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Beer-spoilage Bacterial Strains in Large and Small Corporations in Breweries

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ABSTRACT

Bacteria-spoilage strains are the root cause of unwanted flavors and unaesthetic appearances of beer. This accidental introduction of bacteria is causing economic loss to businesses and is affecting the health and loyalty of the breweries' consumers. The two most studied strains are from the genera Pediococcus and Lactobacillus are a result of either primary or secondary contamination from the production process. Craft beers, such as Hazy Beers, produced by small-scale breweries are more prone to spoilage than commercial beers produced by large-scale breweries. The goal of this study is to test for the presence of bacteria-spoilage strains in small-scale breweries and large-scale breweries to determine if production size has a direct effect on bacterial growth in their final product. Using the spread plate technique, we inoculated two different media with six beer samples in an anaerobic environment and observed growth using the gram staining method to identify the strain by looking at the bacteria's morphology under the microscope. Hazy Lady IPA, produced by Six Rivers Brewery, a small-scale brewery, was the only beer in our sample size that showed significant growth on both WLDs media showing significant Lactobacillus and Pediococcus bacterial contamination. Future studies should continue to explore the relationship between production size and bacterial contamination in larger sample sizes to determine if this correlation is unique to Humboldt County's small-scale brewery, Six Rivers, or all small-scale breweries.

INTRODUCTION

Beer is a product of repute and traditional craftsmanship. It has become a staple beverage with solid and trustworthy authenticity. However, like all manufactured products; beer is not immune from exposure to contaminants. It is important to investigate and pay attention to possible contaminants because they can affect the consumer's health, result in the loss of consumer confidence, and most importantly to the company, cause severe economic loss to breweries [1].

Microbiological contaminants can spoil beer. Primary contaminants are born from raw materials and brewing equipment whereas secondary contaminants occur in the final stages of production; bottling, canning, and packaging. Craft beers made by small-scale breweries are more prone to spoilage than beers produced by large-scale breweries. This is because they are 1) less likely to be pasteurized or sterile-filtered and 2) it is challenging to complete consistent hygiene procedures in small-scale production [2].

Microorganisms that grow in beer change properties by increasing pH and turbidity, as well as create off-putting flavors resulting in what is known as beer spoilage [3]. *L. lindneri*, *L. backi*, *L. paracollinoides, L. brevis,* and *Pediococcus damnosus*, are the most potent beer spoilage gram-positive bacteria [4]. The most hazardous beer-spoilage bacteria belong to the genera *Lactobacillus* and *Pediococcus;* of these two, the most common is *Pediococcus damnosus* in the genera *Pediococcus* [5]. The average pH of beer is 4.5; in the presence of spoilage-causing bacteria, the pH drops to a range of 3.0-5.5 (Table 1). These strains grow during fermentation and create acids that alter the originally intended flavor of the beer produced [6].

Traditionally, the appearance of haze in beers was deemed an unfavorable quality and breweries would try different types of filtering processes to avoid the appearance of floating particulates [7]. In most cases, these particulates which cause the appearance of haze or cloudiness result from a high concentration of proteins, polyphenols, and beta-glucan [8]. Small or microbreweries have come to embrace the natural haze of beers and even coined the term Hazy Beers as a marketed product. Due to its growing popularity, large-scale breweries have adopted Hazy Beers into their own production line. The intentional neglect of hazy beer filtration compared to conventional beers leaves room for beer spoilage bacteria to grow. A critical defining feature of all beer is the presence of hops with some antimicrobial properties [9]. Since hazy beers also contain hops, and in turn contain some antimicrobial properties, the unfiltered nature of hazy beers would be a focal point for studying the introduction of spoilage-causing bacteria.

Table 1.
Beer-spoilage strain characteristics in the genera Pediococcus and Lactobacillus.

Genus Species	Optimum Temperature Growth	Morphology	Grain Stain	Acid Produced	Flavor Alteration
Lactobacillus brevis	30°C	Bacillus	Gram positive	Lactic Acid	Sour and Tart
Pediococcus damnosus	22°C	Cocci	Gram positive	Acetic Acid	Vinegar

Table 2.
Six beer samples and the media used to detect contamination.

Hazy Beer Sample	Brewery	Category	Incubation Temperature	Incubation Period	WLD Media Light	WLD Media Dark
Hazy Lady IPA	Six Rivers	Small-scale	37°C	48 hrs	Lactobacillus	Pediococcus
Hazy IPA	Lost Coast	Small-scale	37°C	48 hrs	Lactobacillus	Pediococcus
Sticky Fingers IPA	Redwood Curtain	Small-scale	37°C	48 hrs	Lactobacillus	Pediococcus
Hazy Lil Thing IPA	Sierra Nevada	Large-scale	37°C	48 hrs	Lactobacillus	Pediococcus
Voodoo Ranger IPA	New Belgium	Large-scale	37°C	48 hrs	Lactobacillus	Pediococcus
Hazy Wonder	Lagunitas	Large-scale	37°C	48 hrs	Lactobacillus	Pediococcus

This study uses three Hazy beers from three different small-scale locally owned breweries in Humboldt County to test for bacteria-spoilage bacteria, specifically Lactobacillus brevis and Pediococcus damnosus. We know that these strains are commonly found and feared in production batches. We aim to investigate if small-scale production batches in Humboldt County produce more spoilage-causing bacteria than large-scale production batches. We expect to see small production batches prone to Lactobacillus and Pediococcus contamination because the production is inconsistent; this leaves room for the introduction of contaminants. The aims of this study are to 1) test for bacteria spoilage strains in small-scale production 2) test for bacteria spoilage strains in large-scale production 3) examine if production sizes increase or decrease the risk of contamination.

