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Yeasts in Fermented Foods and their Probiotic Potential

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Abstract

Fermented foods are sources of microorganisms such as yeasts, which have various beneficial effects in human health and show potential as probiotics. Probiotics have established their efficacy as dietary supplements as they provide benefits to consumers; however they must be carefully selected by *in vitro* and *in vivo* tests before being added into a food product. Probiotic strains must be innocuous, non-toxic to the host, survive the gastrointestinal transit, highly concentrated in the product and maintain viability along shelf-life. Beneficial effects to the host include cholesterol reduction, production of vitamins, enzymes and folates, antibacterial and antioxidant activity, as well as enhancement of the immune system. In the case of yeasts, only two species have been recognized as probiotics: *Saccharomyces cerevisiae* and *S. cerevisiae* var. *boulardii*. Nevertheless, the variety of species found in fermented foods is very large and recent studies reveal other species with probiotic potential, such as *Debaryomyces*, *Pichia*, *Torulaspora*, *Kluyveromyces*, *Hanseniaspora*, *Rhodotorula*, *Wickerhamomyces*, *Candida* and *Williopsis*. Some interesting interactions between probiotic yeasts and bacteria are commented.

Keywords: Probiotics; Yeasts; Beneficial effects; Fermented foods

Abbreviations

AAPH: 2,2'-Azobis(2-amidinopropane) dihydrochloride; CFU: Colony-Forming Unite; DPPH: 2,2-diphenyl-1-picrylhydrazyl; EPS: Exopolysaccharide; IL: Interleukin; IFN: Interferon; IP: Induced Protein; LAB: Lactic Acid Bacteria; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide

Introduction

Microbiota is a system conformed by microorganisms that coexist in equilibrium with the host; it plays an important role in protection against infections. The microbiota is normally in equilibrium with the host; however, when this equilibrium is broken several gastrointestinal disorders may develop, such as antibiotic-associated diarrheas, ulcers, inflammatory bowel disease, irritable bowel syndrome and in some cases more severe ailments such as colon cancer [1].

More than 100 trillion bacteria are found in the human gastrointestinal tract as part of gut microbiota; other microorganisms such as yeasts, archaea, viruses, parasites, or fungi are also harbored in this system [2]. The number of bacteria in the gut microbiota is ten times higher than that of human cells in the body, they include 1,000 different species, mostly anaerobic, and encode 200 times more genes than human genome [3]. The yeast percentage in the microbiota is approximately 0.1% and they are detectable in the gut of about 70% of healthy adults. In the microorganism isolation of the gastrointestinal tract, yeast species such as *Torulopsis glabrata* and *Candida tropicalis* are occasionally recovered at different concentrations along the system; for example, yeast concentration in the stomach is of 10^2 and increases to a maximum of 10^6 in the colon [4]. Nevertheless, the cells size of the bacteria is up to 10 times smaller than yeasts, which may produce changes in the molecular interactions [1].

Recent studies have demonstrated that microbiota controls the development of the immune system, regeneration of the epithelium, inflammatory diseases, metabolic syndrome, stress responses, and has psychiatric and neuro-inflammatory implications [5-9].

The type of foods consumed is a factor affecting gut microbiota, due to food components are substrates for the different microorganisms in the gastrointestinal tract and each microorganism is specialized in the consumption of the substrates. Hence, food matrixes provide promoting and inhibiting factors for specific species [10].

De Filippo, et al. [11] found that the diversity of microorganisms isolated in fecal matter of children from Africa was larger than that of children from developed zones in the EU, this is associated to the consumption of fiber and suggests that microbiota differences are related to dietary differences. Other study explores differences between the type of fecal bacteria and functional genes of people from EU and rural areas of Venezuela [12].

Yeasts and their metabolic products have been used in different forms of food processing and preservation worldwide, mainly for baking and brewing. Nowadays, yeast biotechnology is a part of commercially important sectors, including foods, beverages, pharmaceuticals and industrial enzymes, among others.

