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Beer for live microbe delivery

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ABSTRACT

There is growing interest in the potential of probiotics and other commensal dietary microbes to improve human health. This review will examine beer as a microbe-containing food and the considerations needed when using beer for probiotic and other live microbe delivery to the digestive tract. Although most beers harbor low numbers of live microbes after brewing is complete and the final product is an environmentally stressful environment which impairs long-term microbial survival, commercially-produced Lambic and sour beers can contain live microbes. Recent studies have also tested the viability and impacts of probiotic strains of *Saccharomyces* and lactobacilli strains in beer. The findings show there remains the need to adjust strain use and production practices to enable microbial growth and survival throughout the intended shelf-life. We discuss opportunities to increase microbial survival overall, as well as for strains that confer specific health benefits.

1. Introduction

Brewing is an ancient food fermentation practice, whereby a starch source such as malt is mixed with water, and the resulting sugar-rich liquid (called 'wort') is allowed to ferment using yeasts to yield alcoholic beer (Bamforth, 2006). Although enjoyment is currently the primary reason to drink beer in many parts of the world, beer was historically used for nutrition and consumed in vast quantities as a safe alternative to water (Hornsey, 2003). These brewed beverages also possess bioactive properties beyond basic nutrition, collectively referred to as "functional benefits" (Hornsey, 2003). Hence, beer is similar to other fermented foods and beverages with reported health benefits (Marco et al., 2021). There is also growing interest in the craft brewing industry to create beers that possess both sensory and health-modulatory benefits (Strenk, 2021), much like traditional ales described in historical texts (Hornsey, 2003).

One way to enhance the health benefits of beers is to use them as carriers of live microorganisms to the digestive tract. Live microorganisms in foods and beverages mainly encompass non-harmful bacteria, yeast, and molds (Azcarate-Peril et al., 2019). Whereas moderate numbers of microbes (~10^{10} CFU/g) are present on fresh fruits and vegetables (Finger et al., 2023), this amount can increase 1000-fold in food fermentations (Rezac et al., 2018). Although some fermented foods are pasteurized prior to consumption (for example, bread and soy sauce), other fermented foods are expected to retain live microbes for at least a portion of the product shelf-life (Rezac et al., 2018). Recently, it was shown using the National Health and Nutrition Examination Survey (NHANES) cross-sectional study database, that consumption of microbe containing foods was correlated with reduced blood pressure, plasma insulin, and Body Mass Index, among other metrics of cardiovascular disease risk (Hill et al., 2023; Han and Wang, 2022).

Probiotics are another source of live dietary microbes. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill et al., 2014; Food and Agricultural Organization of the United Nations and World Health Organization, 2001). Probiotics are different from the indigenous microbes in foods because they are specific strains shown to confer a health benefit in controlled human studies. Strains of lactobacilli, bifidobacteria and *Saccharomyces cerevisiae* variant *boulardii* CNCM I-745 are currently the most frequently applied as probiotics (Merenstein et al., 2020). Probiotic intake is associated with a number of health benefits in randomized, controlled trials (RCTs) including, immunomodulatory, anti-obesity, anti-diabetic, and anti-cancer effects (Das et al., 2022). Although the specific doses required for health- promotion likely vary between strains and physiologic end-points, probiotics are typically provided in quantities of at least 10^9 cells per dose (Health Canada, 2009). Probiotics are included in both dietary supplement and (fermented) food formats (Cheng et al., 2019). Fermented dairy foods have received the most

Abbreviations: CFU, Colony Forming Units; EPS, exopolysaccharides; IBU, International Bittering Units; IPA, India Pale Ale; ISAPP, International Scientific Association of Prebiotics and Probiotics; NHANES, National Health and Nutrition Examination Survey.

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attention, and yogurt consumption in particular has been the focus of probiotic RCTs and epidemiological studies (Barengolts et al., 2019; Mousavi et al., 2020; Wagner et al., 2022). Hence, this opens the opportunity to test for potential probiotic effects in beer.

Because wort is a rich source of nutrients, it can be used to support microbial growth and survival (Kanyer et al., 2017). However, there are obvious technical challenges to the incorporation of live microbes and probiotics in beer, an alcoholic drink that is frequently pasteurized and filtered. Additionally, beer is vulnerable to microbially-caused sensory defects. Recent advances in probiotic and prebiotic beer development were recently, comprehensively reviewed (Chan et al., 2021; Zendeboodi et al., 2021). In this review, we broaden the perspective for using alcoholic, malt-based, craft beer for live microbe intake, including the microorganisms needed to make it as well as added probiotic strains. Herein, we present an overview of beer production, describe the microbes needed for brewing and their metabolic and stress-tolerance properties, and include studies examining (putative) probiotics in beer. Challenges and opportunities to increase the viability of the brewing microbes in the final product are discussed.

2. Overview of brewing

The Internal Revenue Code in the US defines the term "beer" as, "beer, ale, porter, stout, and other similar fermented beverages (including sake or similar products) of any name or description containing one-half of one percent or more of alcohol by volume, brewed or produced from malt, wholly or in part, or from any substitute therefor" (Federal Alcohol Administration Act, 2008) Craft beer is further defined as beers brewed by breweries with an annual production of six million barrels or less (Brewers Association, 2023).

The typical brewing process is summarized in Fig. 1. Brewing starts with grinding malt, a barley modified to create free sugars, amino acids as well as flavors and colors of beer. The result, or grist, is then mixed with water at a ratio of between 1:2.5 up to 1:4. Depending upon the beer style, the mixture is heated in a mashing stage (temperature ranges from 45 °C to 70 °C), a step-wise procedure during which a number of enzymes (primarily proteases and amylases) from the malt hydrolyze substrates such as proteins to amino acids and starch to maltose and glucose (Pati & Samantaray, 2022). This results in the sweet wort. The wort is then separated from the malt grist and boiled with the addition of hops for approximately 60 min. Hops contains α-acids such as humulone and adhumulone, which are isomerized during boiling to provide the bitterness in beer (Bamforth, 2006). The resulting wort is cooled and transferred to a fermenter.

The wort undergoes fermentation by a single strain or multiple microbes, once or twice depending on the style of beer. Co-fermentations are also possible. Typically, a strain of *Saccharomyces* and bacteria (for co-fermentation) are inoculated ("pitched") into the fermenter. The microbial pitching rate is determined by the expected strain viability, beer style (ale, lager, or sour), wort volume, and targeted alcohol content. Beer styles are then further distinguished based on their fermentation temperature, time, and pH (Bamforth, 2006). The fermentation process can take anywhere between five to 10 days for ales and lagers and up to 12 months for Lambics, wherein secondary fermentation is required. The beer is frequently filtered to improve clarity and remove residual yeast. For craft beer, most of the yeast flocculate and settle. To speed up this process, "cold crashing" is frequently used wherein the fermenter temperature is dropped to approximately −1 °C or by using

