. doi: 10.1016/j.cyto.2019.154791.

Kabbani, T. A., K. Pallav, S. E. Dowd, J. Villafuerte-Galvez, R. R. Vanga, N. E. Castillo, J. Hansen, M. Dennis, D. A. Leffler, and C. P. Kelly. 2017. Prospective randomized controlled study on the effects of Saccharomyces boulardii CNCM I-745 and amoxicillin-clavulanate or the combination on the gut microbiota of healthy volunteers. *Gut Microbes* 8:17–32. doi: 10.1080/19490976.2016.1267890.

Kabir, M., S. Ishaque, M. Ali, M. Mahmuduzzaman, and M. Hasan. 2011. Role of Saccharomyces boulardii in diarrhea predominant irritable bowel syndrome. *Mymensingh Medical Journal* 20:397–401.

Kelesidis, T., and C. Pothoulakis. 2012. Efficacy and safety of the probiotic Saccharomyces boulardii for the prevention and therapy of gastrointestinal disorders. *Therapeutic Advances in Gastroenterology* 5:111–25. doi: 10.1177/1756283X11428502.

Kelly, C. P., C. Chong Nguyen, L. J. Palmieri, K. Pallav, S. E. Dowd, L. Humbert, P. Seksik, A. Bado, B. Coffin, D. Rainteau, et al. 2019. Saccharomyces boulardii CNCM I-745 modulates the fecal bile acids metabolism during antimicrobial therapy in healthy volunteers. *Frontiers in Microbiology* 10:336. doi: 10.3389/fmicb.2019.00336.

Khaneghah, A. M., K. Abhari, I. Eş, M. B. Soares, R. B. A. Oliveira, H. Hosseini, M. Rezaei, C. F. Balthazar, R. Silva, A. G. Cruz, et al. 2020. Interactions between probiotics and pathogenic

microorganisms in hosts and foods: A review. *Trends in Food Science & Technology* 95:205–18. doi: 10.1016/j.tifs.2019.11.022.

Khatri, I., R. Tomar, K. Ganesan, G. Prasad, and S. Subramanian. 2017. Complete genome sequence and comparative genomics of the probiotic yeast Saccharomyces boulardii. *Scientific Reports* 7 (1):12. doi: 10.1038/s41598-017-00414-2.

Khidhr, K. O., and Z. M. A. Zubaidy. 2014. Isolation and Identification of Saccharomyces cerevisiae var boulardii and its Uses as a Probiotic (in vitro). *Rafidain Journal of Science* 25:1–11.

Kimmey, M. B., G. W. Elmer, C. M. Surawicz, and L. V. McFarland. 1990. Prevention of further recurrences of Clostridium difficile colitis with Saccharomyces boulardii. *Digestive Diseases and Sciences* 35: 897–901. doi: 10.1007/BF01536805.

Kirchhelle, A., N. Frühwein, and D. Tobüren. 1996. Treatment of persistent diarrhea with S. boulardii in returning travelers. Results of a prospective study. *Fortschritte er Medizin* 114:136–40.

Klein, S. M., G. W. Elmer, L. V. McFarland, C. M. Surawicz, and R. H. Levy. 1993. Recovery and elimination of the biotherapeutic agent, Saccharomyces boulardii, in healthy human volunteers. *Pharmaceutical Research* 10:1615–9. doi: 10.1023/A:1018924820333.

Kollaritsch, H., H. Holst, P. Grobara, and G. Wiedermann. 1993. Prevention of traveler's diarrhea with Saccharomyces boulardii. Results of a placebo controlled double-blind study. *Fortschritte er Medizin* 111:152–6.

Kollaritsch, H., P. Kremsner, G. Wiedermann, and O. Scheiner. 1989. Prevention of traveller's diarrhea: comparison of different non-antibiotic preparations. *The International Society of Travel Medicine* 6: 9–17. https://www.epistemonikos.org/en/documents/48dcb727175962d03a2d93c03aaac160633c6e3a.

Koon, H. W., B. Su, C. Xu, C. C. Mussatto, D. H.-N. Tran, E. C. Lee, C. Ortiz, J. Wang, J. E. Lee, S. Ho, et al. 2016. Probiotic Saccharomyces boulardii CNCM I-745 prevents outbreak-associated Clostridium difficile-associated cecal inflammation in hamsters. *American Journal of Physiology* 311:G610–G623. doi: 10.1152/ajpgi.00150.2016.

Kreger-van Rij, N. J. W. 2013. *The yeasts: a taxonomic study*. Amsterdam: Elsevier.

Kuda, T., H. Takahashi, and B. Kimura. 2016. Alcohol-brewing properties of acid-and bile-tolerant yeasts co-cultured with lactic acid bacteria isolated from traditional handmade domestic dairy products from Inner Mongolia. *LWT - Food Science and Technology* 65:62–9. doi: 10.1016/j.lwt.2015.07.065.

Kurugöl, Z., and G. Koturoğlu. 2005. Effects of Saccharomyces boulardii in children with acute diarrhoea. *Acta Paediatrica* 94:44–7. doi: 10.1080/08035250410022521.

Kyriakos, N., K. Papamichael, A. Roussos, I. Theodoropoulos, C. Karakoidas, A. Smyrnidis, E. Archavlis, K. Lariou, and G. J. Mantzaris. 2013. A lyophilized form of Saccharomyces boulardii enhances the Helicobacter pylori eradication rates of omeprazole-triple therapy in patients with peptic ulcer disease or functional dyspepsia. *Hospital Chronicles* 8:127–33.