Yeasts as probiotics

According to the Food and Agriculture Organization (FAO), probiotics are live microorganisms that have a beneficial effect in the health of the host when ingested. Such microorganisms may be autochthonous or allochthonous, the first category appear by the contact of the newborn with the microbiota of the mother, and the second comprises those microorganisms that have been incorporated into the digestive system through the diet [13]. *S. cerevisiae* and *S. boulardii* strains are currently used as probiotic yeast species. From these, the supplementation of *S. cerevisiae* live cultures in animals has been reported to improve growth, health and immune response in the hosts [14]. However, isolation and characterization of yeasts as

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Table 1: Criteria to probiotic evaluation in microorganisms (FAO, 2001).

Identification	*In vitro* tests	Security	*In vivo* tests
· Genera	· Tolerance of acid and bile salts	· Pathogenicity	· Infectivity absence in immunosuppressed animals
· Specie	· Adhesion properties of cell wall	· Antibiotic resistance	· Dose determination
· Probiotic strain	· Adhesion to intestinal mucosa and epithelial cells	· Metabolic activities	· Therapy duration
	· Immunomodulation		· Clinical evaluation

probiotics from natural sources require special considerations. Yeasts can live in different niches such as plants, animals, soil and water and they are associated with the skin, gastrointestinal tract of animals, including aquatic animals, as well as fermented foods [15,16]. FAO has published a guide to evaluate probiotic microorganisms in a systematic manner (Table 1). This evaluation includes the identification, *in vitro* test, safety, and finally *in vivo* tests [17].

Resistance to gastrointestinal conditions

The primary barrier in the stomach is gastric acid, which has an inhibitory action related to its low pH and enzyme presence. The yeasts isolated from fecal matter and kefir are characterized by a high adaptability to conditions of the human gastrointestinal tract: when exposed to pH 2.5 for 8h at 37°C, the survival rate was of 86-97%. At pH 1.5, the survival of yeasts isolated from fecal matter was of 85-92%; kefir isolates showed a higher sensitivity and their survival rate was of 33 and 38% after 8 hours of incubation. Regarding resistance to bile salts, the fecal and kefir isolates demonstrated high resistance: the addition of 0.1 and 1.0% Oxgall did not affect viability of microorganisms [18]. Syal and Vohra (2013) demonstrated tolerance to acid environments of yeasts isolated from Indian bakery products, which achieved a survival rate of 56 to 100% when growing in a medium MYPG with HCl at pH 2. Furthermore, tolerance to bile salts of these organisms was assessed by adding Oxbile in concentrations up to 1%, bearing in mind that the concentration of bile salts in the small intestine is 0.2 to 2.0%. It was observed that the decline in growth was very low, as they reached a high survival rate of 95%. The ability of probiotics to tolerate transit through the gastrointestinal tract depends on the strain, as demonstrated by Chen, et al. [19] when simulating gastrointestinal conditions using 0.3% pepsin and 0.5% NaCl, and incubating for 1.5 hours. This study showed a reduction in viability up to 97%, except for *Geotrichum* spp. *strains* and *Pichia kudriavzevii*.

Adhesion to epithelial cells

Chen, et al. (2010) evaluated the ability of adhesion of yeast cells isolated from cow's raw milk in colon tumor cells HT-29. Adhesion of 62 cells/100 HT-29 cells was observed for *P. kudriavzevii*, and 80 cells/100 HT-29 cells for *Geotrichum* sp. These data were lower than those reported for bifidobacteria species (>350) by Crociani, et al. (1995), and may be due to the size of the cells, since some yeasts require a larger area to adhere to the intestinal surface than bacteria [20]. On the other hand, Kourelis, et al. [22] reported higher adhesion ability of yeasts to Caco-2 cells. In this study, the adhesion of *Candida parapsilosis* was over 300 yeasts per 100 Caco-2 cells. Furthermore, Kumura, et al. [21] studied the impact of digestive proteinases on cell adhesion ability. They isolated several species from blue cheese and kefir in Caco-2 cells treated with proteinase K, pepsin or trypsin. The results showed a 20% reduction in adhesion to Caco-2 cells added with proteinase K, while pepsin and trypsin had no significant effect on this ability. The genus *Kluyveromyces* showed adhesion ability to Caco-2 cells; this was particularly evident for *Kluyveromyces lactis*. On the other hand, *Debaryomyces hansenii* showed adhesion ability comparable to that of *Kluyveromyces marxianus* and *Kloeckera lodderae*; however, the adhesion ability of *Debaryomyces occidentalis* was lower. Other yeasts, including *S. cerevisiae*, *Yarrowia lipolytica*, and *Candida humilis*, were poorly adhesive to Caco-2 cells. These results show that although adhesion relates to the proteins expressed in the cell wall, yeast have a good performance in the presence of intestinal proteinases.