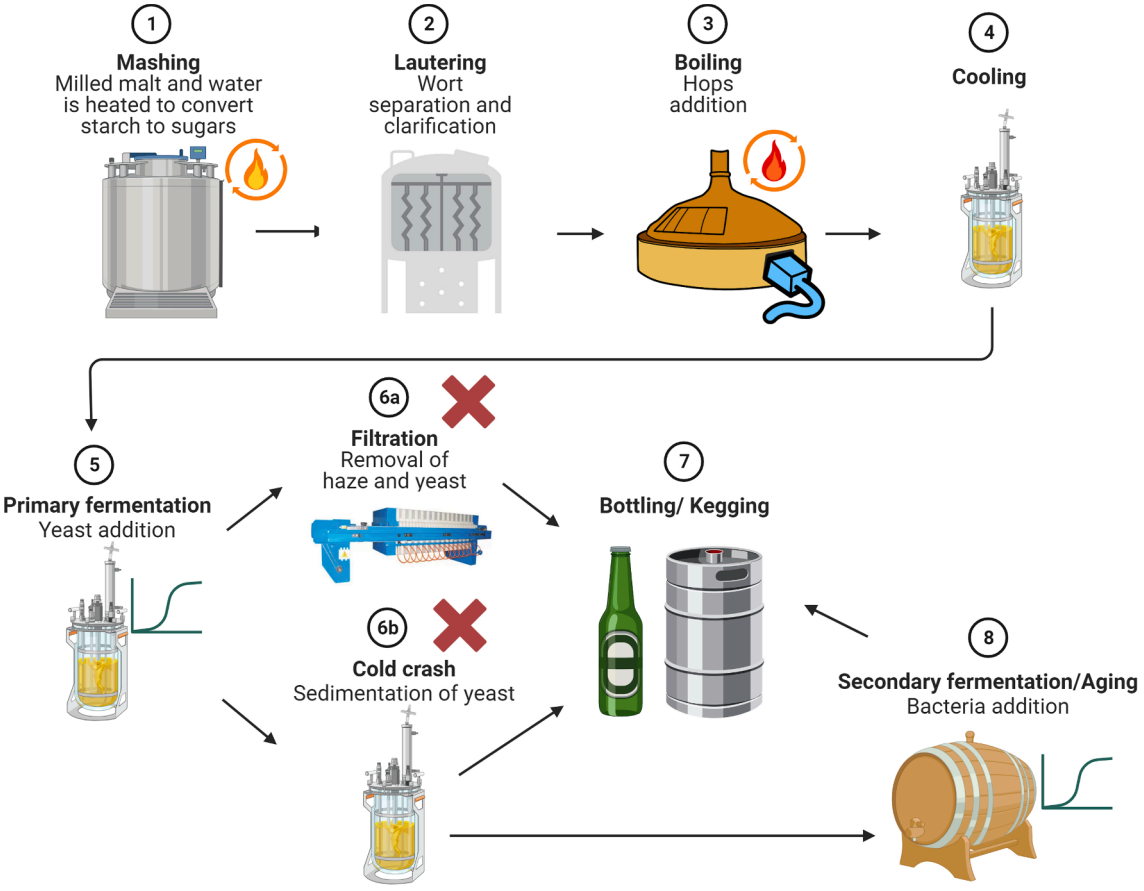

Fig. 1. Overview of the brewing process. Heating steps are indicated by flame symbols. The fermentation steps where microbial count is expected to increase are indicated by green growth curves. In order to maintain the high viable cell-count post primary fermentation, filtration/cold crashing steps need to be avoided, as indicated by the red cross. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

clarifying agents (Thesseling et al., 2019)

Beer styles such as Lambic and sour beers can include a secondary fermentation using lactic acid bacteria (LAB) (Spitaels et al., 2015; Dysvik et al., 2020). This step is completed in casks before the beer is packaged. Secondary fermentation imparts the characteristic sour flavor in these beers. Brewers can also make low alcohol or non-alcoholic beers using dealcoholization (thermal treatments, reverse osmosis etc.) or by arresting fermentation before the alcohol content reaches 0.5% v/v (Salanță et al., 2020).

Beer is finally packaged in bottles, kegs, or cans with an optional pasteurization step. Pasteurization, while commonly employed by industrial-scale, commercial producers, is not typical for craft beer. In the craft brewing industry, yeast can be re-pitched into finished beer to carbonate the beer further and increase the alcohol content in a process referred to as 'bottle conditioning'. The resulting craft beer typically contains between 3.5% and 9% (v/v) alcohol and a pH range of 3.0 to 5.5.

3. Yeast in beer

3.1. Yeast diversity in beer

Although beer can contain diverse yeast species (Table 1), *S. cerevisiae* is the most important microorganism for beer production. Traditionally, in-house *S. cerevisiae* strains were used in breweries (Bamforth, 2006). However, recent analyses have shown that these strains are genetically related and a number of subpopulations have emerged due to hybridization, resulting in specific beer styles (Gallone et al., 2019). A recent phylogenetic study of 35 *S. cerevisiae* strains found beer yeasts belong to three main groups (European dominant, Asian dominant, and African dominant) based on the origin of their allelic content (Saada et al., 2022). This study showed that variable brewery practices and differences in substrates used in those regions have led to genetic adaptation of *S. cerevisiae* strains over time. There are also other genera, *Saccharomyces* species, and variants of *S. cerevisiae* important for beer (Table 1). *S. pastorianus* is used for brewing lager-style beers and is a hybrid between *S. cerevisiae* and *Saccharomyces eubayanus* (). *S. cerevisiae* var. *boulardii* are variants of *S. cerevisiae* and also have reported use in brewing (Edwards-Ingram et al., 2007).

In beer fermentations, *S. cerevisiae* preferentially consumes glucose, sucrose, maltose and maltotriose in that order (Alves-Jr et al., 2007; D'Amore et al., 1989). *S. cerevisiae* ferments these sugars using the glycolytic pathway (Fig. 2), and ethanol is produced as an end-product of energy conservation metabolism (De Deken, 1966). *S. cerevisiae* also assimilates small peptides derived from malt (e.g., hydrophobic peptides derived from storage proteins), to produce distinct flavor compounds such as higher alcohols (alcohols with more than two carbons), organic acids, and esters (Lekkas et al., 2009; Olaniran et al., 2017). *S. cerevisiae* var. *boulardii* and *S. pastorianus* strains possess similar metabolic abilities as *S. cerevisiae* (Fig. 2). However, *S. cerevisiae* var. *boulardii* inefficiently assimilate galactose and substrates with high galactose concentrations are often toxic to these yeast (Liu et al., 2018).

Most *Saccharomyces* used in beer brewing, but not wine-making, are incapable of assimilating larger oligosaccharides called dextrins which

Table 1
Yeast species and their observed abundances in beer.

Yeast group	Source	Inoculum (CFU per mL)	Fermentation time and temperature	Viable cell counts (CFU per mL)	References
Saccharomyces					
Saccharomyces cerevisiae	Beer (ale)	10^5 to 10^7	5–7 days; 20–24 °C	10^4 to 10^8 [a] 10^8 [a,b] 10 [c]	(Wauters et al., 2023; Silva et al., 2020; Yılmaz and Gökmen, 2019)
Saccharomyces pastorianus	Beer (lager)	10^6	5–15 days; 12–20 °C	10^8 [a]	(Salazar et al., 2019; Yılmaz and Gökmen, 2019; Mahanta et al., 2022)
Saccharomyces bayanus	Beer	ND	ND	ND	(Salazar et al., 2019)
Saccharomyces cerevisiae var. *diastaticus*	Beer	ND	ND	ND	(Krogerus & Gibson, 2020)
Saccharomyces cerevisiae var. *boulardii*	Lambic beer/ sour beer	10^5 to 10^6	6–15 days; 20–25 °C	10^6 to 10^9 [a] 10^7 [a,b]	(Capece et al., 2018; Fu et al., 2022; Silva et al., 2020)
Brettanomyces					
Brettanomyces bruxellensis	Lambic beer/ sour beer	ND	3–7 days, 25–28 °C	10^4 to 10^5 [a,b]	(Crauwels, et al., 2015; Serra Colomer et al., 2019)
Brettanomyces custersianus	Lambic beer/ sour beer	ND	ND	ND	(Serra Colomer et al., 2019)
Pichia					
Pichia kluyveri	Lambic beer/ sour beer	10^9	14 days; 22 °C	10^8 [a,b]	(Piraine et al., 2023)
Pichia kudriavzevii	Lambic beer/ Sour beer	ND	ND	ND	(Santos et al., 2022)
Pichia myanmarensis	Lambic beer/ sour beer	ND	ND	ND	(Durga Prasad et al., 2022)
Other yeasts					
Dekkera anomala	Lambic beer/ sour beer	ND	ND	ND	(De Roos et al., 2020)
Candida intermedia	Lambic beer/ Sour beer	ND	ND	ND	(Piraine et al., 2023)
Candida norvegica *Candida atlantica* *Candida friedrichii*	Sour beer	ND	ND	ND	(Bossaert et al., 2021)

ND = Not determined.
[a] indicates cell count at the end of fermentation
[b] indicates cell count in co-cultures or mixed-species communities.
[c] indicates cell count after at least 20 days of storage at 4 °C.

