Universal Quality Control for Brewers

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Abstract

Beer, the product of fermentation that arguably changed the course of human civilization. Though the first brewing process dates back to ancient Mesopotamia, the modern method still presents a host of flaws. There are both many steps of fermentation and many opportunities for contamination. Most breweries will experience some form of spoilage in their lifetime. These invading microorganisms can alter turbidity, coloration, aroma, taste, and the overall quality of the product. This disruption is devastating for commerce. A centralized method of testing would help to eliminate spoiled products and prevent future infections. The following procedure utilizes a combination of polymerase chain reaction and media to detect suspected contaminants.

Introduction

When not being used for sours, dry saisons, or Belgian ales, Diastatic yeast can be quite troublesome. These yeast are a variant of *Saccharomyces cerevisiae* that can invade silently and cause unintended hyperattenuation, both in batch and bottle. The secondary fermentation that occurs after packaging is often associated with a phenomenon known as "Beer Explosion." The chimeric gene common in Diastatic variants, *STA1*, encodes Glucoamylase. This extracellular enzyme hydrolyzes residual dextrin and starch into fermentable sugars, subsequently producing 4-vinyl guaiacol and phenol off flavors. Suspected sources D1-D9 were obtained from the local brewery for analysis. Along with PCR testing, the following consists of the media-based approach: Sabouraud Dextrose Agar (SDA), Lin's Cupric Sulfate Medium (LCSM), and Starch Agar Bromphenol Blue (SABB). *E. coli* and suspected *Rhodotorula* were included in the procedure as controls.

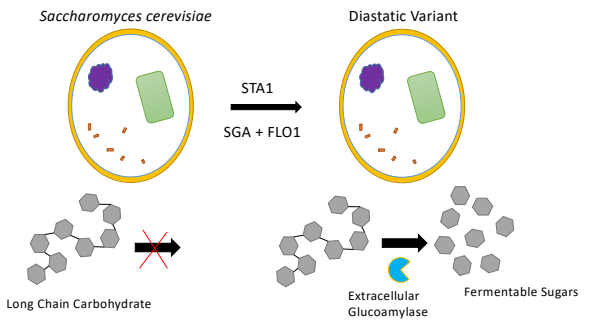

Figure 1. Variant Expression Diagram

Materials and Methods

1. Acquire a fresh specimen.
2. Culture Sabouraud Dextrose Agar plates for selection.
3. Inoculate Lin's Cupric Sulfate Medium and monitor growth over the next week.
4. Inoculate two sets of Starch Agar with Bromophenol Blue, placing one in an anaerobic environment via GasPak. Yellow color indicates the presence of metabolites and a pH drop.
5. Follow the Zymo Research DNA extraction and purification kit.
6. Check for DNA purity and quality through Nanodrop.
7. Perform PCR with selected primer sets.
8. Gel Electrophoresis to confirm product.
9. Sequencing service for matched identity.

Results

The LCSM plates quickly revealed that D1, D2, and D3 showed characteristics of diastatic wild yeast. The SABB plates only added more suspicion. Upon confirmation with PCR and gel electrophoresis via the primer sets SD-5A and SD-6B, 3 individual 900-bp bands appeared. The suspected *STA1* gene presented itself visibly. The sample is in the process of being sequenced by ACGT DNA sequencing services.

Figure 2. Assorted LCSM and SABB Plates

Table 1. Medium Tests

Microorganism	LCSM	Aerobic SABB	Anaerobic SABB
D1	+	+	-
D2	+	+	-
D3	+	+	-
D4	-	+	Weak +
D5	-	+	Weak +
D6	-	+	Weak +
D7	-	+	Weak +
D8	-	+	Weak +
D9	-	+	Weak +

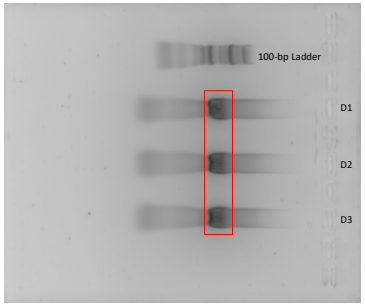

Figure 3. Suspected *STA1* Gel

Discussion

This course of testing can benefit both developing and situated breweries. The tests reveal multiple identities across the different stages of fermentation. Through preventative measures, it can even save the manufacture both money and time. However, there are other methods of testing that exist. A combination of genotypic and phenotypic assessments works best to detect a contaminant that is elusive as diastatic yeast. Such as how the *STA1* gene does not always guarantee the expression of glucoamylase. Observable characteristics such as flocculation, biofilms, spore stains, and sensory tests help to narrow down speculations. Additionally, there are specific primer sets that can be included in PCR for a more diverse array. The *ITS1/ITS4* set can be used as general fungi primers. The *pA/pH* primers identify a conserved sequence in many bacteria. The *FLO1* gene encodes a flocculation protein that is specific to *Saccharomyces cerevisiae*. There are also alternative medias such as Farber Pham Diastaticus Medium (FPDM) and FastOrange Yeast Agar, both of which select for diastatic yeasts. With these methods of quality control, the risk of spoilage and contamination of other brews are severely reduced. Breweries should strive to be as passionate as Sawstone about the quality of their product.

Acknowledgments

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