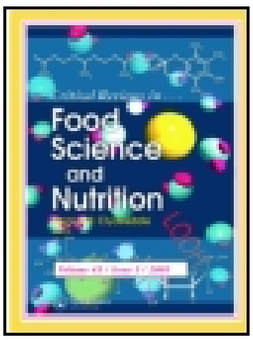

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Overview of craft brewing specificities and potentially associated microbiota

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Abstract

The brewing process differs slightly in craft breweries as compared to industrial breweries, as there are fewer control points. This arguably affects the microbiota of the final product. Beer contains several antimicrobial properties that protect it from pathogens, such as low pH, low oxygen and high carbon dioxide content, and the addition of hops. However, these hurdles have limited power controlling spoilage organisms. Contamination by these organisms can originate in the raw materials, persist in the environment, and be introduced by using flavoring ingredients later in the process. Spoilage is a prominent issue in brewing, and can cause quality degradation resulting in consumer rejection and product waste. For example, lactic acid bacteria are predominately associated with producing a ropy texture and haze, along with producing diacetyl which gives the beer butter flavor notes. Other microorganisms may not affect flavor or aroma, but can retard fermentation by consuming nutrients needed by fermentation yeast. Quality control in craft breweries today relies on culturing methods to detect specific spoilage organisms. Using media can be beneficial for detecting the most common beer spoilers, such as *Lactobacillus* and *Pediococci*. However, these methods are time consuming with long incubation

periods. Molecular methods such as community profiling or high throughput sequencing are better used for identifying entire populations of beer. These methods allow for detection, differentiation, and identification of taxa.

Keywords

Craft beer, Brewing process, Fermentation, Bacteria, Yeast, Molecular microbiology

1. Introduction

Beer is the third most popular drink worldwide after tea and coffee, and is the most preferred alcoholic beverage (Swot. 2016). In contrast with wine or other spirits, beer (including non-alcoholic types in countries that forbid alcohol consumption and sales) is produced and easily available commercially in most countries (Jernigan. 2000). China produced the largest volume of beer in 2014 at 44,933,300 kiloliters with the United States ranked at number two, producing 22,547,400 kiloliters (Anonymous 2015c). Beer consumption per capita ranges from less than 50 liters to more than 150 liters in Ireland and Czech Republic. The United States consumes around 75 liters per capita. (Alcázar et al. 2002). Overall, the brewing industry is a global business dominated by a few multinational companies and thousands of smaller producers, producing tens of billions of liters and generating several hundred billion dollars in global revenues (Anonymous 2015a,Jernigan. 2009).

Most beer consumed in the United States is produced by large, industrial breweries which rely on very stringent practices to limit spoilage risk and variation in the final product (Vrellas and Tsiotras. 2015). While the economic benefits of these industrial processes are numerous, many consumers have been drawn to craft beer due to their novel organoleptic properties. Increased demand for original beer products has resulted in a drastic increase in the home-brewing and microbrewery markets (Aquilani et al. 2015).

As a fermented beverage, beer inherently relies on microbial metabolism for production. Traditionally, the yeast *Saccharomyces cerevisiae* (or the related hybrid *S. pastorianus*) is almost always the primary fermentation microorganisms involved in ethanol and carbon dioxide production (Lodolo et al. 2008). It is also known that *S. cerevisiae* imparts sensory

characteristics through a variety of other metabolic pathways (Cocolin et al. 2011). Industrial beer production processes, especially pasteurization of the final product, are purported to reduce the presence of other microbes to less than detectable levels (Jeon et al. 2015). On the other hand, craft brewing processes are known to only limit the development of such microbes. Common beer spoilage microbes are relatively well described, but very little is known about the arguably more diverse and variable microbiota associated with craft beer.

The objective of this review is to present the current literature about the craft brewing industry from a microbiology perspective. The craft brewing specificities will be delineated in relation with the potential for uncontrolled microbes' establishment. Potential sources of contamination and strategies to reduce microbial load will be presented. Furthermore, the types of microorganisms and their detection methods will be discussed. This review will emphasize the limited knowledge on craft beer microbiology and the need for further research.

2. Evolution of beer production and craft brewing emergence

Modern beer is an alcoholic beverage (made from four main ingredients: malted grain, water, hops, and yeast) which has been perfected through time (Meussdoerffer. 2009). The origin of fermented beverages is unclear, and it is argued that they may have been consumed by nomadic Neolithic populations. Between the years 2000 and 4000 B.C., the Egyptians and Sumerians developed the process for brewing beverages that more closely resemble modern beer, though a variety of fermented beverages based from different food were independently developed by other civilizations (Correa-Ascencio et al. 2014,McGovern et al. 2004,Paul Ross et al. 2002). Although the discovery of yeast as the fermenter didn't occur until 1860, fermentation

was used as early as 700 BC in China to preserve foods and beverages (McGovern et al. 2004,Sicard and Legras. 2011).

Beer brewing remained largely artisanal until the industrial revolution, with a few European countries (Germany, Belgium, and England) taking the lead in mastering brewing processes and developing specific styles. With the discovery of America, German immigrants brought with them lager beer recipes (Meussdoerffer. 2009). Lager was the preferred beer style because of its light color and flavor (Olson et al. 2014). Many people also distrusted the quality of the water; therefore beer was the preferred beverage (Beuchat. 1978).

Nowadays there is a worldwide increase in popularity of beers with rich flavors and aromas that utilize new ingredients (Aquilani et al. 2015,Canonico et al. 2014). The market share of craft beers has been gaining on that of international and national breweries, with most attention on microbreweries and brew pubs (Murray and O'Neill. 2012). Craft beer does not have a specific definition or clear boundaries, but the Brewer's Association describes a craft brewery as small, independent, and traditional (Anonymous 2016). A craft brewery has an annual production of no more than 6 million barrels of beer. No more than 25% of the brewery can be owned by an alcohol industry member that is not a craft brewer. Finally, a craft brewery is traditional in that most of the beverage alcohol by volume comes from beer brewed with traditional or innovative ingredients and is fermented by yeast (Anonymous 2016). The addition of fruits, herbs, and spices can transform ordinary beer into specialty beer, along with other flavorings and fermentable substrates (Aquilani et al. 2015). Craft breweries are focused on the production of traditional ales, lagers, and even beer styles that do not fit in any of the two main

styles; and compete on the market on the criteria of high quality and diversity (Marongiu et al. 2015).

The craft brewing industry has become increasingly popular in the United States just in the last several years. Craft breweries in the United States are seeing large growth in production, sales, brewing capacity, and employment (Marongiu et al. 2015, Anonymous 2016). There was a 16.2% increase in the number of craft breweries nationally from 2015 to 2016, with a total of 5,234 in 2016. Craft breweries account for 98.7% of the total number of breweries in the United States, as of 2016 (Anonymous 2016).

3. **Craft brewing process specificities**

In this section, the emphasis will be on the differences between craft brewing process compared to industrial-scale processes which are well described and reviewed elsewhere (Beuchat. 1978,Priest and Campbell. 2003).

3.1 Raw ingredients and mashing

Beer has commonly been produced from barley and less often from wheat. However, novel consumer trends have led to the evaluation of different grain types for beer production. To develop gluten-free beers (Hager et al. 2014), sorghum (Agu and Palmer 1998, Owuama. 1997) and rice (Teramoto et al. 2002) are now used by several craft breweries. Other grains or seeds used by craft brewers include rye, millet, spelt, and buckwheat (De Meo et al. 2011, Phiarais et al. 2010). While rhizosphere microbiota (Lindow and Brandl 2003,Bulgarelli et al. 2015) and plant pathogens (Beattie and Lindow 1995,Goodwin et al. 2011) have been studied extensively, there is only limited indirect knowledge on the commensal microbiota associated with cereals and grain crops (Sultan et al. 2016, Duniere et al. 2017, Granzow et al. 2017). It is suspected that

grain associated microbes may end up to a certain extent in final beer products, but this has not been demonstrated.

The grains used contain large amounts of starches and sugars which will later serve as nutrients for brewing yeast and sometimes bacteria (Mascia et al. 2014). Starches are converted into fermentable sugars and polysaccharides through germination enzymes released through grain germination, the main step of malting. Malting consists of steeping (increasing humidity), germination, and kilning (heat treatment to dry malted grains). While industrial beer relies on standard malting processes, a staggering diversity of malts is now produced and made available to home and craft brewers (Anonymous 2015b). The most important variation in malts' processing are the intensity of kilning, which is sometimes described as roasting when very high heat is used (Hämäläinen and Reinikainen. 2007). Steeping and kilning can greatly influence grain-associated microbiota dynamics and composition.

It is also known that barley varieties will strongly influence fermentation and final product properties (Hager et al. 2014, Kihara et al. 1998) and possibly indirectly microbiota. Malts are milled and/or crushed by the malting company, or often on site by craft brewers, and diluted in hot water to become the mash. Milling and crushing may influence grain-associated microbiota, though they should be relatively resilient to coarse mechanical treatments (Manthey et al. 2004).

3.2 Sparging and boiling

After the sugars are made available, the sweet liquid, also known as wort, is separated from the spent grains. During this process, wort is pumped through to the boil kettle as the spent grains are sparged, or sprayed with hot water, to extract any other dissolved substances (Beuchat.

1978), a process that should not be much different between craft and industrial breweries. The wort is then boiled at a temperature between 103 and 110°C for approximately one hour (Ormrod. 1986), but it is commonly accepted that boiling is more intense and controlled for industrial breweries while craft brewers will use boil kettles of varying qualities and properties.. Hops are added during the boil, at different times depending on the desired use of the hops. Boiling isomerizes the hops, causes proteins to coagulate for easy removal, concentrates the liquid, causes Maillard reactions to enhance the color and flavor of the wort, and drives off sulfur componds which could lead to a cooked corn or cabbage aroma in beer if not removed (Beuchat. 1978, Vriesekoop et al. 2012). Even if craft brewers tend to use a wider diversity of hops, this should not have a direct impact on wort microbiota, but would definitely drive the microbiota dynamics after boiling. Boiling should drastically reduce the microbial load in wort to undetectable levels. After boiling, the wort is cooled and microorganisms can increase in abundance due to its high sugar content and lower temperature (Kim et al. 2015).

