

# FIGHTING QUALITY THREATS

## Notable microbiological contaminations of craft beer in the United States

*The U.S. craft beer segment continues to develop at a swift pace, but several notable product recalls have emphasized the threat of microbiological contaminants and subsequent product spoilage. Product recalls due to microbiological contamination in the U.S. craft beer industry have caused economic losses and occasionally have exposed the consumer to risk of injury. Wild yeast like *Saccharomyces cerevisiae* variety *diastaticus* (*S. cer. var. diastaticus*) and beer-spoiling bacteria (e.g. *Lactic acid bacteria*) pose a significant threat to breweries and consumers alike. As the U.S. craft beer industry continues to expand, quality control and quality assurance programs must follow suit.*

### Overview of U.S. craft beer industry

The history of craft beer in the United States commenced in 1965 when Fritz Maytag purchased the failing Anchor Steam Brewery in San Francisco. At that time, the market was dominated by less than one hundred breweries, which were churning out American Lager. Maytag insisted on continuing to brew historic Steam Ale in lieu of the more popular style. His risky decision would shape the future of the craft industry. In 1979, Ken Grossman opened Sierra Nevada Brewing Company, and the renowned Sierra Nevada Pale Ale entered production in 1980. The 1980s were a decade of mod-

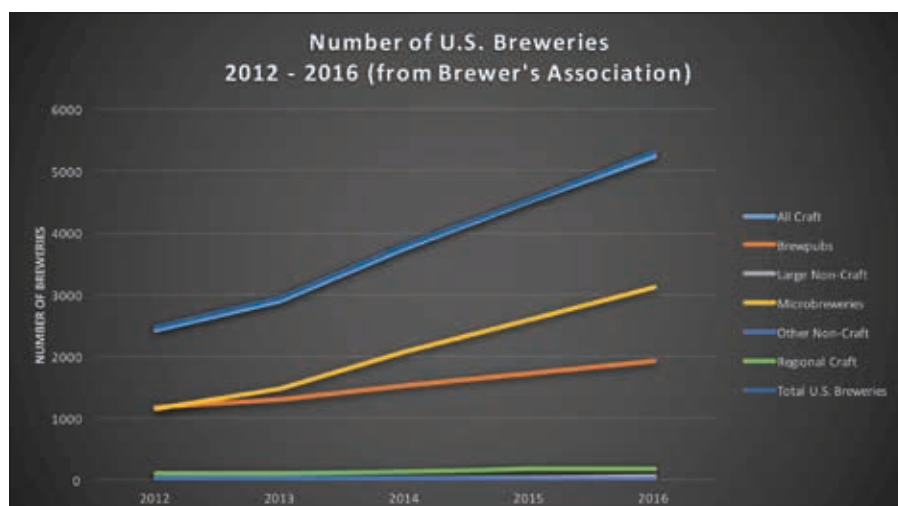
erate but substantial growth in the U.S. craft beer sector. In 1979, U.S. President Jimmy Carter lifted the legal restrictions on home brewing. Before that, it had been illegal to produce alcohol without a license in the United States. In some regions, home brewing had already gained a foothold, as the American Homebrewers Association was formed in 1978 in Boulder, Colorado. The new freedom to produce and enjoy unorthodox beer styles such as ales and stouts at home aided the growth and expansion of U.S. consumers' taste preferences in beer.

The craft beer industry has changed significantly since the 1970s and 1980s. According to the Brewers Association (BA),

the number of craft breweries increased from eight in 1980 to 537 in 1994 (Brewers Association, 2017). In 2016, craft beer accounted for 12.3 percent of the total U.S. beer market (over 28 million hl). A large number of breweries in the U.S. are classified as microbreweries or brewpubs (less than 17,000 hl production) with limited distribution. Today there are over 5,300 craft breweries in the United States, but there remains some debate on what the true definition of a craft brewery is.

### Microbiological threats to beer, including U.S. craft beer

Compared to other manufactured foods and beverages, beer is rather robust and resistant to microbiological contamination and spoilage. A combination of low pH, low oxygen, carbon dioxide, ethanol, and hop acids make beer an inhospitable place for microbes of public health significance like *Salmonella*, *Listeria*, and *Legionella*. After all, pathogens enumerate in environments that are comparable to the human body, and beer is very different. Beer is a product that is susceptible to quality degradation through multiple routes including microbiological spoilage. Because of this, microbiological QA/QC programs aim to detect, identify, and prevent spoilage



Number of breweries in United States by segment

of beer throughout the production process. Perhaps the most notable historical account of microbiological spoilage in fermented beverages occurred in the 1860s when Louis Pasteur was tasked with solving widespread spoilage issues in French beer and wine. The nineteenth century was a time of immense expansion of the French economy, but microbiological contamination was hindering the expansion of wine distribution. Wine and beer producers were in dire need of a solution to prevent wines from succumbing to “la maladie” or the disease that was wreaking havoc on the industry. Pasteur’s efforts eventually led to the development of the germ theory of disease, pasteurization, and a new understanding of fermentation (Ligon, 2002).