Lang, F., and A. Lang. 1975. Acidophilus milk products: little known cultured milks of great potential. *Milk Industry* 77 (3):4–5. https://agris.fao.org/agris-search/search.do?recordID=CZ19760058818.

Lara-Hidalgo, C., L. Dorantes-Álvarez, H. Hernández-Sánchez, F. Santoyo-Tepole, A. Martínez-Torres, L. Villa-Tanaca, and C. Hernández-Rodríguez. 2019. Isolation of yeasts from Guajillo Pepper (Capsicum annuum L.) fermentation and study of some probiotic characteristics. *Probiotics Antimicrob Proteins* 11:748–64. doi: 10.1007/s12602-018-9415-x.

Lazo-Vélez, M., S. Serna-Saldívar, M. Rosales-Medina, M. Tinoco-Alvear, and and M. Briones-García. 2018. Application of Saccharomyces cerevisiae var. boulardii in food processing: A review. *Journal of Applied Microbiology* 125:943–51.

Le, B. L., G. Makhoul, and J. Duhamel. 2010. A multicentric study of a lactose free formula supplemented with Saccharomyces boulardii in children with acute diarrhea. *Archives de Pédiatrie* 17:459–65.

Lee, S. K., H. J. Kim, and S. G. Chi. 2010. Saccharomyces boulardii reduced intestinal inflammation in mice model of 2,4,6-trinitrobencene sulfonic acid induced colitis: Based on microarray. *The Korean Journal of Gastroenterology* 55:33–45. doi: 10.4166/kjg.2010.55.1.33.

Lei, Z., H. Chen, D. Huang, Y. Zhai, and S. Guowei. 2016. Optimization of medium compositions for Saccharomyces boulardii by box-behnken design. Scientific Study and Research: Chemistry and Chemical Engineering. *Biotechnology, Food Industry* 17:405–16.

Leventogiannis, K., P. Gkolfakis, G. Spithakis, A. Tsatali, A. Pistiki, A. Sioulas, E. J. Giamarellos-Bourboulis, and K. Triantafyllou. 2019. Effect of a preparation of four probiotics on symptoms of patients with irritable bowel syndrome: association with intestinal bacterial overgrowth. *Probiotics Antimicrob Proteins* 11:627–34. doi: 10.1007/s12602-018-9401-3.

Lewis, S., L. Potts, and R. Barry. 1998. The lack of therapeutic effect of Saccharomyces boulardii in the prevention of antibiotic-related diarrhoea in elderly patients. *Journal of Infection* 36:171–4. doi: 10.1016/S0163-4453(98)80008-X.

Liboredo, J. C., L. R. Anastácio, M. d C. G. Pelúzio, F. X. Valente, L. C. P. Penido, J. R. Nicoli, and M. I. T. D. Correia. 2013. Effect of probiotics on the development of dimethylhydrazine-induced preneoplastic lesions in the mice colon. *Acta Cirurgica Brasileira* 28: 367–72. doi: 10.1590/S0102-86502013000500008.

Liu, J.-J., I. I. Kong, G.-C. Zhang, L. N. Jayakody, H. Kim, P.-F. Xia, S. Kwak, B. H. Sung, J.-H. Sohn, H. E. Walukiewicz, et al. 2016. Metabolic engineering of probiotic Saccharomyces boulardii. *Applied and Environmental Microbiology* 82:2280–7. doi: 10.1128/AEM.00057-16.

Lourens-Hattingh, A., and B. Viljoen. 2001. Growth and survival of a probiotic yeast in dairy products. *Food Research International* 34: 791–6. doi: 10.1016/S0963-9969(01)00085-0.

Malviya, A., S. Malviya, A. Agarwal, M. Mishra, and V. Dalmida. 2019. Saccharomyces-eukaryotic probiotic for human applications. In *A handbook on high value fermentation products, volume 2: Human welfare*, ed. S. Saran, V. Babu, A. Chaubey, 211. Hoboken: Wiley.

Mansour-Ghanaei, F., N., Dehbashi, K. Yazdanparast, and A. Shafaghi. 2003. Efficacy of Saccharomyces boulardii with antibiotics in acute amoebiasis. *World Journal of Gastroenterology* 9:1832. doi: 10.3748/wjg.v9.i8.1832.

Marshall, D. L., and J. K. Odame-Darkwah. 1995. Influence of pH and NaCl on growth and survival of Propionibacterium freudenreichii subsp. shermanii, Bacillus pumilus, and Saccharomyces cerevisiae in broth media. *LWT - Food Science and Technology* 28:222–6. doi: 10.1016/S0023-6438(95)91554-0.

Marteau, P. 2001. Safety aspects of probiotic products. *Näringsforskning* 45:22–4. doi: 10.3402/fnr.v45i0.1785.

Martins, F. S., A. A. Silva, A. T. Vieira, F. H. Barbosa, R. M. Arantes, M. M. Teixeira, and J. R. Nicoli. 2009. Comparative study of Bifidobacteriumanimalis, Escherichiacoli, Lactobacilluscasei and Saccharomycesboulardii probiotic properties. *Archives of Microbiology* 191:623–30. doi: 10.1007/s00203-009-0491-x.

Maupas, J., P. Champemont, and M. Delforge. 1983. Treatment of irritable bowel syndrome. Double blind trial of Saccharomyces boulardii. *Medecine et Chirurgie Digestives* 12:77–9.