3.3 Fermentation and final stages

The cooled wort is transferred to a fermentation tank in which yeast is added and left to ferment for several days up to one week. In contrast with industrial breweries, yeast is often re-pitched in a craft brewery, meaning the yeast from one batch of beer is used to ferment a future batch. Re-pitching yeast is generally limited to less than ten times to avoid yeast quality degradation (Jenkins et al. 2003). Yeast in better condition will produce less fusel alcohols and more sulfite than old or contaminated yeast (Guido et al. 2004). The practice of re-pitching yeast can cause deterioration by cross contamination with other cultures or wild microorganisms, causing genetic changes to the original culture or causing physiological changes due to stress

(Lodolo et al. 2008). While genetic drift and eventual speciation of novel strains/species could be expected, it has been reported that *Saccharomyces* strains used for brewing are genetically stable (Powell and Diacetis. 2007). Pitching rate of yeast also affects final quality of beer. Higher pitching rates allow for an increased rate of fermentation, but it creates large quantities of yeast biomass. Excessive pitching rates can degrade the health of the yeast culture (Kucharczyk and Tuszyński. 2015).

Most industrial and some craft beers are filtered for clarity before packaging, depending on the brewer's preferences and style. Filtering can be done using cellulose fibers or particles of diatomite as a medium (Gan et al. 2001, Niemsch and Heinrich. 2000). Isinglass can also be used as a fining agent to clarify beer (Walker et al. 2007). Simple filtering removes flocculant yeast but has no effect on reducing bacterial load (Sensidoni et al. 2011). However, more elaborate alternative methods, such as high hydrostatic pressure, have shown potential to reduce microbial load in beer as efficiently as pasteurization (Buzrul et al. 2005). Industrial breweries may use pasteurization to sterilize beer, and fill the beer into sterilized containers (Dilay et al. 2006). In a craft brewery there is usually not a pasteurization process, though. Unpasteurized beer has a more appealing and fresh taste to modern consumers (Asano et al. 2007), but this makes craft beer more prone to bacterial spoilage. For example craft brewers have reported loss of canned beers due to gas production of unidentified microbes (personal communication).

4. Beer parameters and impact on microbial load

4.1 Beer styles defined

Beer is classified in numerous styles based on their properties including alcohol content, color, bitterness, clarity, flavor, and ingredients. Alcohol content is measured in alcohol by

volume (ABV). ABV is calculated using the original and final gravity of the beer. Beer ABV typically ranges from 3 to 14% when normal fermentation is used, but the most commonly consumed styles, especially among mass-produced beers, don't exceed 6%. Alcohol content has traditionally been considered an inherent antimicrobial; however it has become known that several microbes are able to tolerate low to medium alcohol content (Ingram. 1990). Alcohol tolerance in *Saccharomyces* is a trait that has been considered beneficial and sought after, especially in winemaking (Fujita et al. 2006).

Bitterness is measured in International Bitterness Units, or IBUs. IBU is calculated using the percentage of alpha acids, the utilization of iso-α-acids based on the strength of the wort (original gravity), the boil time, and the volume of the recipe (Anonymous 2012). A higher alpha acid hop will result in a more bitter beer and a longer boil will also increase IBUs. Hops also provide antimicrobial properties, to be described in detail in section 4.2. Beer IBUs typically range between 5 and 120, however the vast majority of beer produced at industrial scale in the US have very low IBU (5-15), and the popular use of higher quantities of more bitter hops in craft beers leads to much higher IBU levels (note that some European countries', especially Belgium, tend to produce higher IBU industrial beers).

The color of a beer can be measured by the Standard Reference Method, or the SRM scale. The colors correspond to a number ranging from 1 to 40. The rating is based on the absorbance of turbidity free beer in a ½ inch cell at a wavelength of 430 nm (Anonymous 1958). A light beer such as a lager will have an SRM of 2 to 4. A dark imperial stout beer has an SRM of 40 (Strong and England. 2015). Historically, clear beers with low SRM have been favored and

still dominate the industrial beer market, while darker and cloudier beers are commonly offerent by craft breweries.

Gruit (a mixture of herbs and spices) previously was the distinguishing factor of ales from other fermented beverages, whereas today beers are categorized as ales and lagers by the yeast used for fermentation. Ales are brewed with top-fermenting yeast, typically *Saccharomyces cerevisiae* strains, with fermentation conducted at 20°C (Beuchat. 1978). Common styles in craft breweries include: American Pale Ale, Wheat beers, India Pale Ale (often abbreviated as IPA), American Brown Ale and Belgian Golden Ale (Strong and England. 2015), and ales represent a minor market for industrial breweries.*Saccharomyces pastorianus* (or *Saccharomyces carlsbergensis*) is generally accepted as the fermentation yeast used for lagers and fermentation is carried out at 13°C. Lager yeasts congregate at the bottom of fermentation tanks and result in a lighter, cleaner flavor than ales (Beuchat. 1978), making them the most common industrially produced beers, while craft brewers also show interest on developing their own lagers. Lager and ale yeasts have specific fermentation temperature ranges and an increase in temperature could deteriorate the yeast, reduce foam stability, decrease pH, and reduce bittering compounds (Solgajová et al. 2013).

Ales and lagers are by far the most common beer styles, however, there are several other different variations of the beverage. For example, lambic beers are those that use spontaneous fermentation, rather than inoculation with a yeast strain. These beers are fermented and aged anywhere from one to three years in oak barrels and are native to Belgium. The unique flavors of this style are fruity and sometimes sour (De Keersmaecker. 1996). During the first couple months, *Enterobacteriaceae* are the most prominent bacteria, but disappear later in fermentation.

The first yeast to appear, *Kloeckera*, occurs within the first couple of weeks after wort boiling. This yeast is quickly taken over by *Saccharomyces*, which perform the main fermentation over the next several months (Van Oevelen et al. 1977). Finally, *Brettanomyces* takes over as the last main yeast to impart characteristic flavors and aromas (Van Oevelen et al. 1976). Although lambic beers have a diverse microbiota at the beginning stages of fermentation, the diversity and quantity of microorganisms stabilizes by 18 months (Spitaels et al. 2014).

In the United States, a similar lambic-style beer is being brewed called the American coolship ale. This style is an attempted replica of a lambic beer utilizing spontaneous fermentation and using the same production practices as the brewers in Belgium of lambic beers. The successions found in American coolship ales closely mimic those of the lambic beers, with *Enterobacteriaceae* being the starting bacteria and *Lactobacillaceae* taking over. *Saccharomyces* is the starting yeast with the disappearance of it coinciding with the growth of *Brettanomyces* (Bokulich et al. 2012a).

Barley and wheat are the most common grains used in brewing, but several other fermented beverages are made using different starch sources. Although these beverages are described elsewhere (Blandino et al. 2003), there are notable characteristics of the microbiota of some. For example, ‘cauim’ is a fermented beverage produced in South America made from cassava root. This beverage starts as a porridge and ferments for a couple of days. Typical microbiota of the ‘caium’ beverage is predominately lactic acid bacteria and species belonging to *Enterobacter*, *Serratia*, *Pseudomonas*, and *Streptococcus* genera. Yeast begins playing a role in this product’s fermentation after the first day (Almeida et al. 2007). Chicha beer is another traditional South American beer produced from corn, cassava, or palm. The bacterial community

of chicha beer consists mainly of *Lactobacillus fermentum*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Streptococcus salivarius*, with other bacteria species being less abundant (Freire et al. 2016). A similar microbiota has been shown for a rice-based Brazilian beer, with *Bacillus*, *Enteroccocus*, *Leuconostoc*, and *Lactobacillus* being in highest abundance (Puerari et al. 2015).

4.2 Antimicrobial properties

There are several factors that contribute to the preservation of beer which have been studied extensively. These characteristics include intrinsic factors such as pH and ethanol concentration, and the use of hops.

The two most important intrinsic antibacterial properties of beer are pH and ethanol. Most pathogenic microorganisms prefer a more neutral environment and beer ranges in pH between 3.8 and 4.7 (Jespersen and Jakobsen. 1996). Lower pH values allow for acidification of cells, destroys enzyme systems, and reduces nutrient uptake (Vriesekoop et al. 2012). Alcohol is usually found in a concentration of 0--8% alcohol by volume (ABV) (Jespersen and Jakobsen. 1996). Most microorganisms do not tolerate high ethanol concentrations because it can inhibit cell growth and metabolism (Fujita et al. 2006).

Carbon dioxide that is produced by the yeast and added by the brewers can be an antimicrobial hurdle. Carbon dioxide is typically found in a concentration of 0.5% weight by volume (Jespersen and Jakobsen. 1996). Carbon dioxide helps to provide an anaerobic environment, decreases pH, and has a direct inhibitory effect on cell growth (Vriesekoop et al. 2012). A reduction in CO_2 concentration in beer can ultimately reduce shelf life (Brocklehurst and Lund. 1990).

Fermentation yeasts are often competitive with other microorganisms, thus eliminating the contaminants from the final product. There are only trace amounts of substances for yeast nutrition, so the yeast will consume the sugars before any other bacteria or yeast can (Sakamoto and Konings. 2003,Vriesekoop et al. 2012).