Traditional ale yeast fermentation by way of *Saccharomyces cerevisiae* is preferred by U.S. craft brewers, but a small portion of U.S. craft breweries use lager yeast (*S. pastorianus*) for production. Additionally, mixed and spontaneously fermented beers are growing at moderate rate in the U.S. craft beer industry. The majority of craft beers in the United States is unpasteurized and unfiltered, which is a stark contrast to filtered and pasteurized American Light Lager.

Microbiological spoilage is a significant threat to any beer, and U.S. craft beer is no exception. Beer spoiling bacteria like *Lactobacillus acetotolerans* and wild yeast like *Saccharomyces cerevisiae* var. *diastaticus* (*S. cer.* var. *diastaticus*) are responsible for the vast majority of product spoilage in the industry. It can be difficult to assess the true number of spoilage events in U.S. craft beer because most of the incidents go unreported or are underreported. For example, it is almost unheard of for a brewery to publicize the infection and subsequent destruction of a batch of beer. Additionally, product defects such as gushing or increased turbidity may be incorrectly categorized as production process issues.

## Microbiological beer spoilers

Microbiological beer spoilers are classified into two main groups: bacteria and fungi. Both groups include organisms that are capable

of spoilage throughout the production and distribution process. Contamination can occur anywhere in the process, but the most vulnerable production steps include yeast propagation, wort cooling and transfer, and packaging.

The most noteworthy bacteria that are capable of beer spoilage are Firmicutes and are part of the families Lactobacillaceae and Veillonellaceae. *Pediococcus* and *Lactobacillus* are two of the three genera classified as lactobacillaceates. Two of the most notorious species are *Lactobacillus brevis* and *Pediococcus damnosus*. They are facultative anaerobes that rely on fermentation of simple sugars

as a carbon source, where the end metabolite is lactic acid. Lactobacillaceae are Gram-positive bacilli or cocci that do not form spores. *Megasphaera* and *Pectinatus* are members of Veillonellaceae. These are Gram-negative anaerobes that can create off-flavors and increased turbidity in beer.

The capability of spoiling beer is not necessarily species-dependent, and there is significant interspecies diversity in the microbes of interest to the brewing industry. There are examples of spoilage bacteria strains of *Lactobacillus brevis* that have more hop resistance gene commonalities with organisms of a different genus than other strains



*The microbiological lab of Founders Brewing Company in Grand Rapids, MI*

within the species. For example, not all strains of *Lactobacillus brevis*, which is responsible for the largest proportion of beer spoiling events in modern history, are beer spoilers. This is because the genetic diversity within *Lactobacillus brevis* and *Lactobacillaceae* as a whole is markedly diverse. It is hypothesized that plasmid-based and transposon-based gene transfer of genetic material, including hop resistance genes, is responsible for some of the interspecies diversity within *Lactobacillaceae* (Suzuki, 2006).

Biological classification is used to divide spoilage yeast organisms into two groups (*Saccharomyces* and non-*Saccharomyces* wild yeast). Two important *Saccharomyces* wild yeast include *Saccharomyces bayanus* and *Saccharomyces cerevisiae* var. *diastaticus* (*S. cer. var. diastaticus*). *S. cer. var. diastaticus* is the most dangerous *Saccharomyces* wild yeast because of its ability to wreak havoc on beer during production and distribution.

### Wade Begrow

Microbiologist at Founders Brewing Company in Grand Rapids, MI. He is an avid fan of quality beer and has a strong interest in brewing and fermentation microbiology. He is an expert on traditional and advanced microbiological techniques to detect bacterial and fungal beer spoilers in the brewing process. Before his role at Founders, he was the microbiologist at Bell's Brewery in Comstock, MI. Wade is a member of the Master Brewers Association of the Americas and is a member of the MBAA Food Safety Committee. He presented at the 2016 World Brewing Congress and the 2017 European Brewing Congress.



*Saccharomyces cerevisiae* var. *diastaticus* (*S. cer. var. diastaticus*) was first described in 1943 and was classified as *Saccharomyces diastaticus* in 1965 by R. B. Gilliland of Guinness. The strain was studied extensively because of its ability to ferment starches and dextrins. Additionally, it was responsible for spoiling unpasteurized beers in the United States before the use of pasteurization became widespread. It was later reclassified as a variety within *S. cerevisiae*, but it is still occasionally called *S. diastaticus* or just "diastaticus."