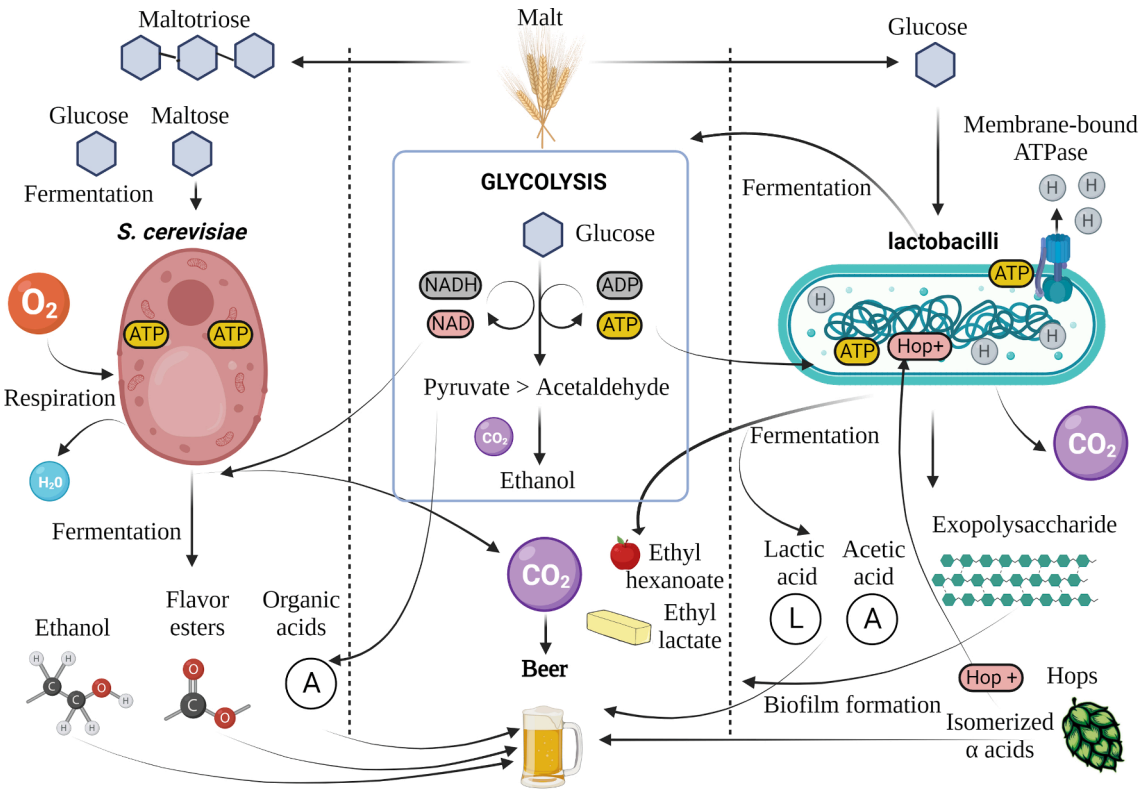

Fig. 2. Beer fermentation metabolism by lactobacilli and *S. cerevisiae*. *S. cerevisiae* strains sequester ATP for respiration, thereby enabling them to initially increase biomass in the fermenter. *S. cerevisiae* strains preferentially consume maltose and maltotriose (majority in malt) while lactobacilli consume glucose. Both *S. cerevisiae* and most lactobacilli (except heterofermentative organisms like *Pediococcus*) conduct fermentation using the glycolysis pathway to produce different by-products. *S. cerevisiae* strains produce ethanol, flavor esters, CO_2 and organic acids. Lactobacilli produce lactic acid, acetic acid and exopolysaccharides. These by-products interact with isomerized hop acids from hops to produce different aromas, haze and flavors in beer. A hop resistance mechanism in some lactobacilli may be due to the overexpression of membrane-bound ATPases, which pump protons from the cytoplasm.

form 20 to 30% of sugars in an all-malt wort (Russell, 2003). These residual dextrins present in beer post fermentation help in providing a good mouthfeel and residual sweetness. However, wild yeast variants like *S. cerevisiae* var. *diastaticus* can assimilate dextrins (Krogerus & Gibson, 2020). These yeast possess an extracellular glucoamylase encoded by *STA* genes that cleaves glucose from the non-reducing ends of oligosaccharides (Latorre-García et al., 2008). *S. cerevisiae* var. *diastaticus* are used in the production of some Belgian style beers (Latorre-García et al., 2008), but they are also notorious contaminants in traditional brewing because they can outcompete *S. cerevisiae* (Krogerus & Gibson, 2020).

Other yeast genera such as *Brettanomyces* spp. and *Pichia* spp. have been used to brew sour, Lambic, and non-alcoholic beers (Durga Prasad et al., 2022; Serra Colomer et al., 2019). These yeasts typically require slightly lower fermentation temperatures (10–15 °C). *Brettanomyces* spp. are more diverse compared to *S. cerevisiae*, produce acetic acid, and are able to assimilate other sugars including dextrins and cellobiose (Serra Colomer et al., 2019). *Pichia* spp. are associated with characteristic, desirable, volatile organic compounds such as ethyl butyrate and octanol which differentiate them from *S. cerevisiae* (Santos et al., 2022).

3.2. Yeast cell numbers and viability in beer

S. cerevisiae strains grow rapidly in wort reaching up to 1×10^8 CFU per mL in 5 days, at 20 °C with pH 7.0 (Silva et al., 2020). *S. pastorianus* pitched into wort at 10^6 CFU per mL can increase to 10^8 CFU per mL in 12 days at 15 °C (Yılmaz & Gökmen, 2019). *S. pastorianus* also reached 10^8 CFU per mL within 5 days at 20 °C during sour beer production with *Lactiplantibacillus plantarum* (Mahanta et al., 2022). To the best of our knowledge, the highest number of *S. cerevisiae* var. *boulardii* in beer was reported to be ~10^9 CFU per mL for strain 17, with the final beer containing 15 International Bittering Units (IBU) and 4 % v/v alcohol (Silva et al., 2020). In co-culture with five different *S. cerevisiae* strains inoculated at equivalent levels, a *S. cerevisiae* var. *boulardii* strain isolated from a commercial product (Codex, Zambon, Italy) was found to be more dominant towards the end of fermentation with cell counts ranging from 8×10^6 to 7×10^7 CFU per mL, ~2-fold higher than the other strains (Capece et al., 2018). However, these high yeast numbers are not necessarily sustained. Out of 115 *S. cerevisiae* strains tested, none persisted in blond ale (8.5 % ABV) for longer than three months post fermentation and packaging. At that time, less than 10 CFU *S. cerevisiae* per mL (0.0005 % survival) were found under refrigerated storage (Wauters et al., 2023).