Antibiotic resistance

Syal and Vohra (2013) evaluated antibiotic tolerance in 20 different yeasts isolated from traditional oriental food. Results showed tolerance to ampicillin (10 and 25 µg/ml), chloramphenicol (30 µg/ml), erythromycin (5 and 15 µg/ml), penicillin (10 µg/ml), streptomycin (25 µg/ml) and tetracycline (30 µg/ml). This is remarkable since the majority of probiotic microorganisms are bacteria, which are mostly non-resistant to these antibiotics. Yeasts show a natural resistance and can be administered to patients undergoing antibiotic treatments [15]. Since potential transfer of plasmid-encoded genes to pathogens or other microorganisms of the intestinal microbiota could lead to disturbance of the microbial balance, strains harboring mobile elements should not be used as probiotics [23]. Kourelis, et al. (2004) tested for the presence of plasmids in *S. cerevisiae*, *S. boulardii*, *K. lactis*, *D. hansenii*, *C. parapsilosis* and *Isaatchenkia orientalis* to exclude the possibility of carrying potentially transmissible plasmid-encoded genes. No plasmid DNA could be isolated in any of the strains evaluated by these authors.

Yeasts in fermented foods

Today, the consumption of fermented vegetables foods has increased by almost 60%, mainly in developed countries, since consumers prefer minimally processed products obtained by natural biochemical methods, such as fermentation. Most fermented products have a long stability and low cost relative to other foods with similar characteristics [24]. Table 2 shows yeast species reported in some fermented foods around the world.

Cassava

In South America, cassava is mainly used to produce starch. "Almidon agrio" or "sour starch" is obtained by a long fermentation (20-30 days) with a final pH between 3.5 and 4 [25]. Spontaneous fermentation is characterized by a succession of microorganisms, mainly LAB and yeasts. Lacerda, et al. [26] detected *Galactomyces geotrichum*, *Issatchenkia* spp. and *Candida ethanolica* in this product, which may contribute to starch hydrolysis. In a study on food-borne yeasts, it was observed that *I. orientalis* is a producer of phytase [27]. Humans do not have the capability of synthesizing

Table 2: Yeasts associated to different fermented foods.

Category	Fermented food	Yeasts associated	References
Vegetables	Cassava	*G. geotrichum*, *I.* spp., *C. ethanolica*	[26]
	Kanji	*R. glutinis*	[63]
	Pulque	*K. marxianus*, *T. delbrueckii*, *P. fermentans, C. diversa*	[75]
Fruits	Chilli pepper	*H. guilliermondii*, *K. ohmeri*, *R.* spp., *D.* spp., *Cryptococcus* spp.	[32,33]
	Olives	*C. krusei*, *C. parapsilosis*, *C. rugose*, *P. anomala*, *P. membranifaciens*, *R. glutinis*, *S. cerevisiae*, *C. boidinii*, *T. delbrueckii*, *K. marxianus*, *D. hansenii*	[45-47]
	Masau	*S. cerevisiae*, *I. orientalis*, *P. fabianii*, *H. opuntiae*, *S. fibuligera*, *C. flavus*, *K. marxianus*, *R. mucilaginosa*	[53]
	Cocoa	*S. cerevisiae*, *H. opuntiae*	[56]
	Tepache	*H. uvarum*, *P. guilliermondii*	[76]
Cereals	Ogi	*P. kudriavzevii*	[36]
	Pozol	*R. minuta*, *R. mucilaginosa*, *D. hansenii*, *G. candidum, C. guilliermondii* and *K. lactis*	[70]
	Sourdough	*C. humilis*, *K. exigua*, *W. anomalus*, *C. famata*, *S. cerevisiae*	[37-40]
Dairy	Kefir	*Kluyveromyces* spp., *Saccharomyces* spp., *Torula* spp., *W. saturnus*var. *saturnus*	[83,88]