MATERIALS AND METHODS

Three large corporation beer samples were collected at a local convenience store in Arcata, CA. Three small corporation beer samples were collected from taps in local breweries around Humboldt County, CA. From each type of beer, we took two replicate samples to determine contamination. For each replicate, we used a spread plate on two light and two dark WLD media. All media were incubated in an anaerobic chamber at 37°C to suppress the growth of mold and yeast and allow for the detection of our target bacterial spoilage species strains, *Lactobacillus brevis,* and *Pediococcus damnosus* (Table 2). The growth observed from the cultures was used for gram-staining procedures to identify the bacterial spoilage species strain through bacterial morphology.

RESULTS

All tests were run concurrently with two replicates of each media: WLD light targeting for spoilage strain *L. brevis* and WLD dark targeting for spoilage strain *P. damnosus.* All large-scale brewery samples were negative for contamination shown by zero colony-forming units (CFUs) on both light and dark WLD media [10]. Two out of the three small-scale breweries

Figure 1.
CFUs counted in all samples.

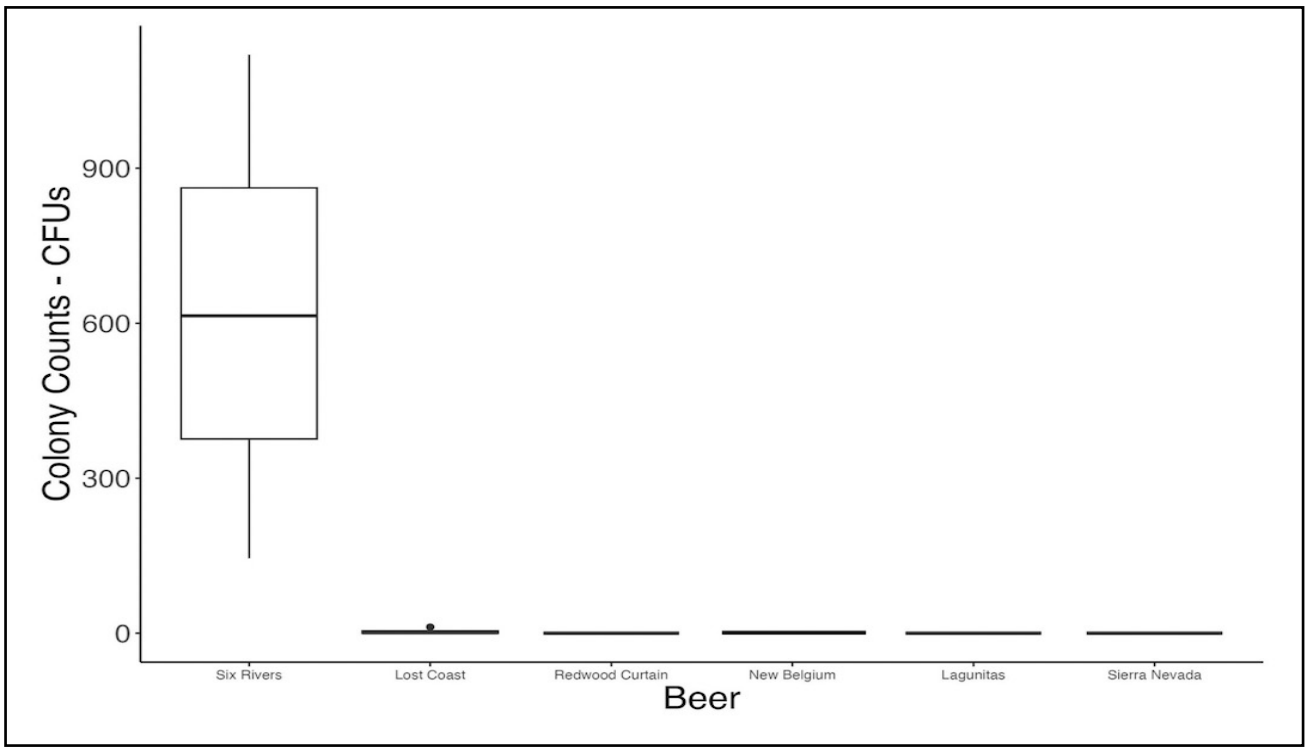
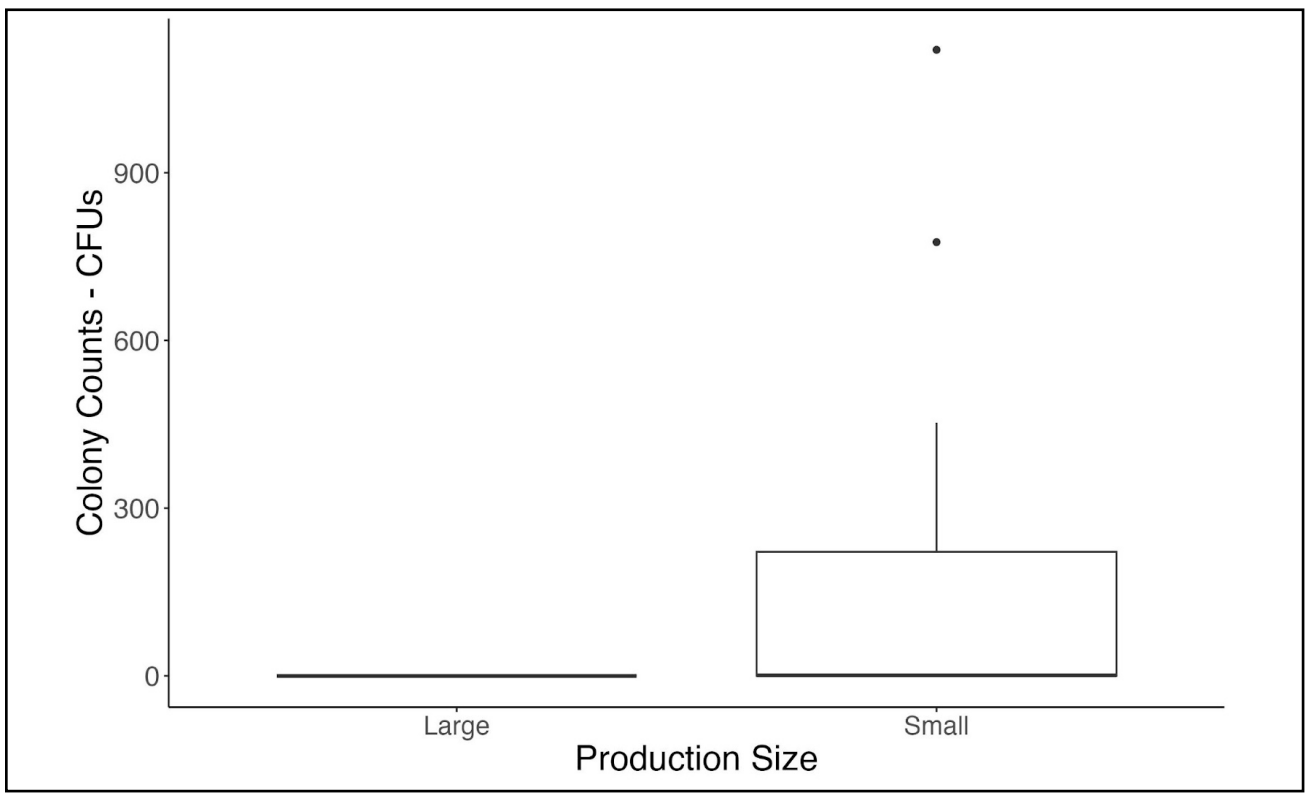