For sour, Lambic, and non-alcoholic beers, *Brettanomyces* spp. was detected in levels of 10^4 to 10^5 CFU per mL (Crauwels, et al., 2015) and *Pichia* spp. in quantities of 10^8 CFU per mL (Piraine et al., 2023). The viability and abundance of *Brettanomyces* and *Pichia* specifically are yet to be explored through beer maturation and/or product shelf life. More generally, after 6 months of Lambic beer ageing, estimated total yeast and bacterial counts were approximately 10^5 CFU per mL, but dropped to approximately 10^2 CFU per mL after 18 months, and to undetectable quantities after two years (De Roos et al., 2018). Yeast cell counts in non-alcoholic beers have not been examined throughout shelf life, but after packaging in glass bottles, yeast cell count was found to be approximately 10^4 CFU per mL (Zendeboodi et al., 2020).

3.3. Factors that influence yeast cell survival in beer

The growth and survival of yeast in beer depends on their capacities to tolerate variations in temperature, acid, ethanol, and antimicrobial

compounds (for example isomerized alpha-acids in hops (Hazelwood et al., 2010)). Fermentation temperatures ranging from 18 to 25 °C and wort pH of 5.3–5.7 were found to be optimal for *S. cerevisiae* ethanolic fermentations (Imai & Ohno, 1995; Torija et al., 2003). After beer is made, it is stored at 4 °C. To this regard, *S. cerevisiae* was found to remain viable up to three months in beer at 4 °C, with little to no growth (Wauters et al., 2023). Additionally, *S. cerevisiae* strain ATCC 2601 remained viable for 24 months in phosphate buffer at neutral pH, when stored between 3 °C and 5 °C (Tanguay & Bogert, 1974).

Yeast should also survive in the quantities of ethanol that they produce. Ethanol has antimicrobial properties causing disruption of cell membranes, thereby increasing permeability and cytosolic acidification (Charoenbhakdi et al., 2016; Chen et al., 2022). Ethanol resistance mechanisms of *S. cerevisiae* strains include vacuole formation, trehalose accumulation, transport of amino acids such as tryptophan and proline, increased expression of chaperone proteins that stabilize denatured proteins, and integration of oleic acid into the membrane (Ramírez-Cota et al., 2021). A transcriptomic and proteomic study on *S. cerevisiae* strain Sc131 showed the mitochondria and endoplasmic reticulum are both primary organelles involved in ethanol stress tolerance (Li et al., 2019). The study also found that G-protein coupled receptor signaling and metal ion regulation, two key biochemical processes for adaptive stress response in yeasts, were activated by the presence of ethanol, indicating an adaptation to ethanol stress. To overcome these challenges, industrial strains of *S. cerevisiae* are often developed with resistance to ethanol and different temperatures. Resistance can be selected for by either genomic hybridization (Wang L et al., 2021) or adaptive evolution facilitated by common brewing techniques such as yeast sequestration (removing yeast solids from beer and re-using in fresh wort) and re-pitching (Gibson et al., 2020).

While *S. cerevisiae* var. *boulardii* and *S. cerevisiae* are highly genetically related (Khatri et al., 2017), they have different stress tolerance levels (Pais et al., 2021) (Fig. 3). In recent studies in beer, *S. cerevisiae* var. *boulardii* strains were found to be more acid and temperature tolerant compared to *S. cerevisiae*. In 0.5 % v/v acetic acid (pH range of 3–4) in laboratory culture medium, *S. cerevisiae* var. *boulardii* CNCM I-1079 reached 2.5 times higher cell numbers compared to *S. cerevisiae* SY (Fu et al., 2022). Exposure of either strain to weak acid stress was associated with a wrinkling of the outer cell wall, leading to budding and release of new yeast cells (Fu et al., 2022). However, CNCM I-1079 exhibited a significant increase in budding in response to acid stress compared to *S. cerevisiae* SY, indicating the capacity of CNCM I-1079 to trigger programmed cell death, rather than succumbing to necrosis due to acetic acid stress (Fu et al., 2022). In another study, *S. cerevisiae* var. *boulardii* ATCC MYA-796 was found to be more tolerant to higher (37 °C) temperatures than *S. cerevisiae* BY4742 (Liu et al., 2018). That study also proposed the thermotolerance could be due to a G1278A mutation in the *PGM2* gene encoding phosphoglucomutase (Liu et al., 2018).

Numerous studies have shown *Brettanomyces* spp. are more stress tolerant than *S. cerevisiae* due to cell wall proteins involved in adhesion, pseudo-hyphal growth, and other functions that enable them to assimilate polyphenols, ethanol, and nitrates, among other compounds for