Hops were originally used in beer because of their bitterness. However, it was eventually discovered that hops also play a crucial role in controlling spoilage. Hops contain alpha acids which isomerize into iso-α-acids during boiling, in concentrations of 17--55 mg, which impart bitterness and antimicrobial properties (Jespersen and Jakobsen. 1996). Hops dissipate the transmembrane pH gradient to prevent spoilage organism growth in beer, acting as protonophores (Simpson. 1993). However, hops have a bactericidal effect on Gram positive bacteria only (Shimwell. 1937). Some lactic acid bacteria have developed resistance to hops and can grow in beer (Richards and Macrae. 1964,Sakamoto and Konings. 2003).

4.3 Heat treatment and sanitation

The overall brewing process affects the microbiological status of beer. Because mashing is a temperature intense process, most microorganisms present in the raw materials are unlikely to be transferred in large numbers to the final product (Couto et al. 2005,Kim et al. 2015). However, aerobic bacteria, lactic acid bacteria, coliforms, *Pseudomonas*, and yeast can still be present in low numbers after the mashing process (O'Sullivan et al. 1999). The boiling process also uses high heat, so pathogens that could be present before boil are not likely to remain post-boil. In one study where *Salmonella* Typhimurium, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus* were inoculated in wort before boiling showed that all pathogens were reduced to undetectable levels by culture dependent methods (Kim et al. 2015).

Many craft breweries often utilize additional ingredients such as fruit juices and flavoring ingredients in innovative beers. These extra ingredients are often heat treated before being added. Many of the fermentation tanks in small breweries require multiple batches to fill. Because of this, the yeast is pitched with the first batch of wort, to protect the beer from bacterial growth (Priest and Campbell. 2003).

The microbiological safety of beer also depends on proper cleaning and sanitation practices in the brewery. Cleaning uses a detergent and removes soil from the substrate, whereas disinfection refers to the destruction of microorganisms to reduce the microbial load to a level that is not harmful to health or quality. Equipment in the brewery is made of stainless steel and the equipment is closed off to the environment (Priest and Campbell. 2003). The equipment is also designed for easy cleaning. For example, the fermentation tanks have a cone shape at the bottom, which is mainly used for harvesting yeast after fermentation, but is also helpful in removing sanitizer and cleaning agents straight out of the bottom (de Oliva Neto et al. 2004). Breweries utilize cleaning-in-place (CIP), cleaning loops, and tank recirculation systems (Bremer et al. 2006,Chen et al. 2012). Cleaning is usually done immediately after use, while sanitization occurs immediately before use to be the most effective. Disinfectants that are used should be compatible with plant materials, tolerant of hard water, non-foaming, nonirritating, economical, and have a low environmental impact. Hot caustic soda is the most common cleaning agent, used in a cycle of pre- rinse, cleaning with caustic, and a post- rinse (Manzano et al. 2011). Little research has been done on the effectiveness of current brewery cleaning practices on reducing/eliminating microbial contamination.

Even with these control measures to prevent spoilage in beer, some bacteria and yeast proliferate in the beverage imparting off-flavors and aromas. This can be desirable or undesirable depending on the style. As mentioned previously, lambic beers thrive on the diverse microbiota and depend on it to provide unique flavors and aromas (Van Oevelen et al. 1977). However, in typical ales and lagers, any microorganisms present in the final product are considered contaminants.

5. Sources of beer microbiota

It is generally accepted that beer is safe of pathogens; however, it is not uncommon for beer to be colonized with undesirable microorganisms. Sources of contamination can be from the raw materials, the process, and from the brewery environment.

5.1 Raw materials

The raw materials used in craft brewing include water, hops, malted grain, and yeast. Due to an increased market for special beers (Yeo and Liu. 2014), some additional ingredients can be used to add unique flavors and aromas to the beer such as fruit additives, spices, and flavoring ingredients. The microbiota of the ingredients is likely to influence the microbiota of the final product.

Barley is the most commonly used grain for brewing beer. In fact, 10% of the world barley crop is used for the production of beer (Kaur et al. 2015). The barley grain is covered in a husk that is normally inhabited by *Eubacteria*, *Actinomycetes*, filamentous fungi, and yeasts (Priest and Campbell. 2003). The grain is malted, milled, and mashed for the starch to convert to sugar to be used for fermentation. Lactic acid bacteria are naturally present on barley, so they can be found throughout the brewing process (Giusto et al. 2006). Cereal grains and fruits used in beer production can be contaminated in the field, during storage, or malting by mycotoxin-producing-

fungi (Kaaya and Kyamuhangire. 2006). Spores can be found in the air when the conditions for temperature, moisture, and oxygen are favorable. The spores then grow and produce mycotoxins. Mycotoxins are generally thermostable and can remain in crops when all signs of the fungi itself have been removed (Inoue et al. 2013).

Craft breweries use pre-malted grain as the starting point for brewing. This grain can be stored for months before use, which can affect the microbiota of the beer. At high water activities (0.8-0.9), visible mold can develop on the malted grain after just one month of storage. At slightly lower water activities (0.693), visible mold will appear after three months of storage. Malted grain can last up to 12 months of storage at low water activities below 0.529 (Hoff et al. 2014).

Many toxins have been shown to be metabolized into less toxic compounds or decrease in concentration due to adsorption of the spent grain during brewing. Zearalenone and patulin are two of the mycotoxins that are metabolized during the beer fermentation process, thus posing little risk to contamination in the final product. Aflatoxins B1 and B2, along with Fusarium1 and Orchatoxin A decreased in residual concentrations to less than 20% during the mashing process when inoculated artificially into the raw materials. This led to the disappearance of the toxins throughout the rest of the brewing process, showing that they are only of small health risk in beer (Inoue et al. 2013). Although these particular mycotoxins were not a threat to the final product in this study, other toxins can be of concern. Furthermore, similar decreases of Aflatoxin B1 and Fumonisin B1 concentrations were reported in another study, demonstrating that mycotoxins are not an issue in beer products, as long as raw grain materials comply with limits set up by national or international regulations (Pietri et al. 2010).

Grain is not the only source of unwanted microorganisms in the raw ingredients. Fruits are sometimes used in brewing because they can be a source of natural yeasts that ancient brewers utilized for fermentation (McGovern et al. 2004, McGovern. 2009). However, in the controlled craft brewing atmosphere today, these natural yeasts can be unwanted contaminants. Yeasts such as *Geotrichum candidus*, *Hanseniaspora guilliermondii*, *Hanseniaspora uvarum*, *Metschnikowia pulcherrima*, *Pichia kluyveri*, *Pichia kudriavzevii*, and *Saccharomyces cerevisiae* are commonly isolated from fruits (Vadkertiova et al. 2012).

Spices can also contain microorganisms that could persist in the brewing environment. Spices can be contaminated from the environment by unsanitary conditions or when hygienic handling is not carried out. High microbial levels in spices are not acceptable to use in ready-to-eat foods, and they can be a source of intoxication when added to foods in which pathogen growth is favorable (Sospedra et al. 2010).

Although the brewing process includes many control steps to eliminate microorganism growth (mashing, boiling, and fermentation), some flavor additives are often added at the final step of craft brewing in the bright tank. Fruit juices, honey, and other flavoring ingredients can be added to craft beer to provide a unique flavor profile, but also their own foodborne microbes (Janisiewicz et al. 2014, Abdelfattah et al. 2016). Some of these may be heat treated before adding, however, the increased amount of sugar could increase the overall susceptibility of the beer to spoilage. After the beer is finished aging in the bright tank, it usually is immediately packaged, thus if there are any microorganisms present in the ingredients added here, it will persist in the finished product.

5.2 Brewery environment

The last major source of contamination in a brewery is the brewery environment. Air, pipework, and equipment can all be potential sources, and colonization by brewery-resident microorganisms is a crucial process in the brewing of lambics and coolship ales (Bokulich et al. 2012a, Spitaels et al. 2014). Remarkably, a study relying on microbial source tracking revealed that cereals and other ingredients microbiota not only seeded the wort and beer microbiota, but also the microbiota of brewery surfaces, while negligible contribution of microbes of environmental and human origins was detected (Bokulich et al. 2015). This observation suggests that appropriate sanitation procedures should limit drastically the risks of transfer of microbial spoilers from the brewery environment.

Seasonality also plays a role in determining microorganism presence in the brewery as well. *Saccharomyces cerevisiae* is primarily found during fermentation and packaging areas in the fall, however, in the spring and summer the yeast is found throughout the entire brewery. *Candida santamariae* was found clustered around the mash and boil steps in fall, but in the cellar during the spring and summer months (Bokulich et al. 2015).

Contaminants within the brewery will play a role on the microbiota of the product during the process. Thermotolerant bacteria and yeast that are present during mashing and boiling could attach and survive on these vessels in a biofilm (Fielding et al. 2007) and thus contaminate other batches. During the mash process, airborne contaminants can drift from the mill to fermentation vessels, bright tanks, and packaging equipment. Microorganisms can also blow in from outside depending on the set up of the brewery (Priest and Campbell. 2003). Contaminated wort could

further infect the pipes that carry the wort throughout the brewery. Leaking or contaminated heat exchangers could cause an unsanitary work environment in the brewery (Bokulich et al. 2015).

6. Types of microbes associated with beer

The craft brewing industry faces spoilage contamination problems similar to those of early brewers in the nineteenth century (Priest and Campbell. 2003). Many different microorganisms can be introduced during the brewing process and cause spoilage. Spoilage in a brewery is defined as any organism not introduced intentionally (Bokulich et al. 2012b). Some microorganisms present may not influence the flavor or taste of the final product, but they can retard the progress of fermentation (Takahashi et al. 2015).

The types of microorganisms found depend on the beer style and process. The microbial community of fermenting beer is often diverse and bacteria could survive in it. In a study designed to trace microbial diversity in a pilot scale brewing process using next generation sequencing and quantitative polymerase chain reaction detected that the bacterial population decreased during boiling, increased at early fermentation, slightly increased at late stage fermentation, and slightly increased again by filtration (Takahashi et al. 2015). These spoilage organisms can be divided into bacteria and fungi, and bacteria further dived by phylum.

