Microbiology of Brewing Beer and Beer Spoilage

An Overview of the Brewing Process and How Things Go Wrong

Author:

David Fang

Emory University

BIOL 370: Microbiology

Dr. Nic Vega

Introduction

Today, beer remains the most popular alcoholic beverage in the world, and the third most popular drink after water and tea (1). Throughout human history, beer has always been a staple beverage. Many historians have even suggested that one reason beer was consumed more than other beverages is due to beer's innate antiseptic properties compared to the difficulty of purifying water. However, this is a common misconception in more ways than one. For one, the human body requires water to survive, so while it was probably scarce and expensive, people must have had some sort of access to clean drinking water. Secondly, beer is not actually immune to contamination. Beer's innate antiseptic properties are attributed to its alcohol content, low pH value, anaerobic atmosphere, limited nutrients, and hop-derived bittering compounds (hops). However, its antiseptic properties are simultaneously more limited and complex than people tend to believe, and some bacteria have adapted to this environment.

Contamination of beer results in the growth of undesirable microbes that can alter the chemical composition of the drink, changing the taste and even making people sick. These microbes, also referred to as bacterial spoilers (BS), can grow at all stages of the brewing process, from the fields in which the grains are grown, to the fermentation process, and even in the pipes and taps that ultimately dispense beer in bars. The presence of bacterial spoilage is surprisingly high as well. A study that randomly sampled 50 breweries in Huston and determined that 20% of the beers they tested showed a significant growth of bacteria when plated on advanced beer-spoilage (ABS) medium (2). ABS media is a newly developed media designed specifically to test for BS by most accurately simulating a beer-based environment. It is made by replacing water with beer when creating de Man, Regosa, and Sharpe (MRS) agar (3). Interestingly, the study also determined that the size of the brewing company did not affect the rate of contamination, suggesting that this issue is brewery specific. The author also suggests that these bacteria are quickly mutating, and that some species have grown so accustomed to the environment that they will only grow in the presence of beer (4). So, what can be done about this issue? Are we doomed to a world of foul-tasting beer? By exploring the microbiology of the brewing process, one can learn about what stages are susceptible to contamination, how spoilage affects the product, and what can be done to improve conditions and prevent it.

Pre-Brewing: Purpose and Vulnerability of Raw Ingredients

Beer is made of four major ingredients: starch, water, yeast, and hops. To put it simply, the yeast microbes ferment starch to create ethanol and carbon dioxide. Water provides a medium to facilitate this interaction, while hops are used to enhance beer's flavor and aroma. Before even reaching the first stage of production, each ingredient has the potential for microbial contamination. If brewers are not careful, a single contaminated ingredient can lead to the production of BS later down the line.

As primary starch sources, wheat and barley encounter their first microbial enemy on crop fields. In damper and colder areas, fusarium head blight (FHB) is a common disease known to wipe out entire fields of cereal crops. Historically, FHB was not predominant in North America, as farmers would combat crop disease by setting infected fields on fire, destroying, and preventing the critical spread of spores. However, once crop burning became illegal, *F. graminearum* eventually got its foot in the door (5). FHB is caused by the fungal plant pathogen *Fusarium graminearum*, also known as *Gibberella zeae*. In wheat, *F. graminearum* alters the amino acid composition, contaminating the rest of the grain with mycotoxins. This compositional shift causes shriveling of kernels and premature bleaching of florets. In barley, mycotoxins cause the heads to darken and brown, sometimes growing pink or orange spores, hence the name, FHB. *F. graminearum* spores can infect nearby plants or spread via infected seeds (6). The central danger of FHB is the production of deoxynivalenol, also known as vomitoxin. Deoxynivalenol inhibits the production of proteins, causing vomiting and liver damage. If used as feed for livestock, infected grains are known to cause birth defects, rendering infected crops entirely unusable. Currently, the long-term effects of low-dose exposure in humans is unknown.