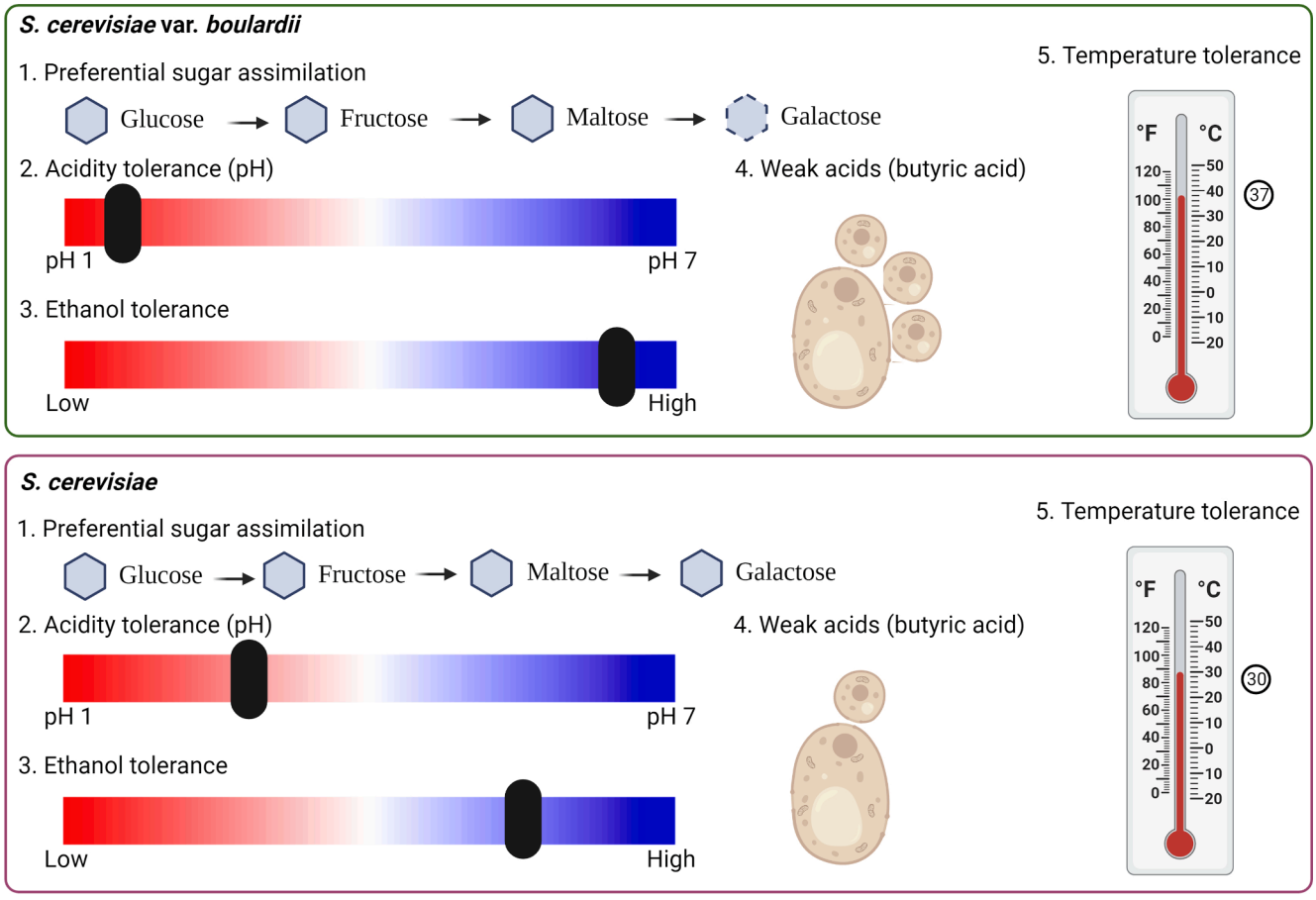

Fig. 3. Comparison of *S. cerevisiae* var. *boulardii* and *S. cerevisiae*. *S. cerevisiae* var. *boulardii* and *S. cerevisiae* strains use the same sugars for growth, except that *S. cerevisiae* var. *boulardii* strains consume galactose ineffectively and in small quantities. Both strains can tolerate acidic environments (up to 4.0), but *S. cerevisiae* var. *boulardii* strains can even tolerate gastric pH of 2.0. Both strains are capable of budding in the presence of weak acids, but *S. cerevisiae* var. *boulardii* strains show more budding in these conditions. Some *S. cerevisiae* var. *boulardii* strains are more ethanol tolerant compared to *S. cerevisiae* strains, and therefore tend to dominate the fermentation community. Finally, certain *S. cerevisiae* var. *boulardii* strains can tolerate human body temperature of up to 37 °C but most *S. cerevisiae* strains can only grow up to 30 °C.

survival (Menoncin & Bonatto, 2019). For example, *Brettanomyces bruxellensis* AW1499 isolated from wine grew in the absence of soluble sugars by utilizing hops-associated nitrogenous compounds as energy sources (Curtin et al., 2012). Examination of the AW1499 genome showed this organism possesses genes encoding transporters and enzymes that may enablenitrate assimilation more effectively compared to *S. cerevisiae* (Curtin et al., 2012). However, because that strain was associated with wine, different stress tolerances could be expected for beer-associated *Brettanomyces*. To that regard, comparisons of the *B. bruxellensis* AW1499 (wine strain) and *B. bruxellensis* ST05.12/22 (beer strain) genomes showed the latter does not possess genes involved in nitrogen metabolism, and correspondingly, was unable to grow on nitrate as a sole nitrogen source (Crauwels et al., 2014).

Lastly, *Pichia* spp., stress tolerance has been sparsely explored in the context of beer. Generally, compared to *S. cerevisiae, Pichia* appears to be less tolerant to ethanol and acid stress (Wang R et al., 2021). The high-osmolarity glycerol mitogen-activated protein kinase (HOG/MAPK) signaling pathway controls adaptation to environmental stress, and plays a vital role in the response of *S. cerevisiae* to hyperosmotic stress (Hohmann, 2015). Although *Pichia pastoris* uses the HOG/MAPK signaling pathway as a stress tolerance mechanism, it does so less effectively (Wang R et al., 2021). Gene transcript quantification for 13 *S. cerevisiae* strains and 18 *P. pastoris* strains upon exposure to osmotic stress showed the *Pichia* strains preferentially utilized stress-induced, damage repair responses rather than the HOG/MAPK pathway (Wang R et al., 2021).

4. Bacteria in beer

4.1. Bacterial diversity in beer

Bacteria are both desirable microorganisms in beer, such as for sour and Belgian Lambic beer fermentations, and important spoilage agents. Traditionally, brewers relied on “wild” or “spontaneous” fermentation practices to produce such beers, whereby the wort was maintained in open vats (Spitaels et al., 2014). In current commercial settings, *Enterobacteriaceae*, lactobacilli, *Pediococcus* and Acetic Acid Bacteria (AAB) have been found to ferment beer wort (Table 2). These bacteria are most important for sour and Lambic beer fermentations and grow in succession in four main stages – an *Enterobacteriaceae* fermentation, followed by a *S. cerevisiae* fermentation, then a secondary fermentation with lactobacilli and AAB, and lastly a maturation phase led by *Pediococcus* (Spitaels et al., 2014; Bongaerts et al., 2021). The fermentation and maturation phases are typically carried out in either a wooden barrel, wherein the microorganisms present in the wood ferment the beer, or a secondary fermentation tank in which microbes are pitched in intervals (De Roos et al., 2018). The diversity and metabolic contributions of *Enterobacteriaceae*, lactobacilli, *Pediococcus* and AAB are described below.

Members of the *Enterobacteriaceae* family such as *Enterobacter aerogenes* (now *Klebsiella aerogenes*) and *Enterobacter kobei* grow rapidly during the first phase of mixed fermentation in sour and Lambic beers together with wild yeasts (Brenner et al., 2005). They produce acetic,

Table 2
Bacterial species and their observed abundances in beer.

Taxa	Source	Inoculum (CFU per mL)	Fermentation time and temperature	Viable cell counts (CFU per mL)	References
Enterobacteriaceae					
Enterobacter aerogenes *Enterobacter cloacae* *Enterobacter hormaechei* *Enterobacter kobei*	Lambic beer and Gueuze beer	ND	ND	ND	(Spitaels et al., 2014)
Klebsiella oxytoca	Lambic beer	ND	ND	ND	(Spitaels et al., 2015)
Escherichia coli	Lambic beer	ND	ND	ND	(Spitaels et al., 2014)
Hafnia alvei *Hafnia paralvei*	Lambic beer	ND	ND	ND	(Spitaels et al., 2014)
Citrobacter freundii	Lambic beer	ND	ND	ND	(Spitaels et al., 2014)
Lactic acid bacteria (LAB)					
Levilactobacillus brevis	Sour beer	10^6–10^7	7 days; 22-30 °C	10^7-10^8 [a] 10^8-10^9 [a,b] 10^7-10^8 [c]	(Dysvik et al. 2020; Fan et al. 2020; Herkenhoff et al. 2023)
Lacticaseibacillus paracasei	Beer, Sour beer	10^6–10^8	8-12 days; 20 °C	10^9 [a] 10^8–10^9 [b,c]	(Bertsch et al., 2019; Chan et al., 2019; Herkenhoff et al., 2023; Loh et al., 2021)
Lacticaseibacillus rhamnosus					
Lactiplantibacillus pentosus	Sour beer	10	2 days; 40 °C	10^9 [a] 10^7 [c]	(Lee et al., 2020)
Lactiplantibacillus plantarum					
Limosilactobacillus fermentum	Sour beer	10	2 days; 40 °C	10^8 [a]	(Lee et al., 2020)
Pediococcus damnosus	Lambic beer, beer	10^7	7 days; 26 °C	~10^5 [a,b]	(Snauwaert et al., 2015; Xu et al., 2022)
Leuconostoc citreum	Malt and beer	10^8	1 hr; 30 °C	10^5 [a,b]	(Choi et al., 2020)
Acetic Acid Bacteria (AAB)					
Acetobacter lambici *Acetobacter orientalis* *Acetobacter pasteurianus*	Lambic beer, beer	ND	24 months; 9-20 °C	10^7 [a,b] (after 3 months); Undetected [a,b] (after 24 months)	De Roos et al. (2018)
Gluconobacter oxydans	Sour beer	10^8	10 days; 20 °C	10^8 [a] 10^6 [c]	(Neffe-Skocińska et al., 2022)

ND = Not determined.
[a] indicates cell count at the end of fermentation
[b] indicates cell count in co-cultures or mixed-species communities.
[c] indicates cell count after at least 20 days of storage at 4 °C.

lactic, and other acids, along with ethanol and carbon dioxide (Bongaerts et al., 2021). *Enterobacteriaeae* can also aid in developing the unique flavor profile of Lambics due to their ability to produce long-chain fatty acids such as linoleic and linolenic acids that can be later metabolized by a *S. cerevisiae*, lactobacilli and AAB (De Roos & De Vuyst, 2019; Spaepen et al., 1978).