these enzymes; however they are needed in the gastrointestinal tract for degradation and dephosphorylation of the phytate complex [28]. Non-degradedphytate lowers the bioavailability of divalent ions and may reduce the functional and nutritional properties of proteins such as digesting enzymes [29]. Phytase activity of microorganisms may be influenced by the strain, enzyme concentration in the biomass and gastrointestinal conditions. In the late gastric and early intestinal phases the degradation of phytase by yeast was insignificant, which may be due to high phytate solubility, high resistance against proteolysis by pepsin, and high cell survival [30]. Phytases are found in various microorganisms including filamentous fungi, Gram-positive and Gram-negative bacteria, as well as yeasts such as *Schwanniomyces castellii*, *Debaryomyces castellii*, *Arxula adeninivorans*, *Pichia anomala*, *Pichia rhodanensis*, *Pichia spartinae*, *Cryptococcus laurentii*, *Rhodotorula gracilis*, *S. cerevisiae*, *Saccharomyces kluyveri*, *Torulaspora delbrueckii*, *Candida* spp. and *K. lactis* have been identified as phytase producers [27]. In a study by Olstorpe, et al. [31] on the ability of different yeast strains (122 strains from 61 species) to utilize phytic acid as sole phosphorus source, strains of *Arxula adeninivorans* and *P. anomala* showed the highest volumetric phytase activities.

Chili peppers

Jalapeño pepper is the most popular variety of *Capsicum* preserved by fermentation. The process takes place at 10-15% salt concentrations, and continues until reaching a pH close to 3 [32]. Regarding yeasts present in this fermentation process, Zhao, et al. [33] reported genre such as *Rhodotorula* (7.4%), *Debaryomyces* (3.94%) and *Cryptococcus* (1.86%); and González-Quijano, et al. [34], found the presence of *Hanseniaspora pseudiguilliermodii* and *Kodamea ohmeri*. This last strain was also identified in fermented broilers' excreta and its probiotic potential was analyzed. García-Hernández (2012) reported its ability of agglutination and adherence of pathogenic bacteria such as *E. coli*, *S. Typhimurium, L. monocytogenes* and *E. faecalis*, which helps form a barrier that prevents colonization by pathogens in the human gut [35].

Red chili powder was utilized as ingredient in the production of a functional food containing a cereal mix (white and red sorghum, pearl millet and wheat). One strain of *P. kudriavzevii* isolated from *ogi* (fermented cereal glue) was inoculated as probiotic yeast. The growth of probiotics was counted at 7.46 to 8.22 Log CFU/mL after 24 h. The fermented functional food scavenged DPPH from 200 μmol/L methanolic solution by 55% [36].

Sourdough

During the California Gold Rush in the USA, sourdough was a key food source for the Northern California population, given its facility to be stored and transported. Nowadays, San Francisco sourdough is very popular in the San Francisco area. Studies indicate that some yeasts trains are responsible for the fermentation of this product, such as *Candida humilis*, *Kazachstania exigua*, *Wickerhamomyces anomalus*, *Candida famata* and *S. cerevisiae,* which occur in a stable association with lactic acid bacteria. Nowadays this baked product is appreciated for its traditional and gastronomic values [37-41]. Some species of *Candida* have been reported as opportunist pathogens in sourdough, such as *C. famata*. This strain was also isolated from chicken feces and Al-Seraih, et al. [42] recognized its probiotic potential, since it showed cytotoxicity in Caco-2 cells with null hemolytic activity and good hydrophobicity and survival in gastric and intestinal environments, similar to *S. boulardii*.