6.1 Firmicutes

Firmicutes are a phylum of Gram positive bacteria. Gram positive bacteria are classified by their thick single layer of peptidoglycan, which stains purple by performing a Gram stain. The two classes of Firmicutes commonly reported in beer are Bacilli and Negativicutes.

Lactic acid bacteria belong to the Bacilli class and can cause spoilage characterized by silky, turbid aspect and/or a buttery flavor caused by diacetyl production. *Lactobacillus brevis* is

the most common beer spoiler. It is generally hop tolerant and grows at 30°C and between pH 4 and 5 (Sakamoto and Konings. 2003). *Lactobacillus lindneri* has been isolated from lagers and grows at 19°C (Priest and Campbell. 2003). Other spoilage strains include *L. maloefermentans, L. paracbuchnerie* (Farrow et al. 1988) *L. collinoides*, and *L. paracasei* subsp. *paracasei* (Hollerova and Kubizniakova. 2001). *Streptococcus lactis* can produce slime and gas in final products (Banwart. 1979). Spoilage is also characterized by an acidic off-flavor (Storåards et al. 1998).

Pediococci are another genus among Bacilli. In beer, *P. damnousus* and *P. inopinatus* are spoilers that produce diacetyl (Dobson et al. 2002). Other *Pediococci* that have been found in breweries include *P. acidilactici*, *P.dextrinicus*, and *P. halophilus* (Collins et al. 1990). These bacteria can adapt to the brewery environment. *Pediococcus damnousus* is also very resistant to the iso-α-acids in hops. Acid formation and the buttery aroma of diacetyl formation are associated with contaminant strains of *Pediococcuss* in beer. Ropiness is also an unfavorable characteristic caused by *Pediococcus* (Priest and Campbell. 2003). Although *Pediococcus* is responsible for beer spoilage, the incidence of this has decreased recently due to improved sanitation conditions (Sakamoto and Konings. 2003).

The third most common Gram positive bacteria causing spoilage in beer is *Leuconostoc*. This is a heterofermentative cocci or oval, short rod. They are found in pairs or short chains. The natural reservoir for *Leuconostoc* is vegetables and fruits, but they can occur rarely in breweries (Priest and Campbell. 2003). In beer, they are also diacetyl producers (Speckman and Collins. 1968).

Pectinatus and *Megasphaera* are two beer spoilers among the Negativicutes. *Pectinatus frisingensis* has been isolated from pitching yeast. *Pectinatus* can grow in beer with ethanol concentrations lower than 5% ABV and in pH above 4.3 (Jespersen and Jakobsen. 1996, Lee et al. 1980). *Megasphaera* causes cloudiness and unpleasant odors. Both genera also form butyric acid, but are sensitive to alcohol production and low pH. Because modern brewery practices include reduction of oxygen to as low as possible, these aerobic bacteria are not as prominently found in beer today (Jespersen and Jakobsen. 1996).

6.2 Proteobacteria

A major phylum of Gram negative bacteria is the Proteobacteria. Gram negative bacteria, rather than a thick layer of peptidoglycan, have a multilayered envelope that contains a thin layer of peptidoglycan and a hydrophobic outer membrane (Priest and Campbell. 2003). Acetic acid bacteria are a large group of Gram negative bacteria that are rod shaped and can convert ethanol into acetic acid. They can grow in and spoil beer, but only under aerobic conditions (Sakamoto and Konings. 2003). Acetic acid bacteria are used in the food industry to make vinegar, soft drinks, and alcoholic beverages (Camu et al. 2007, Wu et al. 2012). In general, acetic acid bacteria spoil beer by producing acid, off-flavors, turbidity, and ropiness. They are resistant to hops, low pH, and ethanol. This group of bacteria is further divided into *Acetobacter* and *Gluconobacter*. *Acetobacter* can oxidize ethanol into acetate, CO_2, and water. *Gluconobacter* is similar to *Acetobacter* but reduces ethanol to acetic acid (Priest and Campbell. 2003) and is responsible for ropy texture in beer (Banwart. 1979).

Another class of Gram negative spoilers is Enterobacteriaceae, which are facultative anaerobic rods. They are indirect beer spoilers because they are not normally found in the

finished product but can cause negative characteristics if present throughout the process. Characteristics of spoilage by Enterobacteriaceae include fermentation retardation or acceleration and off-flavor and aroma production (Prest et al. 1994).

Enterobacteriaceae include *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsielia*, *Obesumbacterium*, *Proteus*, *Rahnella*, and *Serratia* that have all been isolated from breweries. *Obesumbacterium proteus* is a bacterium that has only been isolated from brewery environments and is often found in pitching yeast (Koivula et al. 2006). This Enterobacteriaceae can result in a beer with a high final specific gravity and pH and can give fruity odors or flavors (Keevil et al. 1979). *Rahnella aquatilis* can grow well in hopped or unhopped wort. It also survives the brewing process when the wort has normal gravity (Hamze et al. 1991). *Rahnella aquatilis* can increase levels of acetaldehyde and methyl acetate and can give a fruity, milky, or sulfur taste and aroma (Priest and Campbell. 2003). *Hafnia protea* is found strictly in breweries (Priest et al. 1974).

Other characteristics of anaerobic Gram negative spoilage include acetic acid and propionic acid production (Priest and Campbell. 2003). Anaerobic bacteria incidence has increased due to the practice of non-pasteurized beer and improved technology to reduce oxygen in the brewery (Jespersen and Jakobsen. 1996).

6.3 Other bacterial phyla

Although Firmicutes and Proteobacteria are the most common of the brewery phyla, some others have been detected. *Micrococcus*, belonging to the Actinobacteria phylum, has been reported in breweries (Sakamoto and Konings. 2003). Using next generation sequencing to detect microorganisms in beer, other phyla besides Firmicutes and Proteobacteria have been identified

in beer. Acidobacteria, Actinobacteria, Bacteroidetes, Chlamydiae, and Planctomycetes can all be present in low numbers throughout the brewing process (Takahashi et al. 2015).

6.4 Fungi

The third category of spoilage microorganisms found in beer is fungi, which include yeasts and molds. Before the boiling process, yeasts are of little concern to the brewery because yeast are not thermotolerant and cannot survive even the shortest of boiling procedures. There are four separate groups that wild yeasts can fall into: fermentative contaminants, killer yeasts, wrong type of culture yeast, or non-fermentative yeasts (Priest and Campbell. 2003).

Contamination yeasts sometimes grow slightly faster than the pitching yeast and will take-over the pitching yeast culture through successive fermentations. In a study that aimed at detecting wild yeast in lager breweries, wild yeasts were detected in 41 out of 101 brewery yeast samples (van der Aa Kühle, A. and Jespersen. 1998). Killer yeast attack sensitive yeast cultures and become the dominant yeast in fermentation. It is unlikely that these yeasts will be detected in a brewery until the killer yeast has completely taken over the pitching yeast. Contamination yeast cultures can affect the rate of fermentation, final attenuation, and the production of flavor by-products.

Typically, contamination yeasts are divided into *Saccharomyces* and non-*Saccharomyces* wild yeasts. Non-*Saccharomyces* wild yeasts include a variety of species. *Brettanomyces* produces acetic acid (Coton et al. 2006, Gray et al. 2011) and has a high level of resistance to carbonation (Ison and Gutteridge. 1987). *Pichia* and *Williposis* can produce esters in beer. The most common characteristics of a spoiled beer by yeast is off-flavor, turbidity due to the nonflocculent properties of wild yeast, production of surface film, and granular deposits (Priest

and Campbell. 2003). *Candida* is another contamination yeast that can produce fruity off-flavors and turbidity (Banwart. 1979). The fermentation yeast can also be considered a contaminant after the beer is filtered (Manzano et al. 2011).

Mold and toxins can sometimes be found on raw materials and negatively impact the barley. Usually, fungi cause deterioration of grain which results in discoloration, decreased germination, formation of mycotoxins, and mustiness (Banwart. 1979). Mycotoxins are produced by Fusarium and are fairly heat stable. They are common contaminants of corn, wheat, sorghum, and fruits (Shale et al. 2012). More information on the types of mold and toxins found on the raw material can be found in the sources of contamination section.

7. Microbiota detection techniques

In the quality control department of breweries, if they have one, the main tasks are to confirm sterility, determine that the microbiological count does not exceed the limit to cause spoilage, and examine for presence of specific organisms (Priest and Campbell. 2003). Analysis in the brewery is predominately retrospective, meaning that the brewers typically expect a quality product and the objective is to confirm this.

7.1 Culture dependent methods

Culturing is a method of using specific media to grow and enumerate bacteria. It is the preferred method used by craft breweries to detect spoilage microorganisms; however it does not provide specificity and sensitivity (Jespersen and Jakobsen. 1996, Manzano et al. 2011). Species-specific media have been developed to detect beer microorganisms (Manzano et al. 2011), but there is not one single media that can be used to detect all beer spoilage specific microorganisms (Jespersen and Jakobsen. 1996). MRS (de Man, Rogosa and Sharpe) agar can be used to detect

Lactobacillus and *Pediococcus* bacteria and is often supplemented with cycloheximide to prevent yeast and mold growth. The detection of *Pectinatus* and *Megasphaera* can be accomplished with a beer enrichment step and using one or more types of media such as Universal Beer Agar (UBA), Nachweismedium für bierschädliche Bakteriën agar (NBB), and Raka-Ray media (Sakamoto and Konings. 2003). UBA has been used to isolate *Enterobacter agglomerans* from lager beer (van Vuuren et al. 1978). Some media, along with detecting the desired microorganisms, can also detect non-spoilage species. Although there are compounds that can be added for selectivity, this could require longer incubation times (Sakamoto and Konings. 2003).