McCullough, M. J., K. V. Clemons, J. H. McCusker, and D. A. Stevens. 1998. Species identification and virulence attributes of Saccharomyces boulardii (nom. inval). *Journal of Clinical Microbiology* 36:2613–7. doi: 10.1128/JCM.36.9.2613-2617.1998.

McFarland, L. 2017. Common organisms and probiotics: Saccharomyces boulardii. In *The Microbiota in Gastrointestinal Pathophysiology*, ed. M. H. Floch, Y. Ringel, and W. Allan Walker, 145–64. Amsterdam: Elsevier.

McFarland, L. V. 1996. Saccharomyces boulardii is not Saccharomyces cerevisiae. *Clinical Infectious Diseases* 22:200–1. doi: 10.1093/clinids/22.1.200.

McFarland, L. V. 2009. Evidence-based review of probiotics for antibiotic-associated diarrhea and Clostridium difficile infections. *Anaerobe* 15:274–80. doi: 10.1016/j.anaerobe.2009.09.002.

McFarland, L. V. 2010. Systematic review and meta-analysis of Saccharomyces boulardii in adult patients. *World Journal of Gastroenterology* 16:2202. doi: 10.3748/wjg.v16.i18.2202.

McFarland, L. V. 2015. From yaks to yogurt: The history, development, and current use of probiotics. *Clinical Infectious Diseases* 60: S85–S90. doi: 10.1093/cid/civ054.

McFarland, L. V., and P. Bernasconi. 1993. Saccharomyces boulardii. A review of an innovative biotherapeutic agent. *Microbial Ecology in Health and Disease* 6:157–71. doi: 10.3109/08910609309141323.

McFarland, L. V., C. M. Surawicz, R. N. Greenberg, G. W. Elmer, K. A. Moyer, S. A. Melcher, K. E. Bowen, and J. L. Cox. 1995. Prevention of b-lactam-associated diarrhea by Saccharomyces boulardii compared with placebo. *American Journal of Gastroenterology* 90:439–48.

McFarland, L. V., C. M. Surawicz, R. N. Greenberg, R. Fekety, G. W. Elmer, K. A. Moyer, S. A. Melcher, K. E. Bowen, J. L. Cox, and Z. Noorani. 1994. A randomized placebo-controlled trial of Saccharomyces boulardii in combination with standard antibiotics for Clostridium difficile disease. *JAMA* 271:1913–8. doi: 10.1001/jama.1994.03510480037031.

Menezes, A. G. T., C. L. Ramos, G. Cenzi, D. S. Melo, D. R. Dias, and R. F. Schwan. 2019. Probiotic potential, antioxidant activity, and phytase production of indigenous yeasts isolated from indigenous fermented foods. *Probiotics and Antimicrobial Proteins* 12(1): 280–288.

Metchnikoff, E. 1907. Lactic acid as inhibiting intestinal putrefaction. In *The prolongation of life optimistic studies*, ed. P. Chalmers Mitchell, 161–83. London: W. Heinemann.

Micklefield, G. J. 2014. Saccharomyces boulardii bei Antibiotika-assoziierter Diarrhöe. *MMW - Fortschritte Der Medizin* 156:18–22. doi: 10.1007/s15006-014-2878-0.

Mirzaei, H., H. Pourjafar, and A. H. Rad. 2011. The effect of microencapsulation with calcium alginate and resistant starch on the Lactobacillus acidophilus (La5) survival rate in simulated gastrointestinal juice conditions. *Journal of Veterinary Research* 66:337–77.

Mitterdorfer, G., H. Mayer, W. Kneifel, and H. Viernstein. 2002. Clustering of Saccharomyces boulardii strains within the species S. cerevisiae using molecular typing techniques. *Journal of Applied Microbiology* 93 (4):521–30. doi: 10.1046/j.1365-2672.2002.01710.x.

Moayyedi, P., A. C. Ford, N. J. Talley, F. Cremonini, A. E. Foxx-Orenstein, L. J. Brandt, and E. M. Quigley. 2010. The efficacy of probiotics in the treatment of irritable bowel syndrome: A systematic review. *Gut* 59:325–32. doi: 10.1136/gut.2008.167270.

Mohammadi, R., and A. Mortazavian. 2011. Technological aspects of prebiotics in probiotic fermented milks. *Food Reviews International* 27 (2):192–212. doi: 10.1080/87559129.2010.535235.

Monteiro, E., J. P. Fernandes, M. R. Vieira, J. P. Correia, J. M. Caetano, T. Ribeiro, A. B. Antunes, R. Noronha, F. Batista, L. Reis, et al. 1981. Double blind clinical trial on the use of ultra-levure in the prophylaxis of antibiotic induced gastro-intestinal and mucocutaneous disorders. *Acta Médica Portuguesa* 3 (2):143–5.

Moradi, R., R. Nosrati, H. Zare, T. Tahmasebi, H. Saderi, and P. Owlia. 2018. Screening and characterization of in-vitro probiotic criteria of Saccharomyces and Kluyveromyces strains. *International Journal of Microbiology* 10:123–131.

Moslemi, M., R. Mazaheri Nezhad Fard, S. Hosseini, A. Homayouni-Rad, and A. M. Mortazavian. 2016. Incorporation of propionibacteria in fermented milks as a probiotic. *Critical Reviews in Food Science and Nutrition* 56(8):1290–312. doi: 10.1080/10408398.2013.766584.

Mousa, A. H., G. Wang, and H. Zhang. 2019. Morphological and molecular tools for identification of Saccharomyces boulardii isolated from active dry yeast. *International Journal of Agriculture Innovations and Research* 7 (4):2319–1473.