Olives

Alkaline fermentation is an essential step in olive processing to remove the bitterness of the fruit, due to a substance known as oleuropein. This process is carried out in brine for 24-48 days. Both Spanish-style green olives and Greek-style black olives are fermented with natural microbiota [43,44]. The most representative yeasts isolated from Spanish green olives are *Candida krusei, C. parapsilosis, Candida rugose, P. anomala, Pichia membranifaciens, R. glutinis* and *S. cerevisiae*. For directly brined green or black olives the most representative yeast species are *Candidab oidinii, P. anomala, P. membranifaciens, T. delbrueckii*, *K. marxianus* and *D. Hansenii* [45-47]. These last two species have shown beneficial effects in *in vitro*/*in vivo* studies. *D. hansenii* has exhibited a probiotic potential in the stimulation of enzymes production. A strain isolated from the gut of rainbow trout was tested as a supplement in the diet of sea bass larvae (*Dicentrarchus labrax*); it raised amylase and trypsin activities 1.5 times as compared to the control group. This is relevant since some diarrheas are associated to a decrease of the intestinal disaccharidase activities. The increased disaccharidase activities mentioned above could be mediated by endoluminal release of polyamines (spermine and spermidine) produced by live yeast [48]. Another yeast species, *K. marxianus*, isolated from cow's milk whey induces the production of the proinflammatory cytokines IL-1β, TNF-α, IFN-γ and IL-6 in peripheral blood mononuclear cells. This species decreased the values of TNF-α and IL-6 in cells undergoing an inflammatory process induced by lipopolysaccharaides, until reaching similar values to those observed in the control group (cells with no lipopolysaccharide stimulation). Furthermore, in co-stimulated Caco-2 cells, *K. marxianus* decreased the concentrations of IFN-γ and IL-12 and chemokines IP-10 and IL-8 [49]. The action of yeast cell wall material on the complement system has been known for a long time. Generally speaking, this action is related to the presence of glucans in the inner part of the yeast cell wall, which are formed of main chains of beta-(1-3)-linked D-glucose molecules attached to linear side chains of beta-(1-6) linked residues. These macromolecules have the ability to stimulate certain aspects of the immune system [50]. Since leukocytes and extravascular macrophages have a specific glucan receptor which is activated by β-glucan yeasts, the interactions mediated by macrophages and macrophage-derived products such as cytokines occur by stimulation [51]. On the other hand, Silva, et al. [52] isolated yeasts from Portuguese brined olives and analyzed their production of vitamins from the B-complex. These authors determined that thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin and folic acid can be produced by *P. membranaefaciens* and *P. fermentans*. They also suggested that yeasts release compounds able to promote lactic acid bacterial growth by symbiosis, as acid lactic released by lactic acid bacteria serves as a substrate for yeasts.

Masau

Ziziphus mauritiana fruit is called masau fruit in Zimbabwe, and is fermented spontaneously for 6-7 days by yeasts and LAB that originate in the surface of the fruit. Yeast species such as *S. cerevisiae*, *I. orientalis*, *Pichia fabianii*, *Hanseniaspora opuntiae*, *Saccharomycopisis fibuligera*, *Cryptococcus flavus* and *Rhodotorula mucilaginosa* take place in this process. In togwa fermentation, the presence of *I. orientalis*, *P. anomala*, *S. cerevisiae* and *K. marxianus* has also have been reported, these species demonstrated ability to produce folates [53]. Folates (Vitamin B_9) are essential cofactors in the biosynthesis of nucleotides and therefore crucial for cellular replication and growth [54]. Yeasts and some bacterial species can produce natural folates thought their biosynthesis pathway, but humans lack the capacity to synthesize these compounds [55]. The role of folate in the prevention of neural tube defects in the fetus has been established and sufficient folate intake may reduce the risk of cardiovascular disease, cancer and even Alzheimer's disease [27].

Cocoa

Fermentation and drying are key steps in the production of dry cocoa beans. In the fermentative process, the carbohydrates and citric acid of the cocoa pod mucilage are degraded by yeasts, LAB and acetic acid bacteria [56]. The number of *S. cerevisiae* and other non-*Saccharomyces* increased up to 7 log CFU/g in the product [57,58]. *S. cerevisiae* is commonly used as probiotic, it has showed anti-inflammatory activities in induced colitis in mice [59] and in a randomized clinical trial it was able to reduce abdominal pain in patients suffering from irritable bowel syndrome [60]. Etienne-Mesmin, et al. [61] investigated the survival of *Escherichia coli* O157:H7 against *S. cerevisiae* CNCM I-3856 using a dynamic gastrointestinal model; they observed a high bacterial mortality in the stomach and duodenum, probably due to the production of ethanol. In a study, a *S. cerevisiae* strain isolated from peach fermentation colonized the intestines of *Epinephelus coioides* and improved the feeding efficiency and growth rate of this fish, the optimal dose was of 10^7cfu/kg [62].