It is understood that less than 1% of microbiota in high diversity environments can be cultured using these traditional methods (Amann et al. 1995, Torsvik et al. 1990). Cultivating the microorganisms can also be a long and tedious process (Manzano et al. 2011). It can take a week or more for bacteria to form visible colonies on agar plates or to develop turbidity in liquid broths (Sakamoto and Konings. 2003). Detection is also difficult because microorganisms present in beer are found in low numbers (Jespersen and Jakobsen. 1996). Product that is found to have been contaminated with a spoilage organism has likely already been released for sale, which can lead to recalls and economic damages to a brewery (Sakamoto and Konings. 2003). Therefore, there is a need for the development of faster methods to detect microorganisms in beer. Other reasons for a need of improved methods of detection include an increased awareness of the consumer in the area of product quality, tightened government regulations, increased competition among brewers (in particular, craft breweries of the same region), growing market volumes for non-pasteurized beer in cans and bottles, more low or non-alcoholic beers,

increasing numbers of flavored sweetened type beverages, and technological advancements (Priest and Campbell. 2003).

7.2 Culture Independent Methods

Several molecular methods have been developed to detect and characterize spoilage organisms in beer, as reviewed elsewhere (Esmaeili et al. 2015, Schifferdecker et al. 2014) .Molecular methods involve analysis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins, or lipids. Nucleic acids are informational macromolecules that have defined sequences which serve as blueprints for the cells (Priest and Campbell. 2003). Polymerase chain reaction (PCR) methods have been developed for the use of fast spoilage microbe detection and can be used to detect *Megasphaera* and *Pectinatus* in beer (Satokari et al. 1998). Real time PCR can be used for early detection and quantification of contaminant yeast species, such as *Dekkera*, during fermentation and testing in final beer and beverage products (Gray et al. 2011). Anaerobic beer spoilage *Clostridia* bacteria have been targeted and detected in beer using real time PCR methods (Juvonen et al. 2008). Random Amplification of Polymorphic DNA polymerase chain reaction (RAPD PCR) has been used to develop primers and genetic markers to distinguish between beer spoilage and non-spoilage strains of *Lactobacillus* (Fujii et al. 2005). Hop-resistant *Lactobacillus* strains can be detected by PCR or real time PCR of the plasmid-bound horA, horC and hitA genes (Bergsveinson et al., 2012, Bergsveinson et al., 2015). Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) in combination with Biotyper software was shown to allow rapid identification of bacterial and yeast spoilage organisms (Turvey et al. 2016, Wieme et al. 2014) Temperature Gradient Gradient Electrophoresis (TGGE) and Denaturing Gradient Gel Electrophoresis (DGGE) are different

fingerprinting methods that can be performed after PCR (Manzano et al. 2011). DGGE and TGGE separate sequences of DNA according to different melting conditions along gradients on a polyacrylamide gel (Fischer and Lerman. 1983, Muyzer and Smalla. 1998). TGGE and DGGE have been used to compare microbiota of beer before and after a cleaning process, and also to distinguish different strains of *Saccharomyces* (Manzano et al. 2011). Terminal restriction fragment length polymorphism (TRFLP) is also a fingerprinting method that is used for rapid profiling of complex microbial populations. This method has been used to compare barley microbiota from different geographical regions (Kaur et al. 2015). Microarrays can be used as a fast, sensitive, and specific method to identify different bacterial species in a sample. For example, one study used this method to detect viable spoilage bacteria in beer (Weber et al. 2008).

Whole Genome Sequencing (WGS) gives the most complete understanding of the genetic information of a single microorganism, such as the beer fermentation yeast *Saccharomyces cerevisiae*, and can provide the most in-depth comparisons between related species (Chen et al. 2016). High throughput sequencing (HTS) has been proven to profile highly complex and diverse communities from a wide variety of sources, such as those of fermentation products (Reuter et al. 2015). Sequencing techniques have the accuracy of a using digital system. HTS uses publicly available databases which are continually enhanced (Priest and Campbell. 2003). Sequencing methods have been used to determine potential hop resistance genes in order to develop new methods of detecting beer spoilage *Lactobacilli* (Sami et al. 1997).

In a study that evaluated the microbial diversity in a brewing process by culture dependent and independent methods, culture dependent methods detected 88 genera from the most diverse

sample of beer. Almost all bacteria that were recovered belonged to Proteobacteria or Firmicutes. However, more than 190 different genera belonging to several phyla were detected using culture independent methods. The most predominate genera belonged to the Firmicutes and Proteobacteria phyla (Takahashi et al. 2015). The specificity, sensitivity, and time reduction of molecular methods is preferred over the cost efficient and ease of culturing techniques.

8. Conclusions

Beer is a microbiological product, but diversity and abundance of microbes is typically considered a defect. In contrast with industrial brewing, craft brewing is characterized by less stringent processes to limit microbial load. A few limited studies have confirmed that craft or micro brewed beer harbor relatively diverse and abundant fungal and bacterial microbiota. These observations challenge the common belief that the combination of antimicrobial properties such as alcohol content, acidity and the use of hops should significantly limit microbial load, especially in the final product.

There is a need for further research studies to better understand the normal and detrimental impacts of microbes in the craft brewing industry. The microbiota of malted grain and hops that are ready for use by breweries is virtually unknown. Although some research has been done on the flora of raw barley, brewers do not know the microbiological status of pre-malted grain and the potential differences in microbiota imparted by different varieties, grain types, or malting processes. Hops are known to be inhibitive of Gram positive bacteria, but it is theoretically possible that they are harboring their own distinct microbiota. There is also little information on the potential for seeding of brewery-resident microbes to brewing products at different production stages. Finally, the impact of the different brewing steps on microbiota dynamics is

largely unknown with the exception of the intuitive microbial load reduction incurred by wort boiling.

For economic reasons, small-scale brewers are limited to culture-dependent tests to confirm the safety of beer for consumption and potentially track back the origin of recurrent spoilage. However, such methods are not sufficient to study the full microbiota profiles and dynamics along the brewing process, which may play a role in the organoleptic characteristics and shelf life of the beer. There is a clear need for more culture-independent studies, especially using HTS to explore the role of this microbiota. The few studies conducted on specific beer styles have demonstrated that very diverse bacterial and fungal communities are present along the brewing process. However, the sources of microbes and the parameters driving microbial dynamics in typical craft brewing are unknown.

References

Brewers Association | Promoting Independent Craft Brewers. (2016) **2017**.

Beer: Global Industry Guide. (2015a).

Crafting in-demand beverages: hops and malts demands impacted by craft beer, spirits. (2015b) *Beverage Industry.* **106**: 64.

Kirin Beer University Report Global Beer Production by Country in 2014. (2015c).

ASBC Methods of Analysis, online. Wort 23. Wort Bitterness. (2012).

ASBC Methods of Analysis, online. Beer 10. Color Spectrophotometric Color Method. (1958).

Abdelfattah, A., Wisniewski, M., Droby, S., andSchena, L. (2016). Spatial and compositional variation in the fungal communities of organic and conventionally grown apple fruit at the consumer point-of-purchase. *Horticulture Research.* **3**: 16047.

Agu, R. C. and Palmer, G. H. (1998). A reassessment of sorghum for lager-beer brewing. *Bioresour Technol.* **66**: 253--261.

Alcázar, A., Pablos, F., Martín, M., andGonzález, A. G. (2002). Multivariate characterisation of beers according to their mineral content. *Talanta.* **57**: 45--52.

Almeida, E. G., Rachid, C. C. T. C., andSchwan, R. F. (2007). Microbial population present in fermented beverage ‘cauim’ produced by Brazilian Amerindians. *Int J Food Microbiol.* **120**: 146--151.

Amann, R. I., Ludwig, W., andSchleifer, K. (1995). Phylogenetic identification and in-situ detection of individual microbial cells without cultivation. *Microbiol Rev.* **59**: 143--169.

Aquilani, B., Laureti, T., Poponi, S., andSecondi, L. (2015). Beer choice and consumption determinants when craft beers are tasted: An exploratory study of consumer preferences. *Food Quality and Preference.* **41**: 214--224.

Asano, S., Suzuki, K., Iijima, K., Motoyama, Y., Kuriyama, H., andKitagawa, Y. (2007). Effects of morphological changes in beer-spoilage lactic acid bacteria on membrane filtration in breweries. *Journal of Bioscience and Bioengineering.* **104**: 334--338.

Banwart, G. J. (1979). Basic food microbiology. AVI Pub., Westport, CT.

Beattie, G. A. and Lindow, S. E. (1995). The secret life of foliar bacterial pathogens on leaves. *Annu Rev Phytopathol.* **33**: 145--172.

Bergsveinson, J., Pittet, V., Ziola, B. (2012). RT-qPCR analysis of putative beer-spoilage gene expression during growth of Lactobacillus brevis BSO 464 and Pediococcus claussenii ATCC BAA-344(T) in beer. *Appl.Microbiol.Biotechnol.* **96, 2**: 461--470

Bergsveinson, J., Baecker, N., Pittet, V., Ziola, B. (2015). Role of plasmids in Lactobacillus brevis BSO 464 hop tolerance and beer spoilage. *Appl.Environ.Microbiol.* **81, 4**: 1234--1241

Beuchat, L. R. (1978). Food and beverage mycology. AVI Pub., Westport. CT.

Blandino, A., Al-Aseeri, M. E., Pandiella, S. S., Cantero, D., andWebb, C. (2003). Cereal-based fermented foods and beverages. *Food Research International.* **36**: 527--543.

Bokulich, N. A., Bamforth, C. W., andMills, D. A. (2012a). Brewhouse-resident microbiota are responsible for multi-stage fermentation of American coolship ale. *PloS one.* **7**: e35507.