AAB encompassing *Acetobacter* or *Gluconobacter* genera have been found to originate from the cask wood and then dominate the mid-stages of Lambic beer fermentation (De Roos & De Vuyst, 2019; Spitaels et al., 2015). Eight different *Acetobacter* species were found in the wooden cask used for secondary fermentation (De Roos et al., 2018). The dominant AAB species changed over time, starting with *Acetobacter orientalis* during the first 3 days, followed by *Acetobacter pasteurianus,* and then *Acetobacter lambici* after 18 months (De Roos et al., 2018). AAB contribute to the acidity in Lambic beers by producing acetic acid during aerobic respiration using ethanol as a substrate (Prust et al., 2005). AAB also produce significant quantities of acetoin (approximately 150–180 mM in beer) and ethyl acetate (approximately 1.4–4 mM), compounds that impart complex flavors to Lambic beers (Kashima et al., 1998; Moens et al., 2014). Acetoin (3-hydroxy-2-butanone) is a flavor compound with a buttery taste and cream odor and ethyl acetate provides fruity notes (Bongaerts et al., 2021).

Lactobacilli comprise a collection of genera in the *Lactobacillaceae* family previously grouped under the genus name *Lactobacillus* (Zheng et al., 2020). The most common lactobacilli species used in beer include *Levilactobacillus brevis*, *Lacticaseibacillus paracasei*, and *L. plantarum* (Table 2). Lactobacilli are generally associated with beer spoilage because they produce lactic acid causing an undesired sour flavor (Umegatani et al., 2022). Some lactobacilli also produce exopolysaccharides (EPS) which are levans and dextrans of high molecular weight (Fig. 2; Umegatani et al., 2022). EPSs can lead to undesirable textures in beers (Fraunhofer et al., 2018). For example, *L. brevis* TMW 1.2112 produces slimy-texture beer as a result of its production of the EPSs composed of β-(1,3–1,2)-linked glucose units (Fraunhofer et al., 2018).

For sour beers, lactobacilli are added either at boiling or at secondary fermentation stages (Neffe-Skocińska et al., 2022). In Lambic beers, lactobacilli grow after the *Enterobacteriaceae* and AAB (De Roos et al., 2018). They metabolize mono- and disaccharides using homo- and hetero-fermentation pathways (Fig. 2) to mainly produce lactic acid and acetic acid (Bongaerts et al., 2021; Wang Y et al., 2021). Lactobacilli can also contribute other flavor compounds, like ethyl hexanoate and ethyl lactate (Fig. 2). These compounds were enriched in *L. brevis* and *S. cerevisiae* co-cultures (Fan et al., 2020).

The final stages of Lambic beer maturation are led by the LAB *Pediococcus* spp. (Bongaerts et al., 2021; Spitaels et al., 2015). A recent shotgun metagenomic study found *Pediococcus damnosus* was the only species present during the malic acid consumption stage of Lambic beer production (De Roos et al., 2020). Notably, the *Pediococcus* species *damnosus*, *inopinatus* and *dextrinicus* have also been implicated to cause undesirable effects in traditional beer by overproduction of diacetyl, a compound with intense buttery flavor (Sakamoto & Konings, 2003).

4.2. Bacterial cell numbers and viability in beer

Enterobacteriaceae can reach numbers up to 10^5 CFU per mL in the first month of Lambic beer fermentation (De Roos & De Vuyst, 2019). In sour beer, *Enterobacteriaceae* dominate at levels of $\sim 10^7$ to 10^8 CFU per mL during the first phase of fermentation (Spitaels et al., 2014). The authors of this study also hypothesized *Enterobacteriaceae* can potentially persist in a viable but non-culturable (VBNC) state through the later stages of Lambic beer production. AAB were found in a range of 10–10^5 CFU per mL in the same study (Spitaels et al., 2014). *L. brevis* strain BSO464 reached approximately 10^8 CFU per mL in mono- and co-culture with *S. cerevisiae* US-05 in 48 hours; numbers essential for sour beer production (Dysvik et al., 2020). In a subsequent study, *L. brevis* BSO464 was found in levels of $\sim 1.5 \times 10^6$ CFU per mL in sour beer for up to 14 days post fermentation, provided the wort was supplemented with 2 % w/v xylooligosaccharides (Dysvik et al., 2020) *P. damnosus* was shown to enter a VBNC state at levels of 10^3 CFU per mL in beer (Xu et al., 2022). The inability to culture these bacteria creates a challenge to detect this potential spoilage microbe. For sour and Lambic beers, bacterial cell numbers during beer aging were found to range from a high of approximately 10^6 CFU per mL after 3 months to a low of 10^2 CFU per mL after 12 months (Spitaels et al., 2014).

4.3. Factors that influence bacterial cell survival in beer

It is generally understood a pH of less than 4.0 and ethanol concentrations greater than 2 % are prohibitive to the viability of most bacteria in beer (Stewart & Priest, 2006). However, AAB and other microorganisms may persist (De Roos & De Vuyst, 2019). AAB in particular are known for their ability to survive in the low pH environment of Lambic beer (De Roos et al., 2018). Recently, acid stress tolerance was compared among six laboratory-adapted strains and three ancestral strains of *A. pasteurianus* (Gao et al., 2023). Upon continuous cultivation in the presence of ethanol (4 % v/v) and acetic acid (20 and 30 g/L) for four months in an experimental evolution study, the beer-associated strain *A. pasteurianus* ATCC 33,445 accumulated the highest number of mutations, indicating a lack of acid-adapted properties prior to incubation in those conditions (Gao et al., 2023). All adapted strains shared mutations in 30 different genes involved in lactate metabolism, stress response, cell membrane biosynthesis, and transposases. However, the relationship between these genes and acid stress tolerance is still unclear (Gao et al., 2023).

Numerous studies have examined the genetic and physiological adaptation mechanisms relating to ethanol and acid stresses in lactobacilli (Chen & Lu, 2018; van Bokhorst-van de Veen et al., 2011; Zhang et al., 2022), but only a few reports have focused on examining these mechanisms in beer. Comparisons between lactobacilli and *S. cerevisiae* (Chan et al., 2019; Dysvik et al., 2020), and lactobacilli and *S. pastorianus* (Mahanta et al., 2022) in beer concluded that lactobacilli are generally not as tolerant as these yeast species to those environmental stresses. Of the three commonly used lactobacilli species in sour beer production, *L. brevis* was the most tolerant to both acid and ethanol stress when compared to *L. plantarum* and *L. buchneri* (Dysvik et al., 2020). *Leuconostoc mesenteroides*, *Oenococcus oeni* and *L. brevis* strains survived in beer containing 4–6 % ethanol (v/v), with viabilities $>$ 10 CFU per mL on day 14 (Ovalle-Marmolejo et al., 2023). However, ethanol stress (>6% v/v), lactic acid stress (4.2 mg/mL), and fast nutrient depletion (reduction of sugars from 11 to 1 mg/mL in first 4 days) was found to stimulate the production of biogenic amines such as histamine and tyrazine by all three lactobacilli, causing unpleasant flavors (Ovalle-Marmolejo et al., 2023). In laboratory culture medium, 8 % v/v ethanol led to physiological changes in *L. plantarum,* including higher levels of citrate consumption, modified cell membrane fatty acid composition, and invaginating septa driven by modulated gene expression (van Bokhorst-van de Veen et al., 2011). A hop resistance mechanism in *L. brevis* may be due to the overexpression of membrane-bound ATPases, which pump protons from the cytoplasm (Fig. 2). A *horA* encoded multi-drug ATP-binding cassette (ABC) resistance transporter was found to prevent the internalization of hop compounds into the cytoplasm (Sakamoto et al., 2001). However, once internalized, the protein levels of ATPase pumps in the membrane were shown to increase and were associated with pumping excessive protons out of the cytoplasm across the membrane (Sakamoto et al., 2002).