Kanji

Radish and carrot are fermented to prepare kanji, a traditional vegetable food. Fermentation is carried out with a 2.5% saline solution at a temperature of 25 °C. Radish fermentation has been used not only for food preparation; Malisorn and Suntornsuk [63] have reported the use of a starter culture based on *Rhodotorula glutinis* from brine obtained as by-product in radish fermentation to produce β-carotene. *R. glutinis* is a very important strain in the food industry due to metabolites production and safety implications. Its major application is in the production of large amounts of carotenoids, used not only as vitamin A precursors, but also as coloring agents [64]. Among the carotenoid pigments produced by yeast are lycopene (most powerful antioxidant from carotenoids) and β-carotene, an orange-yellow pigment able to reduce cell and tissue damage [65,66]. The carotenoid extracts obtained from a strain of *R. glutinis* isolated from fermented carrots were effective as antioxidants, since they were able to inhibitoxidation by 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH). These carotenoid extracts also show antibacterial activity against cultures of *Bacillus subtilis*, *Bacillus cereus* and *Salmonella enteritidis* at a concentration of 10^6cfu/mL [67]. Moreover, [68] optimized exopolysaccharide (EPS) production from *R. glutinis*, reaching a concentration of 1.14 g/L; and evaluated the antitumor activity of this substance by the 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay on human liver cancer cells Hep-G2. The exopolysaccharide extract led to a marginal inhibition in cell growth (IC_{50}, 29.81 μg/mL). However, on colon carcinoma HCT-116 cells, the exopolysaccharide had a strong cytotoxic effect with a low IC_{50} of 14.7 μg/mL. Another physiological benefit is that the EPS is retained longer in the gastrointestinal tract, so that colonization by the probiotic microorganisms can be enhanced. Consequently, EPS producing probiotic cultures can contribute to human health by positively affecting gut microbiota [69].

Pozol

Pozol is a fermented beverage from Southeastern Mexico. This beverage is made by cooking and grinding maize to obtain dough known as nixtamal. Nixtamal may or not be mixed with cocoa, and then is fermented at room temperature for 0.5-4 days [70]. Ben Omar and Ampe [71] suggested that during nixtamal fermentation, microorganisms act in proper succession. Yeast isolated from pozol samples showed different species, such as *Rhodotorula minuta*, *R. mucilaginosa*, *D. hansenii*, *Geotrichumc andidum*, *Candida guilliermondii* and *K. lactis* [70]. *K. lactis* isolated from kefir grains was evaluated and considered a probiotic agent due to its immune modulation effect. Lipopolysaccharides from *E. coli* were administered to Caco-2 cells to evaluate the immunological response in the presence of this yeast, results show a reduction in the secretion of Interleukin-8 as compared to the control group. IL-8 is known as the most representative proinflammatory cytokine and its synthesis by enterocytes can be induced in response to bacterial enteric pathogens. *K. lactis* also showed a reduction capacity of cholesterol in an *in vitro* system, Kourelis, et al. (2010) inoculated this strain in YEGP broth supplement with 0.3% Ox gall and a high cholesterol concentration (400 µg/ml). Results showed a cholesterol reduction of 30%. Chen, et al. (2010) determined the percentage of cholesterol assimilation of some yeasts isolated from raw cow's milk, observing that the metabolism of *P. kudriavzevii* and *Galactomyces* sp. decreased by 44.4 and 40% cholesterol concentration after 72 hours incubation in YPD broth containing 224.2 µg/ml cholesterol and 0.3% bile salts. The authors suggest that yeast cells inhibit cholesterol absorption and thus reduce the risks associated to high levels of this substance. Cholesterol reduction may be attributed to metabolic degradation by yeast cells [72].

