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Spoilage Risk-Based Analysis of Lactobacillus and Pediococcus Brewery Isolates in Beers Having Diverse Properties

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ABSTRACT

- A major challenge in maintaining beer quality is early detection of spoilage microorganisms before they have the ability to produce unintended flavors and aromas. Spoilage organisms can be diverse and present different quality risks based on their potential to thrive in beer and in the brewery. Early detection coupled with risk-based analyses can provide invaluable information to quality-centric brewers.
- A novel molecular diagnostic assay, Veriflow® brewPAL, was developed to provide accurate and sensitive detection of beer-spoiling Pediococcus and Lactobacillus species in under three hours. In this study, Veriflow® brewPAL technology was used to assess bacterial growth in beers having diverse properties. Numerous factors may influence the ability of Lactobacillus and Pediococcus species to metabolize and affect the quality of beer, including levels of hops resistance genes in bacterial isolates and percent ABV, IBU, gravity, malt builds and respective substrates in beer formulations. The effects of these factors on Lactobacillus and Pediococcus growth were evaluated with the ultimate goal of developing a comprehensive, validated model for beer spoilage risk assessment that could be used by breweries to preserve the quality and therefore the taste and value of the beer they produce.
- Lactobacillus and Pediococcus strains were isolated from different locations within a brewery setting. Each isolate was genetically characterized to determine strain identity and the hops resistance gene profile. Following characterization, select strains were grown in beers having distinct properties in order to determine the factors that are major predictors of spoilage risk. Bacterial growth in each beer was measured and quantified using the Veriflow® brewPAL system to determine overall risk of spoilage, which was subsequently correlated to the properties specific to each beer. While ABV and IBU are important factors that can influence the risk of beer spoilage, the results of these studies revealed additional properties are strong modulators of bacterial growth, including the utilization of specialty malts which may contain more dextrins. These findings can be used as a guide to help predict whether conditions within a particular beer are favorable for rapid bacterial growth and subsequent spoilage, thereby providing brewers with the ability to make early and informed decisions to maintain the quality of their products.

BACKGROUND

- Most brewers rest knowing that the iso-alpha acids and alcohol by volume will protect their beer from lactic acid producing bacteria. Not all brewers are aware that there are hop resistance genes that can allow those organisms to grow even in the hoppiest of IPA's.
- " The *horA* gene was shown to encode an ATP dependent multidrug transporter that extrudes hop bitter acids out of bacterial cells. In contrast, the product of the *horC* gene confers hop resistance by presumably acting as a proton motive force (PMF)-dependent multidrug transporter. Strikingly, the homologs of *horA* and *horC* genes were found to be widely and almost exclusively distributed in various species of beer spoilage LAB strains, indicating these two hop resistance genes are excellent species-independent genetic markers for differentiating the beer spoilage ability of LAB" (Koji Suzuki, et al. 2006)
- These two genes can be present in combination or singularly. This study is being conducted to show that iso-alpha acids and ABV are not the only factors that can affect the growth of lactic acid bacteria (LAB). Malts have so many variations and different sugars and nutrients that come from them, that they may play a significant role in LAB growth. This study aims to investigate what happens when you have high IBU and high ABV but you still see growth of these organisms.
- Malts play one of the biggest roles in making a beer. Different malts can produce different sugars, proteins and even dextrins. Dextrins are water-soluble compounds composed of polysaccharides and are generated through the process of starch degradation by the addition of heat, acid, or enzymes. Dextrins contribute to the weight, body, and mouthfeel of beer.
- Additionally, different malt builds can affect the free amino nitrogen (FAN) in the product, which can inhibit or promote growth of certain organisms. Yeast will uptake these nitrogens but some are left behind. Larger beer companies don't have to worry about this affecting growth because they use adjuncts and base 2-row malt which produces very little FAN. Craft brewers using mostly malt grain bills will have this play a role in the growth patterns.

REFERENCES

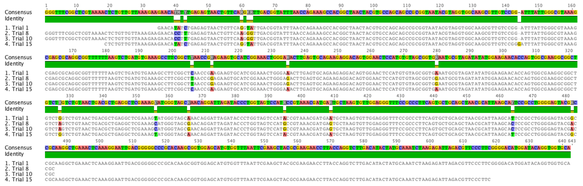
- Suzuki, K., Iijima, K., Sakamoto, K., Sami, M. and Yamashita, H. (2006), A Review of Hop Resistance in Beer Spoilage Lactic Acid Bacteria. Journal of the Institute of Brewing, 112: 173–191. doi:10.1002/j.2050-0416.2006.tb00247.x