*Saccharomyces cerevisiae* var. *diastaticus* (*S. cer. var. diastaticus*) is capable of secreting an extracellular glucoamylase that converts complex carbohydrates to simple sugars through hydrolysis of alpha-1,6 glycosidic bonds. This is a major concern for brewers, cellar operators, packagers, distributors, and consumers because a change in the carbohydrate profile of a product can lead to superattenuation. Superattenuation is the term used to describe continued fermentation that occurs, beyond the time ordinary fermentation appears to have ceased, and it can occur at different stages in the production process.

The most dangerous and detrimental type of superattenuation caused by *S. cer. var. diastaticus* occurs after beer has been packaged. When superattenuation occurs in a closed system (e.g. bottle), sugars are converted to CO<sub>2</sub> and ethanol. The release of extra CO<sub>2</sub> within the package can cause a plethora of issues including gushing, difficulty pouring, dry mouthfeel, and package failure (e.g. leakage or explosion).

Furthermore, an increase in alcohol content after packaging can create regulatory noncompliance and consumer confusion.

*Brettanomyces* (*Dekkera*) are non-*Saccharomyces* wild yeast that can cause spoilage throughout the production process. *Brettanomyces bruxellensis* is capable of outcompeting culture yeast in the propagation system, and it can create off-flavors, including organic acids and phenolic compounds like 4-ethylphenol (barnyard and medicinal flavor/aroma). The inclusion of organic acids and phenolic compounds can be devastating to the beer production process because it is very difficult to blend away or remove the off-flavors. Furthermore, it can be challenging to identify and eliminate non-*Saccharomyces* wild yeast contamination in a brewery without a complete shutdown of the production area and tear-down of all of the equipment in the facility. If *Brettanomyces* contamination occurs during packaging, the undesirable off-flavors may not be produced until weeks or months after distribution.

### Two case studies of contamination in U.S. craft beer

Microbiological contamination can be devastating to any brewing process including U.S. craft producers. Two notable recalls in 2016 are examples of how destructive microbiological contaminants can be to a company. In January 2016, Goose Island Brewery of Chicago, Illinois, USA, announced a major recall of its highly popular Bourbon County Stout because of spoilage from *Lactobacillus acetotolerans*. It was announced that the contamination occurred somewhere in the production process, but it was not discovered until weeks after the beer had been distributed to consumers. The organism was able to survive and spoil the beer in its package state despite its robust 13 percent ABV. The company offered full refunds for all affected products (Noel, 2016).

Left Hand Brewing Company of Longmont, Colorado, USA, issued an enormous recall of its Milk Stout Nitro in September 2016 due to excessive foaming and difficulty pouring. The company announced

that a foreign yeast that caused excessive bottle pressure and foaming had entered its production stream. It went undetected because it looked and behaved like the house ale yeast (The Denver Post, 2016). It is hypothesized that *S. cer. var. diastaticus* caused the spoilage. Over 20,000 cases and an unknown amount of in-process beer were affected by the wild yeast contamination. Left Hand Brewery dropped from the 39<sup>th</sup> largest U.S. craft producer in 2015 to 44<sup>th</sup> in 2016.

## Detection methods

In the U.S. craft industry, the level of microbiological testing for microbiological spoilers varies significantly based on a variety of factors including the size of the operation. Traditional microbiology with selective enrichment media is still dominant in the U.S. craft industry, but modern testing technology like real-time PCR (qPCR) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is used in fully equipped laboratories.

Molecular detection methods for bacterial and fungal beer spoilers have improved dramatically in recent years. Commercially available kits can be used with real-time PCR (qPCR) systems to detect beer-spoiling bacteria from a variety of sample types (e.g. environmental swabs, enriched samples, colonies on microbiological media). DNA is extracted from samples and analyzed with the kits on the qPCR platforms to give results in under two hours. The most comprehensive kits use multiplex master mixes that can give up to four separate results in a single PCR well.

## Summary

Microbiological beer spoilers, including *Lactobacillus acetotolerans* and *Saccharomyces cerevisiae var. diastaticus*, pose a significant threat to the U.S. craft beer industry. The majority of craft beer produced in the U.S. is unfiltered and unpasteurized and is susceptible to microbiological spoilage before, during, and after packaging. Financial repercussions of a widespread recall can be extremely damaging, and risk can be reduced with routine microbiological monitoring. □

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