Pulque

Pulque is a traditional Mexican alcoholic beverage produced by fermentation of the fresh sap known as aguamiel, extracted from some species of *Agave* plants. The fermented product is a slightly acidic liquid beverage with alcohol content of 4 to 7° GL. The microorganisms responsible for the fermentation are naturally occurring during sap accumulation in agave, and later they are incorporated in the collection and manipulation of the plant [73,74]. Páez-Lerma, et al. [75] isolated yeast species from *Agave duranguesis* fermentation in different regions of Mexico, results showed that the plant microbioma is dependent on environment factors and the most predominant species were *K. marxianus*, *T. delbrueckii*, *Pichia fermentans* and *Candida diversa*. Among these, *T. delbrueckii*, also isolated from fermented grape must, showed probiotic characteristics such as resistance to gastric and intestinal conditions, growth at 37°C, and 40% inhibition rate in DPPH activity (Trolox equivalent of 128 µM).

Tepache

Tepache is a beverage consumed in Mexico since pre-Hispanic times. It is mainly prepared from pineapple pulp, which may be mixed with maize, apple and orange. Fermentation of tepache takes place in wooden barrels at room temperature during 1 to 4 days [76]. *Hanseniaspora uvarum* and *Pichia guilliermondii* were the main yeast species observed on pineapples peel as well as in the fresh and fermented juice. *P. guilliermondii* was the dominant species in the early stage of the process, whereas *H. uvarum* became more prevalent at the end of fermentation, with a population concentration increasing from an initial level of 5 log CFU/mL to 8 CFU/mL at the end of the process. *H. uvarum* isolated from the surface of grape demonstrated an inhibitory effect against *Bortrytiscinerea* infections. In a study carried out by Liu, et al. [77] a culture of $1x10^6$ CFU/mL of this yeast decreased grape fruit disease by 25% for 4 days.

The use of antagonistic yeasts to inhibit the growth of pathogenic bacteria is still at an early stage of development. Antagonism of microorganisms by yeasts has been attributed primarily to (1) competition for nutrients, (2) pH changes in the medium as a result of growth-coupled ion exchange or organic acid production, (3) production of high concentrations of ethanol, (4) secretion of antibacterial compounds and release of antimicrobial compounds such as killer toxins or "mycocins" [78]. Mycocins are extracellular proteins or glucoproteins that disrupt cell membrane in susceptible microorganisms [79]. Various genera of yeasts have been reported as mycocinproductors, for example, Hatoum, et al. (2012) characterized anti-listerial hydrophobic peptides secreted by *D. hansenii*, *P. fermentans*, *C. tropicalis* and *Wickerhamomyces anomalus*, isolated from cheese. In experiments using tryptic soy broth, the anti-listerial compounds of *D. hansenii* decreased *Listeria monocytogenes* population by 3 log units. Analysis by Transmission Electronic Microscopy indicated that the anti-listerial compounds induce leakage in bacterial cells and ultimately cause bacterial lysis. More recently, *P. kudriavzevii* showed activity against bacterial pathogens such as *E. coli*, *Enterococcus faecalus*, *Klebsiella* sp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Pseudomonas alcaligenes*. The characterization of the killer toxin by SDS-PAGE showed a single protein band which corresponded to a molecular weight of approximately 39.8 kDa [80].

Water kefir, also known as "tepache de tibicos" is a slightly alcoholic beverage fermented, which is made with sugar cane and some fruits as figs or lemons (usually added to enhance flavor) [81]. The starter cultures used in the fermentation are grains composed of water and polysaccharides, in which living differents microorganisms. Martínez-Torres, et al. [82] described the fermentation process to produce "tepache de tibicos", they found the occurrence of *S. cerevisiae* (responsible of alcoholic fermentation) in the early stage of the process and other yeasts as *Candida californica* and *P. membranefaciens*.

Dairy foods

While the microbiology of dairy products is commonly dominated by lactic acid bacteria, it is now know that yeasts play an important role in the development of sensory characteristics during the fermentation process of products such as kefir [83], which originated in the Northern Caucasus and is acidic, viscous, slightly carbonated and contains small amounts of alcohol [84]. Kefir grains, the starter for producing kefir, comprise a complex microbial symbiotic mixture of microorganisms, among them yeasts (*Kluyveromyces*, *Saccharomyces* and *Torula*) [85]. Several studies investigate the variety of probiotic microorganisms growing in kefir [18,21,86,87].