Pediococci have also been examined for acid and ethanol stress tolerance levels. Genome comparisons between *P. damnosus* LMG 28219, isolated from sour beer, and *P. pentosaceus* ATCC 25745, isolated from plants, revealed 30 unique genes in LMG 28219 potentially corresponding to hop-acid resistance, folate biosynthesis and EPS production (Snauwaert et al., 2015). Similar to lactobacilli, the *horA*, *hitA* and/

or *horC* genes encoding transporters were associated with hop-resistance in both *P. damnosus* LMG 28219 (Snauwaert et al., 2015) and another beer strain *P. claussenii* ATCC BAA-344^T (Pittet et al., 2013).

5. Probiotic yeasts and bacteria in beer

The potential for making probiotic beer was first nicely shown when probiotic *Bifidobacterium lactis* BB-12 and *Lactobacillus acidophilus* LA-5 were sustained in non-alcoholic beer at levels ~10^6 CFU per mL after 20 days of refrigerated storage, when added after heat inactivation of the residual yeast (Sohrabvandi et al., 2010). Subsequent studies have shown similar possibilities and challenges for including probiotics in beers, both during and after the brewing process. These aspects were systematically reviewed by (Chan et al., 2021). Thus, we contribute to this dimension of live microbe delivery, by addressing more recent findings on the inclusion of (putative) probiotic strains in beer production.

There have been a few studies investigating probiotic *S. cerevisiae* var. *boulardii* CNCM I-745 in beer (Mohammadi & Saris, 2022; Ramírez-Cota et al., 2021). *S. cerevisiae* var. *boulardii* CNCM I-745 was first isolated from mangosteen fruit and has a dossier of health benefits from clinical studies (Abdel-Kareem et al., 2019; Mourey et al., 2020; Silva et al., 2021; Sougioultzis et al., 2006), some of which include improvement of the inflammatory bowel disease Ulcerative Colitis (Duysburgh et al., 2021) and the prevention and treatment of diarrhea (Mourey et al., 2020). This strain was tested in commercial nutraceuticals and fermented foods (Capece et al., 2018; Goktas et al., 2021; Senkarcinova et al., 2019; Swieca et al., 2019).

In beer, *S. cerevisiae* var. *boulardii* CNCM I-745 maintained stable numbers (~10^7 CFU per mL) for up to 60 days post bottling of ale-style beer fermented at 20 °C (Mohammadi & Saris, 2022). Notably, a biofilm was found on the inside of the bottle glass, leaving a haze-like appearance. It was hypothesized biofilm formation could be due to CNCM I-745 cell stress during brewing. In another report, CNCM I-745 was compared to *S. cerevisiae* US-05 in beer (Ramírez-Cota et al., 2021), and it was concluded the former can tolerate higher concentrations of ethanol, and higher temperatures (Fig. 3**)**. CNCM I-745 was more resistant to alcohol concentrations between 6 and 8 % v/v at 28 °C and up to 4 % v/v at 37 °C (Ramírez-Cota et al., 2021). CNCM I-745 also had a shorter lag phase, higher growth rate, and reached higher cell numbers compared to US-05 at all ethanol concentrations and temperatures tested. Furthermore, after incubation in 6 % ethanol, CNCM I-745 had a 40 % increase in cell wall thickness and exhibited vacuole enlargement (Ramírez-Cota et al., 2021). Vacuolar enlargement was found previously to increase the number and overall action of H^+-ATPases to prevent ethanol-induced cytosolic acidification (Charoenbhakdi et al., 2016). It Such H^+-ATPases are also associated with resistance to acid stress (Hazelwood et al., 2010). These findings may also relate to strain survival in the gut because comparative transcriptome wide analysis of *S. cerevisiae* CNCM I-745 and BY4741 strains in simulated intestinal conditions showed that 578 stress response genes were upregulated in strain CNCM I-745 compared to BY4741, potentially enabling CNCM I-745 to survive better in the human digestive tract (Pais et al., 2021). Lastly, a mutant of CNCM I-745 modified to produce the anti-listerial bacteriocin leucocin C (Ran Li et al., 2021) was successfully applied to make a beer with anti-listerial activity that was stable for at least 38 days (Ran Li et al., 2021).

Other putatively probiotic strains of *S. cerevisiae* var. *boulardii* was shown to have similar stress tolerance as CNCM I-745. Compared to *S. cerevisiae* strain SY, *S. cerevisiae* var. *boulardii* CNCM I-1079 survived better at the gastric pH of 2.0 in saline medium with varying concentrations of acetic acid, butyric acid and lactic acid (Fu et al., 2022). *S. cerevisiae* var. *boulardii* ATCC MYA-796 was found to use the same sugars as *S. cerevisiae* BY4742 for growth, except that the latter consumed galactose inefficiently (Liu et al., 2018).

A few recent studies demonstrated feasibility of using probiotic lactobacilli as starter cultures in beer. *Lacticaseibacillus paracasei* L26 was shown to inhibit bacterial pathogens such as *Streptococcus* in a double blind, randomized, human clinical trial (Mortazavi & Akhlaghi, 2012) and inhibit angiotensin-converting enzyme *in vitro* (Donkor et al., 2005). This strain was used in beer fermentations containing *S. cerevisiae* S-04 and reached cell numbers of up to 10^8 CFU per mL (Chan et al., 2019). However, growth was only tested in un-hopped wort and *iso*-alpha acids were adding after fermentation during refrigerated storage. In another report, probiotic strains of *L. paracasei* F19 and 431 shown to improve immune function (Sjödin et al., 2023; Rizzardini et al., 2012), were used to make sour beer (Herkenhoff et al., 2023). These strains were first individually cultured in sour beer for 24 h at 37 °C, followed by secondary fermentation with *S. cerevisiae* US-05 for 8 days at 15 °C. After 30 days of storage at 4 °C, *L. brevis* numbers ranged between 10^7 to 10^8 CFU per mL (Herkenhoff et al., 2023).

Besides adding individual probiotic strains to beer, a recent study reported combining the probiotics *L. paracasei* Lpc-37 and *S. cerevisiae* CNCM I-3856 to ferment un-hopped beer wort (Loh et al., 2021). *L. paracasei* strain Lpc-37 was previously shown to reduce stress-induced blood pressure and psychological markers of stress in human trials (Patterson et al., 2020). Similarly, *S. cerevisiae* CNCM I-3856 was shown to reduce constipation (Spiller et al., 2016). Upon combining the two strains for beer fermentation, bioactive tryptophan metabolites including phenyllactic acid, hydroxyphenyllactic acid and indole-lactic acid were produced (Loh et al., 2021). At the end of the fermentation (day 12), *L. paracasei* Lpc-37 and S. *cerevisiae* CNCM I-3856 were at cell numbers of ~10^9 and 10^8 CFU per mL, respectively.