Bokulich, N. A., Bamforth, C. W., andMills, D. A. (2012b). A Review of Molecular Methods for Microbial Community Profiling of Beer and Wine. *Journal of the American Society of Brewing Chemists.* **70**: 150--162.

Bokulich, N. A., Bergsveinson, J., Ziola, B., andMills, D. A. (2015). Mapping microbial ecosystems and spoilage-gene flow in breweries highlights patterns of contamination and resistance. *eLife.* **4**.

Bremer, P. J., Fillery, S., andMcQuillan, A. J. (2006). Laboratory scale Clean-In-Place (CIP) studies on the effectiveness of different caustic and acid wash steps on the removal of dairy biofilms. *Int J Food Microbiol.* **106**: 254--262.

Brocklehurst, T. F. and Lund, B. M. (1990). The influence of pH, temperature and organic acids on the initiation of growth of Yersinia enterocolitica. *J Appl Bacteriol.* **69**: 390.

Bulgarelli, D., Garrido-Oter, R., Münch, P. C., Weiman, A., Dröge, J., Pan, Y., McHardy, A. C., andSchulze-Lefert, P. (2015). Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell host & microbe.* **17**: 392--403.

Buzrul, S., Alpas, H., andBozoglu, F. (2005). Effects of high hydrostatic pressure on shelf life of lager beer. *European Food Research and Technology.* **220**: 615--618.

Camu, N., Winter, T. D., Verbrugghe, K., Cleenwerck, I., Vandamme, P., Takrama, J. S., Vancanneyt, M., andVuyst, L. D. (2007). Dynamics and Biodiversity of Populations of Lactic Acid Bacteria and Acetic Acid Bacteria Involved in Spontaneous Heap Fermentation of Cocoa Beans in Ghana. *Appl Environ Microbiol.* **73**: 1809--1824.

Canonico, L., Comitini, F., andCiani, M. (2014). Dominance and influence of selected Saccharomyces cerevisiae strains on the analytical profile of craft beer refermentation. *J Inst Brewing.* **120**: 262--267.

Chen, L., Chen, R., Yin, H., Sui, J., andLin, H. (2012). Cleaning in place with onsite-generated electrolysed oxidizing water for water-saving disinfection in breweries: Cleaning in place with onsite-generated electrolysed oxidizing water. *J Inst Brewing.* **118**: 401--405.

Chen, P., Dong, J., Yin, H., Bao, X., Chen, L., He, Y., Chen, R., Wan, X., Zhao, Y., andHou, X. (2016). Genome comparison and evolutionary analysis of different industrial lager yeasts (Saccharomyces pastorianus). *J Inst Brewing.* **122**: 42--47.

Cocolin, L., Campolongo, S., Gorra, R., Rolle, L., andRantsiou, K. (2011). Saccharomyces cerevisiae Biodiversity During the Brewing Process of an Artisanal Beer: A Preliminary Study. *J Inst Brewing.* **117**: 352--358.

Collins, M. D., Williams, A. M., andWallbanks, S. (1990). The phylogeny of Aerococcus and Pediococcus as determined by 16S rRNA sequence analysis: description of Tetragenococcus gen. nov. *FEMS Microbiol Lett.* **58**: 255.

Correa-Ascencio, M., Robertson, I. G., Cabrera-Cortés, O., Cabrera-Castro, R., andEvershed, R. P. (2014). Pulque production from fermented agave sap as a dietary supplement in Prehispanic Mesoamerica. *Proc Natl Acad Sci U S A.* **111**: 14223--14228.

Coton, M., Coton, E., Levert, D., Casaregola, S., andSohier, D. (2006). Yeast ecology in French cider and black olive natural fermentations. *Int J Food Microbiol.* **108**: 130--135.

Couto, J. A., Neves, F., Campos, F., andHogg, T. (2005). Thermal inactivation of the wine spoilage yeasts Dekkera/Brettanomyces. *Int J Food Microbiol.* **104**: 337--344.

De Keersmaecker, J. (1996). The mystery of lambic beer. *Scientific American.* **275**: 56.

De Meo, B., Freeman, G., Marconi, O., Booer, C., Perretti, G., andFantozzi, P. (2011). Behaviour of Malted Cereals and Pseudo-Cereals for Gluten-Free Beer Production. *J Inst Brewing.* **117**: 541--546.

de Oliva Neto, P., Amorim Ferreira, M., andYokoya, F. (2004). Screening for yeast with antibacterial properties from an ethanol distillery. *Bioresour Technol.* **92**: 1--6.

Dilay, E., Vargas, J. V. C., Amico, S. C., andOrdonez, J. C. (2006). Modeling, simulation and optimization of a beer pasteurization tunnel. *J Food Eng.* **77**: 500--513.

Dobson, C. M., Deneer, H., Lee, S., Hemmingsen, S., Glaze, S., andZiola, B. (2002). Phylogenetic analysis of the genus Pediococcus, including Pediococcus claussenii sp. nov., a novel lactic acid bacterium isolated from beer. *Int J Syst Evol Microbiol.* **52**: 2003--2010.

Duniere, L., Xu, S., Long, J., Elekwachi, C., Wang, Y., Turkington, K., Forster, R., andMcAllister, T. A. (2017). Bacterial and fungal core microbiomes associated with small grain silages during ensiling and aerobic spoilage. *BMC Microbiology.* **17**.

Esmaeili, S., Mogharrabi, M., Safi,F., Sohrabvandi,S., Mortazavian,A. M., Bagheripoor-Fallah, N. (2015). The common spoilage microorganisms of beer: occurrence, defects, and determination-a review. Carpathian Journal of Food Science & Technology **7, 4**: 68--73,

Farrow, J., Phillips, B., andCollins, M. (1988). Nucleic acid studies on some heterofermentative lactobacilli: Description of Lactobacillus malefermentans sp.nov. and Lactobacillus parabuchneri sp. nov. *FEMS Microbiol Lett.* **55**: 163--167.

Fay, J. C. and Benavides, J. A. (2005). Evidence for Domesticated and Wild Populations of Saccharomyces cerevisiae: e5. *PLoS Genetics.* **1**.

Fielding, L. M., Hall, A., andPeters, A. C. (2007). An evaluation of ozonated water as an alternative to chemical cleaning and sanitisation of beer lines. *Journal of Foodservice.* **18**: 59.

Fischer, S. G. and Lerman, L. S. (1983). DNA fragments differing by single base-pair substitutions are separated in denaturing gradient gels: correspondence with melting theory. *Proc Natl Acad Sci U S A.* **80**: 1579--1583.

Freire, A. L., Zapata, S., Mosquera, J., Mejia, M. L., andTrueba, G. (2016). Bacteria associated with human saliva are major microbial components of Ecuadorian indigenous beers (chicha). *PeerJ.* **4**: e1962.

Fujii, T., Nakashima, K., andHayashi, N. (2005). Random amplified polymorphic DNA-PCR based cloning of markers to identify the beer-spoilage strains of Lactobacillus brevis, Pediococcus damnosus, Lactobacillus collinoides and Lactobacillus coryniformis. *J Appl Microbiol.* **98**: 1209--1220.

Fujita, K., Matsuyama, A., Kobayashi, Y., andIwahashi, H. (2006). The genome- wide screening of yeast deletion mutants to identify the genes required for tolerance to ethanol and other alcohols. *FEMS Yeast Research.* **6**: 744--750.

Gan, Q., Howell, J. A., Field, R. W., England, R., Bird, M. R., O'Shaughnessy, C. L., andMeKechinie, M. T. (2001). Beer clarification by microfiltration — product quality control and fractionation of particles and macromolecules. *J Membr Sci.* **194**: 185--196.

Gil, G., del Mónaco, S., Cerrutti, P., andGalvagno, M. (2004). Selective antimicrobial activity of chitosan on beer spoilage bacteria and brewing yeasts. *Biotechnol Lett.* **26**: 569--574.

Giovenzana, V., Beghi, R., andGuidetti, R. (2014). Rapid evaluation of craft beer quality during fermentation process by vis/NIR spectroscopy. *J Food Eng.* **142**: 80--86.

Giusto, C., Iacumin, L., Comi, G., Buiatti, S., andManzano, M. (2006). PCR-TTGE and RAPD-PCR techniques to analyze Saccharomyces cerevisiae and Saccharomyces carlsbergensis isolated from craft beers. *J Inst Brewing.* **112**: 340--345.

Goodwin, S. B., Ben M'Barek, S., Dhillon, B., Wittenberg, A. H. J., Crane, C. F., Hane, J. K., Foster, A. J., van der Lee, T. A. J., Grimwood, J., Aerts, A., Antoniw, J., Bailey, A., Bluhm, B., Bowler, J. M., Bristow, J., van der Burgt, A., Canto-Canché, B., Churchill, A. C. L., Conde-Ferràez, L., Cools, H. J., Coutinho, P. M., Csukai, M., Dehal, P., de Wit, P., Donzelli, B., Geest, H. G., van Ham, R. C. H., Hammond-Kosack, K. E., Henrissat, B., Kilian, A., Kobayashi, A. K., Koopmann, E., Kourmpetis, Y., Kuzniar, A., Lindquist, E., Lombard, V., Maliepaard, C., Martins, N., Mehrabi, R., Nap, J. P. H., Ponomarenko, A., Rudd, J. J., Salamov, A., Schmutz, J., Schouten, H. J., Shapiro, H., Stergiopoulos, I., Torriani, S. F. F., Tu, H., de Vries, R. P., Waalwijk, C., Ware, S. B., Wiebenga, A., Zwiers, L. H., Oliver, R. P., Grigoriev, I. V., andKema, G. H. J. (2011). Finished Genome of the Fungal Wheat Pathogen Mycosphaerella graminicola Reveals Dispensome Structure, Chromosome Plasticity, and Stealth Pathogenesis. *PLoS Genetics.* **7**: 1002070-1002070.