Nagashima, A. I., P. E. Pansiera, M. M. Baracat, and R. J. H. C. Gómez. 2013. Development of effervescent products, in powder and tablet form, supplemented with probiotics Lactobacillus acidophilus and Saccharomyces boulardii. *Food Science and Technology (Campinas)* 33:605–11. doi: 10.1590/S0101-20612013000400002.

Nami, Y., R. Vaseghi Bakhshayesh, H. Mohammadzadeh Jalaly, H. Lotfi, S. Eslami, and M. A. Hejazi. 2019. Probiotic properties of Enterococcus isolated from artisanal dairy products. *Frontiers in Microbiology* 10:300. doi: 10.3389/fmicb.2019.00300.

Namkin, K., M. Zardast, and F. Basirinejad. 2016. Saccharomyces boulardii in Helicobacter Pylori eradication in children: a randomized trial from Iran. *Iranian Journal of Pediatrics* 26 (1):e3768. doi: 10.5812/ijp.3768.

Naumova, E., A. Z. Sadykova, N. Martynenko, and G. Naumov. 2013. Molecular genetic characteristics of Saccharomyces cerevisiae distillers' yeasts. *Microbiology* 82:175–85. doi: 10.1134/S0026261713020112.

Nevitt, T., J. Pereira, D. Azevedo, P. Guerreiro, and C. Rodrigues-Pousada. 2004. Expression of YAP4 in Saccharomyces cerevisiae under osmotic stress. *Biochemical Journal* 379:367–74. doi: 10.1042/BJ20031127.

Niamah, A. 2017. Physicochemical and microbial characteristics of yogurt added with Saccharomyces Boulardii. *Current Research in Nutrition and Food Science Journal* 5:300–7. doi: 10.12944/CRNFSJ.5.3.15.

Niamah, A. K., A. J. Al-Manhel, and S. T. G. Al-Sahlany. 2018. Effect microencapsulation of Saccharomyces boulardii on viability of yeast in vitro and ice cream. *The Carpathian Journal of Food Science and Technology* 10:111–8. http://chimie-biologie.ubm.ro/carpathian_journal/index.html.

Niamah, A. K., A. A. Sahi, and A. S. Al-Sharifi. 2017. Effect of feeding soy milk fermented by probiotic bacteria on some blood criteria and weight of experimental animals. *Probiotics Antimicrob Proteins* 9: 284–91. doi: 10.1007/s12602-017-9265-y.

Norouzi, S., H. Pourjafar, F. Ansari, and A. Homayouni. 2019. A Survey on the survival of Lactobacillus paracasei in fermented and non-fermented frozen soy dessert. *Biocatalysis and Agricultural Biotechnology* 21:101297. doi: 10.1016/j.bcab.2019.101297.

Offei, B., P. Vandecruys, S. De Graeve, M. R. Foulquié-Moreno, and J. M. Thevelein. 2019. Unique genetic basis of the distinct antibiotic potency of high acetic acid production in the probiotic yeast Saccharomyces cerevisiae var. boulardii. *Genome Research* 29 (9): 1478–94. doi: 10.1101/gr.243147.118.

Ozkan, T., E. Sahin, G. Erdemir, and F. Budak. 2007. Effect of Saccharomyces boulardii in children with acute gastroenteritis and its relationship to the immune response. *Journal of International Medical Research* 35:201–12. doi: 10.1177/147323000703500204.

Pandiyan, C., and G. Kumaresan. 2013. Utilization of probiotic yeast Saccharomyces Boulardii in dairy products-a overview. In *Probiotics in sustainable food production: Current status and future prospects - probiotic foods*, ed. A. Ramanathan and T. Senthivel, 57–60. Coimbatore: Bonfring.

Pandiyan, C., R. A. Villi, G. Kumaresan, B. Murugan, and T. Gopalakrishnamurthy. 2012. Development of synbiotic ice cream incorporating Lactobacillus acidophilus and Saccharomyces boulardii. *International Food Research Journal* 19:1233.

Pardo, S., M. A. Galvagno, and P. Cerrutti. 2009. Studies of viability and vitality after freezing of the probiotic yeast Saccharomyces boulardii: physiological preconditioning effect. *Revista Iberoamericana de Micologia* 26:155–60.

Paula, B. P. D., D. W. H. Chávez, L. Junior, W. J. Fernandes, A. F. Guerra, M. F. D. Corrêa, K. S. Pereira, and M. A. Z. Coelho. 2019. Growth parameters and survivability of Saccharomyces boulardii for probiotic alcoholic beverages development. *Frontiers in Microbiology* 10:2092. doi: 10.3389/fmicb.2019.02092.

Peng, M., P. Patel, V. Nagarajan, C. Bernhardt, M. Carrion, and D. Biswas. 2019. *Feasible options to control colonization of enteric pathogens with designed synbiotics Dietary Interventions in Gastrointestinal Diseases*, 135–49. Amsterdam: Elsevier.

Peng, M., Z. Tabashsum, M. Anderson, A. Truong, A. K. Houser, J. Padilla, A. Akmel, J. Bhatti, S. O. Rahaman, and D. Biswas. 2020. Effectiveness of probiotics, prebiotics, and prebiotic-like components in common functional foods. *Comprehensive Reviews in Food Science and Food Safety* 19 (4):1908–33. doi: 10.1111/1541-4337.12565.

Pettipher, G. 1991. Preliminary evaluation of flow cytometry for the detection of yeasts in soft drinks. *Letters in Applied Microbiology* 12: 109–12. doi: 10.1111/j.1472-765X.1991.tb00517.x.