Finally, putative probiotic strain *L. paracasei* DTA-81, found to exhibit anti-cancer and anti-microbial properties *in vitro* (Tarrah et al., 2019), was used to make a sour beer with *S. cerevisiae* S-04 and the beer tested for effects on behavior in a mouse model of depression (Silva et al., 2021). Mice consumption of the probiotic sour beer with DTA-81 had less anxious behaviors compared to those fed the control beer (Silva et al., 2021). Mice given the probiotic beer also exhibited less depressant behavior in a tail suspension test (Silva et al., 2021).

6. Challenges and opportunities for the delivery of live microbes and probiotics in beer

While beer is a beverage that was likely traditionally consumed with live microbes present, maintaining cell numbers while sustaining desirable product sensory attributes remains a significant challenge in commercial beer production today. Yet, there remains numerous opportunities to address this issue.

Firstly, overall microbial viability could be improved during the primary and secondary fermentation steps. At the end of primary fermentation, this may be possible by avoiding filtration which is usually done to remove yeast residue to improve beer clarity (Fig. 1). Additionally, viability losses due to hop-acid stress may be avoided by initially allowing the yeast and/or lactobacilli to ferment un-hopped beer, and then adding hop-acids post fermentation (Chen & Lu, 2018; van Bokhorst-van de Veen et al., 2011; Zhang et al., 2022).

Probiotic bacteria may be co-cultivated together with beer yeast. To avoid antagonistic interactions between the microbes, a semi-separated system may be used. For example, the microorganisms can be immobilized in hollow fiber membranes made from natural polymers like lignocellulose (Cui et al., 2021; Nguyen et al., 2019). The use of additives like sodium nitrate (Suastes-Rivas et al., 2020) or filter membranes (Cui et al., 2021; Nguyen et al., 2019) have been successfully used to co-culture other microorganisms in non-food sources, but haven't been tested to brew beer. Increased viability in a coculture system may also be possible using sequential inoculations whereby one organism (either the yeast or the probiotic strain) is pitched into the wort first, followed by the second organism pitched after a few days of fermentation. For example, when *S. cerevisiae* S-04 was first inoculated followed by *L. paracasei* DTA-81, the viability of DTA-81 was significantly higher (~10^4 CFU per mL), compared to when both organisms were pitched

together (~10^2 CFU per mL) (Silva et al., 2020). Notably, this study did not measure viability of these organisms post fermentation during cold storage.

Lambic beers wherein multiple yeasts and bacterial species co-exist in mixed communities add another opportunity for live microbe delivery. However, because these beers can be aged between six months and three years, they also have additional complexity due to extended aging times (Bongaerts et al., 2021; Mendes et al., 2013). Previous research has shown that lactobacilli that are part of mixed-species communities in Lambic beers, have higher stress tolerance levels than bacterial or yeast monocultures (Bongaerts et al., 2021; Fan et al., 2020). While synergistic interactions between bacterial and yeast species and strains have been demonstrated in other fermented foods (Adesulu-Dahunsi et al., 2020; Carbonetto et al., 2020), this relationship is yet to be assessed in Lambic style beers.

Microbial viability throughout beer shelf-life has not been explored in detail to date except one yeast-focused study (Wauters et al., 2023) and one bacterial-focused study (Ovalle-Marmolejo et al., 2023). If viability were to be sustained, the beer should be monitored for souring and quality defects (Djameh et al., 2019) and strains tested to confirm their metabolic and enzymatic activity are compatible with the desired sensory attributes of the final product. Alternatively, beer may be produced to eliminate viable yeast after the fermentation is complete and instead provide probiotics, as was shown for *B. lactis* BB-12 and *L. acidophilus* LA-5 in non-alcoholic beer (Sohrabvandi et al., 2010).

Of the probiotic strains tested to date, *S. cerevisiae* var. *boulardii* CNCM I-745 shows particular promise for utilization in beer. CNCM I-745 can tolerate high levels of ethanol (6–8 % v/v) and produces similar flavor compounds to *S. cerevisiae* strains (Ramírez-Cota et al., 2021). *S. cerevisiae* var. *boulardii* CNCM I-745 is able to assimilate maltose and can survive in intestinal conditions (Hossain et al., 2020; Liu et al., 2018). Future research could be focused on the utilization of malt as a prebiotic in conjunction with CNCM I-745, to make a potentially synbiotic beverage.

Probiotic lactobacilli such as *L. paracasei* strains L26, and Lpc-37 have also been explored to make probiotic beer, but their sensitivity to hop acids remains a limitation (Chan et al., 2019; Loh et al., 2021; Silva et al., 2021). One opportunity to overcome hop sensitivity is through mutagenesis or experimental evolution approaches. For example, overexpressing ATP-binding cassette transporter genes such as *rbsA*, *rbsB*, *msmK*, and *dppA* in *Lactococcus lactis* improved its survival in acidic (pH 4.0) conditions between 5-fold to 200-fold, depending on which transporters were over-expressed (Zhu et al., 2019). Co-cultures of *S. cerevisiae* var. *boulardii* CNCM I-745 and probiotic lactobacilli may also provide a new approach for probiotic beer. This was exemplified for green tea fermentations wherein CNCM I-745 and *L. plantarum* 299 V were used (Wang R et al., 2022). The strains survived at levels of ~10^7 CFU per mL when measured after 87 days of storage at 25 °C (Wang R et al., 2022). It was proposed that viability was maintained because CNCM I-745 consumed lactic acid produced by *L. plantarum* (lactic acid concentration dropped from ~ 5 g/L in monoculture vs. ~ 3 g/L in coculture), thereby avoiding a low pH.

Aside from established probiotic strains, there may be opportunities for other bacteria with potential health-promoting properties to be added to beer. *Gluconobacter oxydans* H32 isolated from kombucha was shown to persist in sour beer at high numbers (~10^6 CFU per mL) after 6 months of storage (Neffe-Skocińska et al., 2022). This strain was found to express anti-oxidant and oxidative stress inhibition properties *in vitro* (Choi et al., 2023).

Development of any probiotic beer will ultimately have to respond to the fact it is an alcoholic drink. Alcohol content one of the biggest areas of concern for the brewing industry because of the negative effects of alcohol on health (Anderson et al., 2021). Currently in the US and Europe, beer with alcohol content greater than 0.5 % v/v (Code of Federal Regulations, 2023), and greater than 1.2 % v/v (EC Regulations, 2007), respectively, cannot contain nutritional or health related claims. As underscored in a prior evaluation (Chan et al., 2021), strict adherence to local regulations is paramount not only for legal compliance but, more crucially, as an unwavering commitment to safeguarding consumer health. Consequently, the utilization of probiotic, non-alcoholic brews can help promote the brewing industry's role in beneficial live microbe delivery. These drinks are gaining interest among consumers and the brewing industry (Anderson et al., 2021). Low-alcohol and non-alcoholic beers also do not have the same regulatory constraints associated with nutritional labeling as alcoholic beers (Code of Federal Regulations, 2023).

7. Conclusions

There are many properties of beer that are beneficial for providing live dietary microbes. Because microorganisms have evolved for growth and survival in wort, there is significant opportunity to develop next generation beers that deliver physiologically relevant numbers of live microbes to the digestive tract. Among documented probiotic strains, the yeast *S. cerevisiae* var. *boulardii* CNCM I-745, alone and in co-culture with probiotic lactobacilli, currently offers unique opportunities for health benefits because of its capacity to produce beer with desired sensory and shelf-life qualities. Ultimately, with this approach and exploration of alcohol-free beers, it will be possible to expand options for health promotion using brewed beverages, with sensory profiles desired by a wide-variety of consumers.

8. Ethics statements

This article does not contain any experiments with human or animal participants.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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