Figure 2.
CFUs in all the samples based on small or large-scale production classification.



tested positive for contamination: Six Rivers Brewery and Lost Coast Brewery. Hazy Lady IPA produced by Six Rivers Brewery showed growth in both light and dark WLD media. Hazy Lady IPA colonies grown on light WLD were examined and classified as gram-positive and bacilli shaped, exhibiting a positive test for contamination of *L. brevis*. Hazy Lady IPA colonies grown on dark WLD media were examined and classified as gram-positive and cocci shaped, exhibiting a positive test for contamination of *P. damonous*. Unlike Hazy Lady by Six Rivers Brewery, Hazy IPA produced by Lost Coast Brewery only showed growth in the dark WLD media. Growth from the dark WLD media was determined gram-positive and cocci shaped, exhibiting a positive test for contamination of *P.damnosus.* To analyze total contamination in small-scale samples, targetted bacteria spoilage strains were grouped. We found that small-scale breweries did have higher rates of contamination compared to large-scale breweries (Fig. 2, H=4.32, p=0.03, Kruskal-Wallis)

DISCUSSION

Our results show supporting evidence (Fig. 2) that there is a relationship between production size and bacterial contamination, however, we do not know the strength of this relationship. There is a distinct lack of evidence of contamination from other small-scale breweries, therefore, we cannot definitively say that contamination is due to the size of the brewery or an outside factor. Future studies should expand the sample size of both large and small-scale breweries to give stronger support for a production size-bacterial contamination relationship. Along with a larger sample size of breweries, testing multiple batches consistently over a period of time would provide beneficial data. In addition, conducting another study with the sole purpose of pinpointing why Six Rivers Brewery's Hazy Lady IPA is more susceptible to contamination would be worthwhile. With that said, further testing could prove difficult for anyone without proper licensing and credentials as breweries are unforthcoming about their recipes and practice.

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