Piatek, J., H. Sommermeyer, A. Ciechelska-Rybarczyk, and M. Bernatek. 2019. In-vitro pathogen inhibition: Comparing the inhibitory effects of a complex multistrain synbiotic with simple probiotics containing the yeast Saccharomyces boulardii or Lactobacillus rhamnosus bacteria. *BioRxiv 543819*. doi: 10.1101/543819..

Picó-Monllor, J. A., and J. M. Mingot-Ascencao. 2019. Search and selection of probiotics that improve mucositis symptoms in oncologic patients. *Nutrients* 11:2322. doi: 10.3390/nu11102322.

Plein, K., and J. Hotz. 1993. Therapeutic effects of Saccharomyces boulardii on mild residual symptoms in a stable phase of Crohn's disease with special respect to chronic diarrhea–a pilot study. *Zeitschrift ur Gastroenterologie* 31:129–34.

Postollec, F., H. Falentin, S. Pavan, J. Combrisson, and D. Sohier. 2011. Recent advances in quantitative PCR (qPCR) applications in food microbiology. *Food Microbiology* 28:848–61. doi: 10.1016/j.fm.2011.02.008.

Pourjafar, H., N. Noori, H. Gandomi, and A. A. Basti. 2016. Study of protective role of double coated beads of calcium alginate-chitosan-eudragit S100 nanoparticles achieved from microencapsulation of Lactobacillus acidophilus as a predominant flora of human and animals gut. *Journal of Veterinary Research* 71 (3):311–20. doi: 10.22059/JVR.2016.58729.

Pourjafar, H., N. Noori, H. Gandomi, A. A. Basti, and F. Ansari. 2018. Stability and efficiency of double-coated beads containing lactobacillus acidophilus obtained from the calcium alginate-chitosan and eudragit S100 nanoparticles microencapsulation. *International Journal of Probiotics & Prebiotics* 13:77–84.

Pourjafar, H., N. Noori, H. Gandomi, A. A. Basti, and F. Ansari. 2020. Viability of microencapsulated and non-microencapsulated Lactobacilli in a commercial beverage. *Biotechnology Reports* 25: e00432. doi: 10.1016/j.btre.2020.e00432.

Pozzoni, P., A. Riva, A. G. Bellatorre, M. Amigoni, E. Redaelli, A. Ronchetti, M. Stefani, R. Tironi, E. E. Molteni, D. Conte, et al. 2012. Saccharomyces boulardiifor the prevention of antibiotic-associated diarrhea in adult hospitalized patients: A single-center, randomized, double-blind, placebo-controlled trial. *American Journal of Gastroenterology* 107:922–31. doi: 10.1038/ajg.2012.56.

Qamar, A., S. Aboudola, M. Warny, P. Michetti, C. Pothoulakis, J. T. LaMont, and C. P. Kelly. 2001. Saccharomyces boulardii stimulates intestinal immunoglobulin a immune response to clostridium difficiletoxin a in mice. *Infection and Immunity* 69:2762–5. doi: 10.1128/IAI.69.4.2762-2765.2001.

Ragavan, M. L., and N. Das. 2017. Molecular identification of probiotic yeast strains and their characterization. *Asian Journal of Pharmaceutical and Clinical Research* 10:339–43. doi: 10.22159/ajpcr.2017.v10i10.20052.

Rajkowska, K., A. Kunicka-Styczyńska, and A. Rygala. 2012. Probiotic activity of Saccharomyces cerevisiae var. boulardii against human pathogens. *Food Technology and Biotechnology* 50:230–6.

Rattray, J., A. Schibeci, and D. K. Kidby. 1975. Lipids of yeasts. *Bacteriological Reviews* 39 (3):197–231. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC413914/. doi: 10.1128/MMBR.39.3.197-231.1975.

Reethu Narayanan, N. R. K., and P. J. J. Ch. 2012. Evaluation of probiotic potential of stress tolerant Saccharomyces cerevisiae and development of economically viable media for maximum growth. *Journal of Food Processing & Technology* 3 (2)https://agris.fao.org/agris-search/search.do?recordID=US2016B00418.

Riaz, M., S. Alam, A. Malik, and S. M. Ali. 2012. Efficacy and safety of Saccharomyces boulardii in acute childhood diarrhea: a double blind randomised controlled trial. *European Journal of Gastroenterology & Hepatology* 79:478–82. doi: 10.1007/s12098-011-0573-z.

Ribeiro, M., D. Oliveira, F. Oliveira, M. Caliari, F. Martins, J. Nicoli, M. Torres, M. Andrade, V. Cardoso, and M. Gomes. 2018. Effect of probiotic Saccharomyces boulardii in experimental giardiasis. *Beneficial Microbes* 9:789–97. doi: 10.3920/BM2017.0155.

Roos, T. B., C. M. de Moraes, R. T. Sturbelle, L. A. Dummer, G. Fischer, and F. P. L. Leite. 2018. Probiotics Bacillus toyonensis and Saccharomyces boulardii improve the vaccine immune response to Bovine herpesvirus type 5 in sheep. *Research in Veterinary Science* 117:260–5. doi: 10.1016/j.rvsc.2017.12.022.

Roostita, R., and G. Fleet. 1996. The occurrence and growth of yeasts in Camembert and blue-veined cheeses. *International Journal of Food Microbiology* 28:393–404. doi: 10.1016/0168-1605(95)00018-6.