Despite water purification tactics and the innate lack of nutrients, some organisms still manage to survive in water and contaminate breweries. *Enterobacteriaceae* is one of the most common families of bacteria to contaminate beer. When introduced in the fermentation process, *Enterobacter agglomerans* caused increased levels of acetaldehyde, methyl acetate, diacetyl, 2,3-pentanedione, and dimethyl sulfide. While the increased consumption of these chemicals is not harmful to the body, they can elongate the fermentation process and slightly raise the pH of the product (7). A less common, but more deadly contaminant is *Legionella*. In 2013, a brewery in Germany had an outbreak right before Oktoberfest that infected 165 people and killed two (8). This bacterium proliferates in stagnant water and can cause Legionnaires' pneumonia if introduced into the lungs. This is extremely relevant and concerning in today's COVID-19 pandemic, as many small breweries have shut down, rendering water systems inactive and at optimal temperatures for growth.

The fermentation of starch extracts by yeast is the single most important microbial process in the brewing process. Nearly all the world's beer is brewed using one of two strains: *Saccharomyces cerevisiae* (ale yeast) and *Saccharomyces pastorianus* (lager yeast). Ale yeast is a simpler and naturally occurring organism, compared to lager yeast, which is more complex and not readily found in nature. The most significant difference between the two is lager yeast's ability to hydrolyze melibiose, using an alternate biochemical pathway for more unique flavors (5). Most contaminant strains are alternate strains of *Saccharomyces*, which can cause excess ester production (referred to as phenolic off-flavor production, or POF), sediment formation, or hyper-attenuation, resulting in increased alcohol and carbon dioxide production. Another common strain of contaminant yeast are *Brettanomyces*, which create the phenolic volatile compounds 4-ethylguaiacol and 4-ethylphenol. These compounds create a smell like that of used bandages and sweat (9). Some breweries intentionally use *Brettanomyces* yeast to brew certain beers, most typically Lambic Belgian beers.

Hops are the primary ingredient of beer that give beer its antibacterial properties. Without them, pH- and ethanol-resistant Gram-positive bacteria would be able to freely grow in beer. Hops provide the medium with an important compound, iso-alpha-acids. Iso-alpha-acids can carry protons across the cell membrane, causing a decrease in cytoplasmic pH that eliminates the cell's proton gradient. Without this gradient, enzymatic activity, and nutrient transport ceases, causing the cell to die. Despite this mechanism, bacteria have evolved plasmids that carry hop-resistant genes, *horA*, *ORF5,* and *horC*. *HorA* and *ORF5* can transport iso-alpha-acids back out of the cell to maintain their gradient, while *horC* works to maintain the proton gradient on its own (10). However, on the fields where the plant grows, they are vulnerable to verticillium wilt. This fungus lies dormant in soil, germinating when the roots of a susceptible plant grows close enough to infect. The fungus encroaches up the roots and spreads to the rest of the plant, "plugging" the leaves and limiting water circulation. Another disease that affects hops plants is downy mildew. This disease lies dormant in the buds and crowns of the plant to produce infected shoots during the spring. Once infected, spores form on the underside of the leaves that spread with the wind and rain. Infected crowns can produce uninfected shoots as well. This can make detecting and tracking downy mildew difficult. Although uncommon, affected hops can be accidentally incorporated into the brewing process, compromising the antimicrobial nature of the brewing medium.

Brewing: Presence and Impact of Contamination during Brewing and Distribution

Generally, the brewing process is split up into nine different steps: milling, malting, mashing, lautering, boiling, fermenting, conditioning, filtering, and packaging -and each step has the potential for spoilage.

Before stainless steel was invented, many brewing companies used wooden barrels and repurposed dairy equipment to brew beer. Today, breweries spend millions of dollars on top-of-the-line industrial-grade stainless steel equipment that have reduced oxygen exposure and improved temperature control and sterility throughout the process. Aerobic, Gram-negative acetic acid bacteria used to be a major hurdle for breweries, but the reduction of oxygen exposure throughout the process has nearly eliminated this form of contamination.