Yeast as enhancer of bacterial survival

The interactions between yeasts and bacteria are very convoluted. For example, competition for nutrients, commensalism, mutualism, or antagonism may be beneficial or harmful [88]. These interaction mechanisms are still poorly understood.

Carvalho, et al. [89] reported the presence of *Lactococcus lactis* and *S. cerevisiae* in the fermentation process of cachaça (a sugar cane spirit produced in Brazil), and the analysis showed that *S. cerevisiae* was not negatively influenced by bacteria, however the growth of *L. lactis* decreased 2 log CFU/mL, which can be due to the competition for nutrients, or to the alcohol produced by the yeast. On the contrary, [90] observed that the production of metabolites such as lactic and acetic acids by LAB in sourdough had a negative effect in the maximum specific growth rate and ethanol production of *S. cerevisiae*; however the presence of yeast resulted in a significant increase in the maximum specific growth rate of *L. sanfranciscensis*. The investigations of [91] in the fermentation of water kefir describe an example of symbiosis between the yeasts *Zygotorulaspora florentina* and *S. cerevisiae*, and the bacteria *Lactobacillus hordei* and

Lactobacillus nagelii. These authors observed an increase of cell yield for all microorganisms, showing that the acidification of the medium by LAB allowed the growth of *Z. florentina*, whereas the growth of lactobacilli is improved by the production of amino acids and vitamin B_6 by the yeasts.

Proteins in the microbial cell wall may support the colonization of probiotics in the gut. Katakura, et al. [92] demonstrated that mannoprotein from *S. cerevisiae* and cytosolic proteins of *L. lactis* such as DnaK increased the aggregation between both the bacteria and yeast at pH 4. In a different study, strain-specific lectin-like activity of S-layer proteins from *L. kefir* was found to be responsible of the co-aggregation with *S. lypolitica* [93]. Hirayama, et al. [94] deleted the genes related to mannanbyosinthesis in *S. cerevisiae* and observed that co-aggregation activities decreased with *L. plantarum* isolated from Fukuyama pot vinegar. The authors indicated that surface proteins on bacteria recognize mannose residues of side chains on mannan as lectin-like protein.

Recently, interactions of two strains isolated from kefir were studied in an *in vitro* model and the results showed that some characteristics from *Lactobacillus paracasei* were enhanced by the interaction with *S. cerevisiae*. Yeast counts in the range of 3 to 5 log CFU/mL increased up to 1 log cycle the viability of *L. paracasei* in gastric juice at pH 2 and intestinal juice at pH 8, respectively. Co-aggregation and adhesion to Caco-2 cells by bacteria showed the same trend. This could be due to the proteins of the bacterial cell surface and polysaccharides in yeast cell wall, which play important roles in the different probiotic abilities [95]. Another study on fermented milk tried to improve the stability of *Lactobacillus rhamnosus* by using yeast *Williopsis saturnus var. saturnus*. The results showed that this yeast enhanced *Lactobacillus* stability in the fermented milk for 8 days as compared to the control. This positive interaction is generally attributed to the excretion of nutrients such as peptides, amino acids and vitamins by the yeast [96].

A more recent study evaluated the addition of *S. cerevisiae* or supernatant without viable yeast cells to *L. rhamnosus* during milk fermentation, the authors discovered that the yeast supernatant was able to enhance the survival of bacteria in the process. This suggests that yeast metabolites played an important role in enhancing *L. rhamnosus* survival. The same study found that these metabolites are mostly above 3,500 Dalton [97].

Conclusions

This review aims to promote the consumption of fermented foods as an alternative for a healthy diet and focus on the importance of yeasts as beneficial microorganisms. Further investigations by *in vivo* models can be considered to assess the use of yeast as probiotics and its commercialization in the world market. The morphological and physiological properties are very different for each species; therefore it is important to conduct a detailed study of each particular strain in order to find the best application. Supplementation of foods with yeasts to improve the quality of life is profitable, since these microorganisms show good tolerance to stress, being able to develop in restricted environments.

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