Rozee, K., D. Cooper, K. Lam, and J. Costerton. 1982. Microbial flora of the mouse ileum mucous layer and epithelial surface. *Applied and Environmental Microbiology* (6)43:1451–63. doi: 10.1128/aem.43.6.1451-1463.1982.

Ruyssen, T., G. Vlaemynck, M. Uyttendaele, P. Van Assche, and T. Le Trung. 2007. *Flow cytometry as a rapid tool for microbiological analysis in the food industry: Potentials and restrictions rapid methods for food and feed quality determination*, 85–101. Wageningen: Wageningen Academic Publishers.

Ryan, J., D. Hanes, M. Schafer, J. Mikolai, and H. Zwickey. 2015. Effect of the probiotic Saccharomyces boulardii on cholesterol and lipoprotein particles in hypercholesterolemic adults: a single-arm, open-label pilot study. *Journal of Alternative and Complementary Medicine* 21:288–93. doi: 10.1089/acm.2014.0063.

Ryan, E. P., A. L. Heuberger, T. L. Weir, B. Barnett, C. D. Broeckling, and J. E. Prenni. 2011. Rice bran fermented with Saccharomyces boulardii generates novel metabolite profiles with bioactivity. *Journal of Agricultural and Food Chemistry* 59:1862–70. doi: 10.1021/jf1038103.

Saint-Marc, T., H. Blehaut, C. Musial, and J. Touraine. 1995. AIDS-related diarrhea: A double-blind trial of Saccharomyces boulardii. *Semaine es Hopitaux de Paris* 71:735–41.

Salimi, M., and M. Mahzonieh. 2015. Preparation of proper culture medium for Saccharomyces cerevisiae var. boulardii with Molasses and Animal Serum. *Journal of Medical Microbiology and Infectious Diseases* 3:18–22.

Santino, I., A. Alari, S. Bono, E. Teti, M. Marangi, A. Bernardini, L. Magrini, S. Di Somma, and A. Teggi. 2014. Saccharomyces cerevisiae fungemia, a possible consequence of the treatment of Clostridium difficile colitis with a probioticum. *International Journal of Immunopathology and Pharmacology* 27:143–6. doi: 10.1177/039463201402700120.

Sardonini, C. A., and D. DiBiasio. 1987. A model for growth of Saccharomyces cerevisiae containing a recombinant plasmid in selective media. *Biotechnology and Bioengineering* 29:469–75. doi: 10.1002/bit.260290410.

Schlotterer, M., P. Bernasconi, F. Lebreton, and D. Wassermann. 1987. Value of Saccharomyces boulardii in the digestive acceptability of continuous-flow enteral nutrition in burnt patients. *Nutrition Clinique et Métabolisme* 1:31–4. doi: 10.1016/S0985-0562(87)80016-X.

Schneider, S. M., F. Girard-Pipau, J. Filippi, X. Hébuterne, D. Moyse, G. C. Hinojosa, A. Pompei, and P. Rampal. 2005. Effects of Saccharomyces boulardii on fecal short-chain fatty acids and microflora in patients on long-term total enteral nutrition. *World Journal of Gastroenterology* 11:6165–9. doi: 10.3748/wjg.v11.i39.6165.

Schwenzer, B. 1998. Saccharomyces boulardii. *Deutsche Apotheker-Zeitung* 138:75–7.

Seddik, H., H. Boutallaka, I. Elkoti, F. Nejjari, R. Berraida, S. Berrag, K. Loubaris, S. Sentissi, and A. Benkirane. 2019. Saccharomyces boulardii CNCM I-745 plus sequential therapy for Helicobacter pylori infections: a randomized, open-label trial. *European Journal of Clinical Pharmacology* 75:639–45. doi: 10.1007/s00228-019-02625-0.

Shah, N. P. 2000. Probiotic bacteria: selective enumeration and survival in dairy foods. *Journal of Dairy Science* 83:894–907. doi: 10.3168/jds.S0022-0302(00)74953-8.

Shan, L.-S., P. Hou, Z.-J. Wang, F.-R. Liu, N. Chen, L.-H. Shu, H. Zhang, X.-H. Han, X.-X. Han, X.-X. Cai, et al. 2013. Prevention and treatment of diarrhoea with Saccharomyces boulardii in children with acute lower respiratory tract infections. *Beneficial Microbes* 4: 329–34. doi: 10.3920/BM2013.0008.

Sharif, M. R., H. H. Kashani, A. T. Ardakani, D. Kheirkhah, F. Tabatabaei, and A. Sharif. 2016. The effect of a yeast probiotic on acute diarrhea in children. Probiotics and antimicrobial proteins. *Probiotics and Antimicrobial Proteins* 8:211–4. doi: 10.1007/s12602-016-9221-2.

Silley, P., and S. Forsythe. 1996. Impedance microbiology—a rapid change for microbiologists. *Journal of Applied Bacteriology* 80: 233–243..

Silva, J. F. M. d., J. M. Peluzio, G. Prado, J. E. G. C. Madeira, M. O. Silva, P. B. de Morais, C. A. Rosa, R. S. Pimenta, and J. R. Nicoli. 2015. Use of probiotics to control aflatoxin production in peanut grains. *The Scientific World Journal* 2015:959138. doi: 10.1155/2015/959138.

Silveira, M. M., F. R. Conceição, M. Mendonça, G. M. S. G. Moreira, C. E. P. Da Cunha, N. L. Conrad, P. D. de Oliveira, D. D. Hartwig, P. M. M. De Leon, and Â. N. Moreira. 2017. Saccharomyces boulardii improves humoral immune response to DNA vaccines against leptospirosis. *Journal of Medical Microbiology* 66:184–90. doi: 10.1099/jmm.0.000414.