Granzow, S., Kaiser, K., Wemheuer, B., Pfeiffer, B., Daniel, R., Vidal, S., andWemheuer, F. (2017). The Effects of Cropping Regimes on Fungal and Bacterial Communities of Wheat and Faba Bean in a Greenhouse Pot Experiment Differ between Plant Species and Compartment. *Frontiers in Microbiology.* **8**.

Gray, S. R., Rawsthorne, H., Dirks, B., andPhister, T. G. (2011). Detection and enumeration of Dekkera anomala in beer, cola, and cider using real-time PCR: D. anomala real-time detection. *Lett Appl Microbiol.* **52**: 352--359.

Grønbæk, M., Deis, A., Thorkild I. A. Sørensen, Becker, U., Schnohr, P., andJensen, G. (1995). Mortality Associated With Moderate Intakes Of Wine, Beer, Or Spirits. *BMJ: British Medical Journal.* **310**: 1165--1169.

Guido, L. F., Rodrigues, P. G., Rodrigues, J. A., Gonçalves, C. R., andBarros, A. A. (2004). The impact of the physiological condition of the pitching yeast on beer flavour stability: an industrial approach. *Food Chem.* **87**: 187--193.

Hager, A., Taylor, J., Waters, D., andArendt, E. (2014). Gluten free beer – A review. *Trends Food Sci Technol.* **36**: 44--54.

Hämäläinen, J. J. and Reinikainen, P. (2007). A Simulation Model for Malt Enzyme Activities in Kilning. *J Inst Brewing.* **113**: 159--167.

Hamze, M., Mergaert, J., van Vuuren, H. J., Gavini, F., Beji, A., Izard, D., andKersters, K. (1991). Rahnella aquatilis, a potential contaminant in lager beer breweries. *Int J Food Microbiol.* **13**: 63.

Hoff, S., Lund, M. N., Petersen, M. A., Jespersen, B. M., andAndersen, M. L. (2014). Quality of pilsner malt and roasted malt during storage. *J Inst Brewing.* **120**: 331--340.

Hollerova, I. and Kubizniakova, P. (2001). Monitoring Gram positive bacterial contamination in Czech breweries. *J Inst Brewing.* **107**: 355--358.

Ingram, L. O. (1990). Ethanol tolerance in bacteria. *Crit Rev Biotechnol.* **9**: 305.

Inoue, T., Nagatomi, Y., Uyama, A., andMochizuki, N. (2013). Fate of Mycotoxins during Beer Brewing and Fermentation. *Biosci Biotechnol Biochem.* **77**: 1410--1415.

Ison, R. W. and Gutteridge, C. S. (1987). Determination of the carbonation tolerance of yeasts. *Lett Appl Microbiol.* **5**: 11--13.

Janisiewicz, W. J., Jurick, W. M., Peter, K. A., Kurtzman, C. P., andBuyer, J. S. (2014). Yeasts associated with plums and their potential for controlling brown rot after harvest: Plum yeasts. *Yeast.* **31**: 207--218.

Jenkins, C. L., Kennedy, A. I., Hodgson, J. A., Thurston, P., andSmart, K. A. (2003). Impact of Serial Repitching on Lager Brewing Yeast Quality. *Journal of the American Society of Brewing Chemists.* **61**.

Jeon, S. H., Kim, N. H., Shim, M. B., Jeon, Y. W., Ahn, J. H., Lee, S. H., Hwang, I. G., andRhee, M. S. (2015). Microbiological Diversity and Prevalence of Spoilage and Pathogenic Bacteria in Commercial Fermented Alcoholic Beverages (Beer, Fruit Wine, Refined Rice Wine, and Yakju). *J Food Prot.* **78**: 812--818.

Jernigan, D. H. (2009). The global alcohol industry: an overview. *Addiction.* **104**: 6--12.

Jernigan, D. H. (2000). Applying commodity chain analysis to changing modes of alcohol supply in a developing country. *Addiction.* **95**: S465-S475.

Jespersen, L. and Jakobsen, M. (1996). Specific spoilage organisms in breweries and laboratory media for their detection. *Int J Food Microbiol.* **33**: 139--155.

Juvonen, R., Koivula, T., andHaikara, A. (2008). Group-specific PCR-RFLP and real-time PCR methods for detection and tentative discrimination of strictly anaerobic beer-spoilage bacteria of the class Clostridia. *Int J Food Microbiol.* **125**: 162--169.

Kaaya, A. N. and Kyamuhangire, W. (2006). The effect of storage time and agroecological zone on mould incidence and aflatoxin contamination of maize from traders in Uganda. *Int J Food Microbiol.* **110**: 217--223.

Kaur, M., Bowman, J. P., Stewart, D. C., andEvans, D. E. (2015). The fungal community structure of barley malts from diverse geographical regions correlates with malt quality parameters. *Int J Food Microbiol.* **215**: 71--78.

Keevil, W. J., Hough, J. S., andCole, J. A. (1979). The influence of a coliform bacterium on fermentation by yeast. *J Inst Brewing.* **85**: 99--102.

Kihara, M., Kaneko, T., andIto, K. (1998). Genetic variation of beta-amylase thermostability among varieties of barley, Hordeum vulgare L., and relation to malting quality. *PLANT BREEDING.* **117**: 425--428.

Kim, S. A., Jeon, S. H., Kim, N. H., Kim, H. W., Lee, N. Y., Cho, T. J., Jung, Y. M., Lee, S. H., Hwang, I. G., andRhee, M. S. (2015). Changes in the Microbial Composition of Microbrewed Beer during the Process in the Actual Manufacturing Line. *J Food Prot.* **78**: 2233--2239.

Knights, D., Kuczynski, J., Charlson, E. S., Zaneveld, J., Mozer, M. C., Collman, R. G., Bushman, F. D., Knight, R., andKelley, S. T. (2011). Bayesian community-wide culture-independent microbial source tracking. *Nature methods.* **8**: 761--763.

Koivula, T. T., Juvonen, R., Haikara, A., andSuihko, M. - . (2006). Characterization of the brewery spoilage bacterium Obesumbacterium proteus by automated ribotyping and development of PCR methods for its biotype 1. *J Appl Microbiol.* **100**: 398--406.

Kucharczyk, K. and Tuszyński, T. (2015). The effect of pitching rate on fermentation, maturation and flavour compounds of beer produced on an industrial scale. *J Inst Brewing.* **121**: 349--355.

Lee, S. Y., Madee, M. S., Jangaard, N. O., andHoriuchi, E. K. (1980). Pectinatus, a new genus of bacteria capable of growth in hopped beer. *J Inst Brewing.* **86**: 28--30.

Lindow, S. E. and Brandl, M. T. (2003). Microbiology of the phyllosphere. *Appl Environ Microbiol.* **69**: 1875--1883.

Lodolo, E. J., Kock, J. L. F., Axcell, B. C., andBrooks, M. (2008). The yeast Saccharomyces cerevisiae– the main character in beer brewing. *FEMS Yeast Research.* **8**: 1018--1036.

Manthey, F. A., Wolf-Hall, C. E., Yalla, S., Vijayakumar, C., andCarlson, D. (2004). Microbial Loads, Mycotoxins, and Quality of Durum Wheat from the 2001 Harvest of the Northern Plains Region of the United States. *J Food Prot.* **67**: 772--780.

Manzano, M., Iacumin, L., Vendrame, M., Cecchini, F., Comi, G., andBuiatti, S. (2011). Craft Beer Microflora Identification Before and After a Cleaning Process. *J Inst Brewing.* **117**: 343--351.

Marongiu, A., Zara, G., Legras, J., Del Caro, A., Mascia, I., Fadda, C., andBudroni, M. (2015). Novel starters for old processes: use of Saccharomyces cerevisiae strains isolated from artisanal sourdough for craft beer production at a brewery scale. *J Ind Microbiol Biotechnol.* **42**: 85--92.

Mascia, I., Fadda, C., Dostálek, P., Olšovská, J., andDel Caro, A. (2014). Preliminary characterization of an Italian craft durum wheat beer. *J Inst Brewing.* **120**: 495--499.

McGovern, P. E. (2009). Uncorking the past: the quest for wine, beer, and other alcoholic beverages. **In:**, pp. 60--104, University of California Press, Berkeley.

McGovern, P. E., Zhang, J., Tang, J., Zhang, Z., Hall, G. R., Moreau, R. A., Nuñez, A., Butrym, E. D., Richards, M. P., Wang, C., Cheng, G., Zhao, Z., Wang, C., andBar-Yosef, O. (2004). Fermented Beverages of Pre- and Proto-Historic China. *Proc Natl Acad Sci U S A.* **101**: 17593--17598.

Meussdoerffer, F. G. (2009). Chapter 1. A Comprehensive History of Beer Brewing. In:Handbook of Brewing: Processes, Technology, Markets. Eßlinger, H. M., Ed., Wiley-VCH Verlag GmbH & Co. KGaA.

Murray, D. W. and O'Neill, M. A. (2012). Craft beer: penetrating a niche market. *Br Food J.* **114**: 899--909.

Muyzer, G. and Smalla, K. (1998). Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie Van Leeuwenhoek.* **73**: 127--141.

Niemsch, K. and Heinrich, T. (2000). Raible-Test for Evaluation of Filtration Properties. *J Inst Brewing.* **106**: 277--286.

Olson, E. D., Murphy, K. S., andRo, H. (2014). An Exploratory Study of Home Brewers' Motivational Factors. *Journal of Foodservice Business Research.* **17**: 228--241.

Ormrod, I. (1986). Modern brewhouse design and its impact on wort production. *J Inst Brewing.* **92**: 131--136.

O'Sullivan, T., Walsh, Y., O'Mahony, A., Fitzgerald, G., andSinderen, D. (1999). A comparative study of malthouse and brewhouse microflora. *J Inst Brewing.* **105**: 55--61.