The second step of the brewing process, malting, has one the greatest potentials for beer spoilage. Malting has three main steps: steeping, germination, and kilning. These steps simulate the grain's natural germination cycle. During steeping and germination, the grain is moisturized, heated, and aerated, allowing for the rapid multiplication of microbial cells due to dissolved nutrients. During kilning, the temperature is increased to dry the malt to prevent further germination. Even though kilning reduces the viable count of microbes, the prolonged exposure to air and high availability of nutrients makes malting vulnerable to spoilage (9). A major concern is *F. graminearum*. As previously discussed, *F. graminearum* produces deoxynivalenol which has detrimental physiological effects, but FHB also produces hydrophobin, an intensely hydrophobic polypeptide. This compound provides a nucleation site for carbon dioxide in beer, resulting in spontaneous gas release once exposed to the atmosphere. When already packaged in cans or bottles, this can be as harmless as opening a shaken-up soda, but in larger scale storage containers it can cause explosions (5).

During worting, the last step of mashing, the malted grain and water is heated to certain temperatures that allow the plant enzymes to break down starches into maltose sugar. Here, bacterial growth of *Bacillus* can reduce nitrate to nitrite, leading to excessive acidification and nitrosamine formation in the beer, making the final product taste extremely sour. Additionally, the growth of *Clostridium* in wort will produce butyric acid, making the final product taste like cheese (11).

During boiling, the fifth overall step, the wort is boiled for a long period of time. While this sterilizes the wort, some bacteria can still contaminate the wort given the right conditions. The most prominent wort spoilers are *Klebsiella*, *Citrobacter*, *Enterobacter*, *Obesumbacterium*, and *Escherichia* (12). These bacteria will produce dimethyl sulfide, 2,3-butanediol, and various organic acids that will make the product smell vegetal and taste unpleasantly fruity.

While many of the brewing steps, such as boiling, fermentation, and conditioning are done in air-tight tanks, several steps require natural aeration to proceed. At any point during exposure to air, airborne lactic acid bacteria (LAB) can contaminate the malt. While over 20 strains of LAB have evolved hop tolerance and have reported growth in beer, not all these strains confer a high rate of beer spoilage. Among those, two strains stand out for both their rate of occurrence and spoilage: *Lactobacillus brevis*, and *Pediococcus damnosus*. Together, these two pose the greatest threat to beer spoilage, accounting for over 50% of all BS cases (13). *L. brevis* is very commonly found in nature and is associated with plants such as barley and wheat. It can travel through both air and water, meaning that its spread within breweries is inevitable (14). LAB can spoil beer by acidification, sediment formation, and diacetyl production, which can give beer an unpleasant buttery flavor. Many strains of LAB will also produce exopolysaccharides, which can alter the texture of beer by making it more oily or slimy (14).

On the other hand, *P. damnosus* can contaminate beer via unmaintained or dirty pipes. This means that *P. damnosus* can contaminate at any point in the brewing process, from unkempt pipes in a brewery to the taps and drafts at your local bar. *P. damnosus* causes an increase in vicinal diketones (VDKs), which are an important component for creating flavors in beer. Normally, yeast will consume any naturally produced VDKs during the fermentation process, but any addition afterwards will result in an undesired popcorn or butterscotch flavoring to the product (5).

Conclusion

By exploring the brewing process, it is easy to imagine the countless instances of possible beer contamination as the wheat and barley grown on fields eventually finds its way into our glasses. Nearly every raw ingredient and brewing stage has its own unique possible risk of contamination, yet only a handful of contaminants pose any real pathological threat. For this reason, it is important to record and have knowledge of the various contaminants that can survive in beer, something that the scientific community surrounding brewing has done an excellent job of. Most of the time, these “contaminants” will only alter the taste or smell of the final product, and some are not even contaminants at all, as they serve crucial roles in the biochemical brewing process. Different strains of yeast, which used to be considered spoilers, are now being used to create alternative brews in many other countries. Because of research like this, brewing has become a lot safer and more creative over time. Who knows, it might only be a matter of time before buttery beer becomes the next big thing.