Song, M. J., D. I. Park, J. H. Park, H. J. Kim, Y. K. Cho, C. I. Sohn, W. K. Jeon, and B. I. Kim. 2010. The effect of probiotics and mucoprotective agents on PPI-based triple therapy for eradication of Helicobacter pylori. *Helicobacter* 15:206–13. doi: 10.1111/j.1523-5378.2010.00751.x.

Soukoulis, C., I. D. Fisk, and T. Bohn. 2014. Ice cream as a vehicle for incorporating Health-Promoting Ingredients: Conceptualization and overview of quality and storage stability. *Comprehensive Reviews in Food Science and Food Safety* 13:627–55. doi: 10.1111/1541-4337.12083.

Speck, M. L. 1984. *Compendium of methods for the microbiological examination of foods*. Washington, DC: American Public Health Association. https://ajph.aphapublications.org/doi/book/10.2105/MBEF.0222.

Stadie, J., A. Gulitz, M. A. Ehrmann, and R. F. Vogel. 2013. Metabolic activity and symbiotic interactions of lactic acid bacteria and yeasts isolated from water kefir. *Food Microbiology* 35:92–8. doi: 10.1016/j.fm.2013.03.009.

Stier, H., and S. Bischoff. 2017. Saccharomyces boulardii CNCM I-745 influences the gut-associated immune system. *MMW - Fortschritte der Medizin* 159:1–6. doi: 10.1007/s15006-017-9802-3.

Sulik-Tyszka, B., E. Snarski, M. Niedźwiedzka, M. Augustyniak, T. N. Myhre, A. Kacprzyk, E. Swoboda-Kopeć, M. Roszkowska, J. Dwilewicz-Trojaczek, W. W. Jędrzejczak, et al. 2018. Experience with Saccharomyces boulardii probiotic in oncohaematological patients. *Probiotics Antimicrob Proteins* 10:350–5. doi: 10.1007/s12602-017-9332-4.

Sultana, A., A. Miyamoto, Q. L. Hy, Y. Tanaka, Y. Fushimi, and H. Yoshii. 2017. Microencapsulation of flavors by spray drying using Saccharomyces cerevisiae. *Journal of Food Engineering* 199:36–41. doi: 10.1016/j.jfoodeng.2016.12.002.

Surawicz, C. M., L. V. McFarland, G. Elmer, and J. Chinn. 1989. Treatment of recurrent Clostridium difficile colitis with vancomycin and Saccharomyces boulardii. *American Journal of Gastroenterology* 84:1285–7.

Surawicz, C. M., L. V. McFarland, R. N. Greenberg, M. Rubin, R. Fekety, M. E. Mulligan, R. J. Garcia, S. Brandmarker, K. Bowen, D. Borjal, et al. 2000. The search for a better treatment for recurrent Clostridium difficile disease: use of high-dose vancomycin combined with Saccharomyces boulardii. *Clinical Infectious Diseases* 31:1012–7. doi: 10.1086/318130.

Suriyarachchi, V., and G. Fleet. 1981. Occurrence and growth of yeasts in yogurts. *Applied and Environmental Microbiology* (4)42:574–9. doi: 10.1128/aem.42.4.574-579.1981.

Suryavanshi, A., A. Agarwal, A. Kaler, U. Bihade, J. Kaur, K. Tikoo, and U. Banerjee. 2013. Comparative studies on the antioxidant potential of vanillin-producing Saccharomyces boulardii extracts. *Oxidants and Antioxidants in Medical Science* 2 (3):201–9. doi: 10.5455/oams.190413.or.040.

Syal, P., and A Vohra. 2013. Probiotic potential of yeasts isolated from traditional Indian fermented foods. *International Journal of Microbiology Research* 5:390–8. doi: 10.9735/0975-5276.5.2.390-398.

Szajewska, H., and J. Mrukowicz. 2005. Meta-analysis: non-pathogenic yeast Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea. *Alimentary Pharmacology & Therapeutics* 22 (5): 365–72. doi: 10.1111/j.1365-2036.2005.02624.x.

Tabrizi, A., L. Khalili, A. Homayouni-Rad, H. Pourjafar, P. Dehghan, and F. Ansari. 2019. Prebiotics, as promising functional food to patients with psychological disorders: A review on mood disorders, sleep, and cognition. *NeuroQuantology* 17 (6):1–9. doi: 10.14704/nq.2019.17.6.2189.

Tang, C., and Z. Lu. 2019. Health promoting activities of probiotics. *Journal of Food Biochemistry* (8)43:e12944 doi: 10.1111/jfbc.12944.

Tanner, R., C.-J. Wei, and J. Woodward. 1981. *The development of a semi-solid fermentation system for the production of lysine-enriched yeast and ethanol Scientific and Engineering Principles*, 323–8. Amsterdam: Elsevier.

Tempe, J., A. Steidel, H. Blehaut, M. Hasselmann, P. Lutun, and F. Maurier. 1983. Prevention of diarrhea administering *Saccharomyces boulardii* during continuous enteral feeding. La semaine des hopitaux: organe fonde par. *l'Association d'enseignement medical des hopitaux de Paris* 59:1409–12. https://pubmed.ncbi.nlm.nih.gov/6306827/.

Tempest, D., J. Meers, and C. Brown. 1970. Influence of environment on the content and composition of microbial free amino acid pools. *Journal of General Microbiology* (2)64:171–85. doi: 10.1099/00221287-64-2-171.