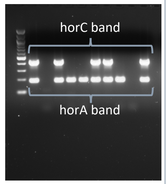
OBJECTIVES AND METHODS

1. Isolate and culture lactic acid bacteria from different parts of a brewery

Organism	Location of Origen	Beer of Origen
1	Environmental	Blower (not brand specific)
8	Racking Port	Pale Ale
10	Bright Tank	IPA
15	Keg	Milk Stout

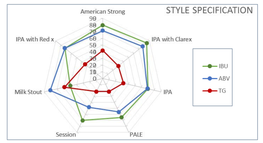
2. Genetically identify species using 16S sequencing and characterize hop resistance genes by PCR and gel electrophoresis





3. Test isolates in multiple beer styles to assess growth

Organism	Species	Hops Resistance Genes
1	L. plantarum	horA
8	L. brevis	horA and horC
10	L. brevis	horA and horC
15	L. plantarum	horA



Aliquot 25 ml beer into 6 sterile tubes for each brand. Inoculate one tube of each brand with organism. Incubate for 24 hours. Vortex and serially dilute spiked sample into 4 other tubes of 25 ml beer, leaving one negative control. 4 isolates were tested in each of 7 beer styles.

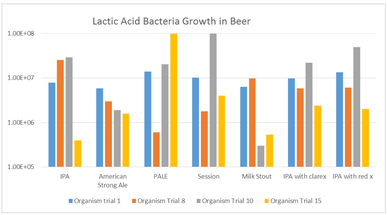
4. Quantify organism growth using Veriflow brewPAL PCR

Centrifuge samples, resuspend pellets, and perform PCR using Veriflow brewPAL

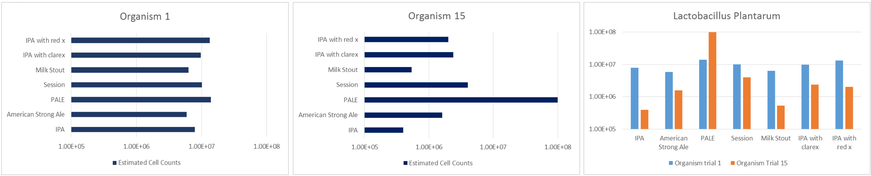
Analyze results using Veriflow cassettes to detect positives and negatives. The intensity of the test line is proportional to bacterial concentration

Quantify organisms in each dilution tube using Veriflow Cassette Optical Reader. Correlate growth results to beer properties

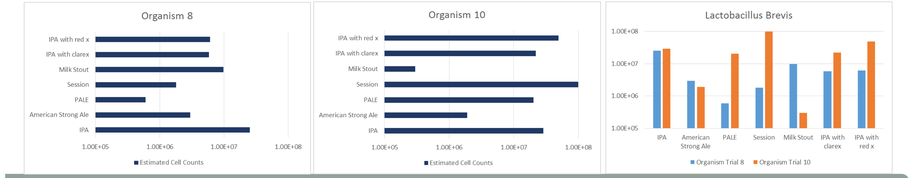
RESULTS



L. plantarum isolates



L. brevis isolates



CONCLUSIONS

- The results indicate that lactic acid bacteria species is not a primary predictor of growth and spoilage risk, as distinct growth patterns are observed between isolates of the same species. The commonly accepted spoilage risk factors including hops, alcohol, and bacterial strain are insufficient to explain the observed growth patterns.
- Other factors, including beer properties and bacterial genetic factors, must be responsible. Efforts are underway to determine relative hops resistance gene copy number in each strain using a recently-developed quantitative PCR assay to investigate whether copy number is correlated to growth.
- Organism 1, a horA-containing L. plantarum, exhibited robust growth in each of the beers tested. This growth was on the same order of growth observed with L. brevis strains, indicating that L. plantarum strains pose a significant growth and spoilage risk. Organism 15, another horA-containing L. plantarum, exhibited reduced growth in most formulations tested, except for the Pale Ale.
- Organisms 8 and 10, both horA/horC-containing L. brevis isolates, had unique growth patterns consisting of a mixture of robust and attenuated growth in different beers.
- Organisms 10 and 15 showed the most inhibition in Milk Stout, a brand that has very little bitterness, higher ABV, but more nutrients, while the other two strains grew well. This suggests that Organisms 10 and 15 are more sensitive to ABV than IBU and the additional nutrients did not help growth.
- Organism 8 and 15 showed inhibited growth in Session likely due to less FAN availability because it has very simple malt build, mostly base 2row. This beer has a higher IBU, but the other two organisms did not have the same inhibition, despite the fact that they are the same species and hop resistance genotype as the other organisms. This would indicate the malt could be a factor in the organism growth.

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