References

1. "Volume of World Beer Production". European Beer Guide. Archived from the original on 28 October 2006.
2. Goodman, Mary, et al. "Isolation of Beer-Spoiling Bacteria from Texas Craft Breweries." *Journal of Culinary Science & Technology*, vol. 18, no. 2, 2018, pp. 116–123., doi:10.1080/15428052.2018.1509754.
3. Suzuki K, Asano S, Iijima K, Kuriyama H, Kitagawa Y. "Development of Detection Medium for Hard-to-Culture Beer-Spoilage Lactic Acid Bacteria." *Journal of Applied Microbiology*, U.S. National Library of Medicine, pubmed.ncbi.nlm.nih.gov/18070034/.
4. Goodman, Mary. "Good Craft Beer Getting Spoiled by Bacteria." ASM Live. New Orleans, Louisiana. June 1st, 2015. Lecture.
5. Bamforth, Charlie. "The Magnificent Microbiology of Beer - Microbiology After Dark." ASM Live. Washington DC, Maryland. October 10th, 2013. Lecture
6. Yang F, Jacobsen S, Jørgensen HJL, Collinge DB, Svensson B and Finnie C (2013) "*Fusarium graminearum* and its interactions with cereal heads: studies in the proteomics era." *Front. Plant Sci.* **4**:37. doi: 10.3389/fpls.2013.00037
7. Vuuren, H.J.J.v., Cosser, K. and Prior, B.A. (1980), "THE INFLUENCE OF *ENTEROBACTER AGGLOMERANS* ON BEER FLAVOUR." J*ournal of the Institute of Brewing*, 86: 31-33. https://doi.org/10.1002/j.2050-0416.1980.tb03952.x
8. Fuchs, Stephen. "Deadly Bacteria Found In Waste Water Of Warsteiner Brewery." *German Pulse*, 15 Sept. 2013

9. Bokulich, Nicholas A, and Charles W Bamforth. "The microbiology of malting and brewing." *Microbiology and molecular biology reviews: MMBR* vol. 77,2 (2013): 157-72. doi:10.1128/MMBR.00060-12

10. Sakamoto K, Konings WN. "Beer spoilage bacteria and hop resistance." *Int J Food Microbiol.* 2003 Dec 31;89(2-3):105-24. doi: 10.1016/s0168-1605(03)00153-3. PMID: 14623377.

11. Smith, N.A., Smith, P. and Woodruff, C.A. (1992), THE ROLE OF *BACILLUS* spp. IN N-NITROSAMINE FORMATION DURING WORT PRODUCTION. *Journal of the Institute of Brewing*, 98: 409-414. https://doi.org/10.1002/j.2050-0416.1992.tb01124.x

12. Priest, F.G., Cowbourne, M.A. and Hough, J.S. (1974), WORT ENTEROBACTERIA—A REVIEW. *Journal of the Institute of Brewing*, 80: 342-356. https://doi.org/10.1002/j.2050-0416.1974.tb03629.x

13. Geissler AJ, Behr J, von Kamp K, Vogel RF. "Metabolic strategies of beer spoilage lactic acid bacteria in beer." *Int J Food Microbiol*. 2016 Jan 4; 216:60-8. doi: 10.1016/j.ijfoodmicro.2015.08.016. Epub 2015 Sep 5. PMID: 26398285.

14. Sakamoto, K et al. "Hop resistance in the beer spoilage bacterium Lactobacillus brevis is mediated by the ATP-binding cassette multidrug transporter HorA." *Journal of bacteriology* vol. 183,18 (2001): 5371-5. doi:10.1128/jb.183.18.5371-5375.2001