Tiencheu, B., E. Ngongang, B. Fossi, G. Nchanji, D. Shiynyuy, F. Mbiapo, and Z. François. 2016. Isolation and identification of cholesterol lowering probiotic yeast from palm Raffia (Raffia mambillensis) Wine. *Journal of Advances in Biology & Biotechnology* 11:1–17. doi: 10.9734/JABB/2017/30469.

Tomičić, Z., R. Colovic, I. Cabarkapa, D. Vukmirovic, O. Djuragic, and R. Tomicic. 2016. Beneficial properties of probiotic yeast *Saccharomyces boulardii*. *Food and Feed Research* 43:103–10. doi: 10.5937/FFR1602103T.

Trigueros, D., M. Fiorese, A. Kroumov, C. Hinterholz, B. Nadai, and G. Assunção. 2016. Medium optimization and kinetics modeling for the fermentation of hydrolyzed cheese whey permeate as a substrate for Saccharomyces cerevisiae var. boulardii. *Biochemical Engineering Journal* 110:71–83. doi: 10.1016/j.bej.2016.02.014.

Tripathi, M. K., and S. K. Giri. 2014. Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of Functional Foods* 9:225–41. doi: 10.1016/j.jff.2014.04.030.

Trotta, F., G. Caldini, L. Dominici, E. Federici, R. Tofalo, M. Schirone, A. Corsetti, G. Suzzi, and G. Cenci. 2012. Food borne yeasts as DNA-bioprotective agents against model genotoxins. *International Journal of Food Microbiology* (3)153:275–80. doi: 10.1016/j.ijfoodmicro.2011.11.009.

Urganci, N., T. Polat, M. Uysalol, and F. Çetinkaya. 2001. Evaluation of the efficacy of Saccharomyces boulardii in children with acute diarrhoea. *Archives of Gastroenterohepatology* 20:81–3.

Van der Aa Kühle, A., K. Skovgaard, and L. Jespersen. 2005. In vitro screening of probiotic properties of Saccharomyces cerevisiae var. boulardii and food-borne Saccharomyces cerevisiae strains. *International Journal of Food Microbiology* 101:29–39. doi: 10.1016/j.ijfoodmicro.2004.10.039.

Vasiljevic, T., and N. P. Shah. 2008. Probiotics—from Metchnikoff to bioactives. *International Dairy Journal* 18:714–28. doi: 10.1016/j.idairyj.2008.03.004.

Vinderola, C. G., P. Mocchiutti, and J. A. Reinheimer. 2002. Interactions among lactic acid starter and probiotic bacteria used for fermented dairy products. *Journal of Dairy Science* (4)85:721–9. doi: 10.3168/jds.S0022-0302(02)74129-5.

Wang, G., and D. Feng. 2019. Therapeutic effect of Saccharomyces boulardii combined with Bifidobacterium and on cellular immune function in children with acute diarrhea. *Experimental and Therapeutic Medicine* 18:2653–9. doi: 10.3892/etm.2019.7836.

Wang, X., and H. Yang. 2017. Application of modern new technology in food microbiological detection. *Engineering and Technology Research* 652–5. doi: 10.12783/dtetr/mcee2017/15823.

Wei, C.-J., R. D. Tanner, and G. W. Malaney. 1982. Effect of sodium chloride on bakers' yeast growing in gelatin. *Applied and Environmental Microbiology* (4)43:757–63. doi: 10.1128/aem.43.4.757-763.1982.

Whelan, K. 2007. Enteral-tube-feeding diarrhoea: Manipulating the colonic microbiota with probiotics and prebiotics: BAPEN

Symposium 2 on 'Pre-and probiotics. *The Proceedings of the Nutrition Society* 66:299–306. doi: 10.1017/S0029665107005551.

Wilkinson, M. G. 2018. Flow cytometry as a potential method of measuring bacterial viability in probiotic products: a review. *Trends in Food Science & Technology* 78:1–10. doi: 10.1016/j.tifs.2018.05.006.

Wu, X., B. A. Vallance, L. Boyer, K. S. Bergstrom, J. Walker, K. Madsen, J. R. O'Kusky, A. M. Buchan, and K. Jacobson. 2008. Saccharomyces boulardii ameliorates Citrobacter rodentium-induced colitis through actions on bacterial virulence factors. *American Journal of Physiology* 294:G295–G306. doi: 10.1152/ajpgi.00173.2007.

Zamora-Vega, R., J. L. Montañez-Soto, J. Venegas-González, A. Bernardino-Nicanor, L. G. Cruz, and H. E. Martínez-Flores. 2013. Development and characterization of a symbiotic cheese added with Saccharomyces boulardii and inulin. *African Journal of Microbiology Research* 7:2828–34.

Zhao, H., H. Ou-Yang, B. Duan, B. Xu, Z. Chen, J. Tang, and J. You. 2014. Clinical effect of triple therapy combined with Saccharomyces boulardii in the treatment of Helicobacter pylori infection in children. *Chinese ournal of Contemporary Pediatrics* 16:230–3.

Zojaji, H., M. Ghobakhlou, H. Rajabalinia, E. Ataei, S. J. Sherafat, B. Moghimi-Dehkordi, and R. Bahreiny. 2013. The efficacy and safety of adding the probiotic Saccharomyces boulardiito standard triple therapy for eradication of H. pylori: A randomized controlled trial. *Gastroenterology and Epatology from Bed to Bench* 6:S99.