Owuama, C. I. (1997). Sorghum: a cereal with lager beer brewing potential. *World Journal of Microbiology and Biotechnology.* **13**: 253--260.

Paul Ross, R., Morgan, S., andHill, C. (2002). Preservation and fermentation: past, present and future. *Int J Food Microbiol.* **79**: 3--16.

Phiarais, B., Mauch, A., Schehl, B., Zarnkow, M., Gastl, M., Herrmann, M., Zannini, E., andArendt, E. (2010). Processing of a Top Fermented Beer Brewed from 100% Buckwheat Malt with Sensory and Analytical Characterisation. *J Inst Brewing.* **116**: 265--274.

Pietri, A., Bertuzzi, T., Agosti, B., andDonadini, G. (2010). Transfer of aflatoxin B1 and fumonisin B1 from naturally contaminated raw materials to beer during an industrial brewing process. *Food Additives and Contaminants Part A-Chemistry Analysis Control and Risk Assessment.* **27**: 1431--1439.

Powell, C. D. and Diacetis, A. N. (2007). Long Term Serial Repitching and the Genetic and Phenotypic Stability of Brewer's Yeast. *J Inst Brewing.* **113**: 67--74.

Prest, A. G., John R. M. Hammond, andGordon S. A. B. Stewart. (1994). Biochemical and Molecular Characterization of Obesumbacterium proteus, a Common Contaminant of Brewing Yeasts. *Appl Environ Microbiol.* **60**: 1635--1640.

Priest, F., Cowbourne, M., andHough, J. (1974). Wort Enterobacteria—a review. *J Inst Brewing.* **80**: 342--356.

Priest, F. G. and Campbell, I. (2003). Brewing microbiology. Kluwer Academic/Plenum Publishers, New York.

Puerari, C., Magalhães-Guedes, K. T., andSchwan, R. F. (2015). Physicochemical and microbiological characterization of chicha, a rice-based fermented beverage produced by Umutina Brazilian Amerindians. *Food Microbiol.* **46**: 210--217.

Reuter, J., Spacek, D., andSnyder, M. (2015). High-Throughput Sequencing Technologies. *Mol Cell.* **58**: 586--597.

Richards, M. and Macrae, R. (1964). The significance of the use of hops in regard to the biological stability of beer. II. The development of resistance to hop resins by strains of lactobacilli. *J Inst Brewing.* **70**: 484--488.

Ruitenberg, A., van Swieten, J. C., Witteman, J. C., Mehta, K. M., van Duijn, C. M., Hofman, A., andBreteler, M. M. (2002). Alcohol consumption and risk of dementia: the Rotterdam Study. *The Lancet.* **359**: 281--286.

Sakamoto, K. and Konings, W. N. (2003). Beer spoilage bacteria and hop resistance. *International Journal of Food Microbiology*. **89**: 105--124.

Sami, M., Yamashita, H., Kadokura, H., Kitamoto, K., Yoda, K., andYamasaki, M. (1997). A new and rapid method for determination of beer-spoilage ability of Lactobacilli. *Journal of the ASBC.* **55**: 137--140.

Satokari, R., Juvonen, R., Mallison, K., von Wright, A., andHaikara, A. (1998). Detection of beer spoilage bacteria Megasphaera and Pectinatus by polymerase chain reaction and colorimetric microplate hybridization. *Int J Food Microbiol.* **45**: 119--127.

Sensidoni, M., Marconi, O., Perretti, G., Freeman, G., andFantozzi, P. (2011). Monitoring of Beer Filtration Using Photon Correlation Spectroscopy (PCS). *J Inst Brewing.* **117**: 639--646.

Shale, K., Mukamugema, J., Lues, R. J., andVenter, P. (2012). Toxicity profile of commercially produced indigenous banana beer. *Food Additives & Contaminants: Part A.* **29**: 1300--1306.

Schifferdecker, A.J., Dashko, S., Ishchuk, O.P., Piskur, J. (2014). The wine and beer yeast *Dekkera bruxellensis*. *Yeast* **31, 9**: 323--332

Shimwell, J. L. (1937). On the Relation Between the Staining Properties of Bacteria and their Reaction Towards Hop Antiseptic. *J Inst Brewing.* **43**: 111--118.

Sicard, D. and Legras, J. (2011). Bread, beer and wine: yeast domestication in the Saccharomyces sensu stricto complex. *Comptes rendus biologies.* **334**: 229--236.

Simpson, W. J. (1993). Studies on the sensitivity of lactic acid bacteria to hop bitter acids. *J Inst Brewing.* **99**: 405--411.

Solgajová, M., Francáková, H., Dráb, S., andTóth, Z. (2013). Effect of temperature on the process of beer primary fermentation. *The Journal of Microbiology, Biotechnology and Food Sciences.* **2**: 1791--1799.

Sospedra, I., Soriano, J. M., andManes, J. (2010). Assessment of the Microbiological Safety of Dried Spices and Herbs Commercialized in Spain. *Plant Foods for Human Nutrition.* **65**: 364--368.

Speckman, R. A. and Collins, E. B. (1968). Diacetyl Biosynthesis in Streptococcus diacetilactis and Leuconostoc citrovorum. *J Bacteriol.* **95**: 174--180.

Spitaels, F., Wieme, A. D., Janssens, M., Aerts, M., Daniel, H., Van Landschoot, A., De Vuyst, L., andVandamme, P. (2014). The microbial diversity of traditional spontaneously fermented lambic beer. *PloS one.* **9**: e95384.

Storåards, E., Suiiiko, M. -., Pot, B., Vanhonacker, K., Janssins, D., Broomfield, P. L. E., andBanks, J. G. (1998). Detection and identification of Lactobacillus lindneri from brewery environments. *J Inst Brewing.* **104**: 46--54.

Strong, G. and England, K. (2015). Beer Judge Certification Program 2015 Style Guidelines.

Sultan, A., Andersen, B., Svensson, B., andFinnie, C. (2016). Exploring the Plant-Microbe Interface by Profiling the Surface-Associated Proteins of Barley Grains. *Journal of proteome research.* **15**: 1151.

Swot, S. (2016). Top 10 Widely Consumed Drinks in the World.

Takahashi, M., Kita, Y., Kusaka, K., Mizuno, A., andGoto- Yamamoto, N. (2015). Evaluation of microbial diversity in the pilot- scale beer brewing process by culture- dependent and culture- independent method. *J Appl Microbiol.* **119**: 904-904.

Teramoto, Y., Yoshida, S., andUeda, S. (2002). Characteristics of a rice beer (zutho) and a yeast isolated from the fermented product in Nagaland, India. *World Journal of Microbiology and Biotechnology.* **18**: 813--816.

Torsvik, V., Goksøyr, J., andDaae, F. L. (1990). High diversity in DNA of soil bacteria. *Appl Environ Microbiol.* **56**: 782--787.

Turvey, M.E., Weiland, F., Meneses, J., Sterenberg, N., Hoffmann, P. (2016). Identification of beer spoilage microorganisms using the MALDI Biotyper platform. **100, 6**: 2761--2773

Vadkertiova, R., Molnarova, J., Vranova, D., andSlavikova, E. (2012). Yeasts and yeast-like organisms associated with fruits and blossoms of different fruit trees. *Can J Microbiol.* **58**: 1344--1352.

van der Aa Kühle, A. and Jespersen, L. (1998). Detection and identification of wild yeasts in lager breweries. *Int J Food Microbiol.* **43**: 205--213.

Van Oevelen, D., L'Escaille, F. d., andVerachtert, H. (1976). Synthesis of aroma components during the spontaneous fermentation of lambic and gueuze. *J Inst Brewing.* **82**: 322--326.

Van Oevelen, D., Spaepen, M., Timmermans, P., andVerachtert, H. (1977). Microbiological aspects of spontaneous wort fermentation in the production of lambic and gueuze. *J Inst Brewing.* **83**: 356--360.

van Vuuren, H. J. J., Kersters, K., Ley, J. D., Toerien, D. F., andMeisel, R. (1978). ENTEROBACTER AGGLOMERANS—A NEW BACTERIAL CONTAMINANT ISOLATED FROM LAGER BEER BREWERIES. *J Inst Brewing.* **84**: 315--317.

Vrellas, C. G. and Tsiotras, G. (2015). Quality management in the global brewing industry. *International Journal of Quality & Reliability Management.* **32**: 42--52.

Vriesekoop, F., Krahl, M., Hucker, B., andMenz, G. (2012). 125th Anniversary Review: Bacteria in brewing: The good, the bad and the ugly. *J Inst Brewing.* **118**: 335--345.

Walker, S. L., Camarena, M. C. D., andFreeman, G. (2007). Alternatives to Isinglass for Beer Clarification. *J Inst Brewing.* **113**: 347--354.

Weber, D. G., Sahm, K., Polen, T., Wendisch, V. F., andAntranikian, G. (2008). Oligonucleotide microarrays for the detection and identification of viable beer spoilage bacteria. *J Appl Microbiol.* **105**: 951--962.

Wieme, A.D., Spitaels, F., Aerts, M., De Bruyne, K., Van Landschoot, A., Vandamme,P. (2014). Identification of beer-spoilage bacteria using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Int. J. Food Microbiol.*, **185**: 41--50,

Wu, J. J., Ma, Y. K., Zhang, F. F., andChen, F. S. (2012). Biodiversity of yeasts, lactic acid bacteria and acetic acid bacteria in the fermentation of “Shanxi aged vinegar”, a traditional Chinese vinegar. *Food Microbiol.* **30**: 289--297.

Yeo, H. Q. and Liu, S. (2014). An overview of selected specialty beers: developments, challenges and prospects. *International Journal of Food Science and Technology.* **49**: 1607--1618.