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Open-Source PCR and Agar-Based Methods for Cost-Effective Detection of Diastatic Yeast

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ABSTRACT

Diastatic variants of *Saccharomyces cerevisiae* are useful yet problematic in the brewing industry. A cross-contamination event by a diastatic strain in a beer can cause considerable problems for the brewery, especially if the contamination goes undetected until the final packaged product. Stringent quality control procedures are required to detect the presence of unintentional diastatic yeast in beer but are often still insufficient for rapidly detecting low-level contaminations. This paper discusses two cost-effective approaches breweries can use to screen for diastatic yeasts. First, a method of DNA isolation using a commercially available extraction kit is presented that enables polymerase chain reaction testing directly from a highly contaminated beer sample without enrichment, affording same-day results. The second approach is a selective medium that yields fewer false positives than existing approaches and was designed by coupling two well-understood features of diastatic yeast: copper resistance and starch consumption. These methods are simple and cost-effective, making the test more accessible to breweries of all sizes.

Keywords: diastatic, quality control, *STA1*, yeast, fermentation, PCR

Introduction

Cross-contamination by diastatic strains of *Saccharomyces cerevisiae* yeast represents one of the most prevalent quality control issues in the brewing industry with regard to the packaged product. This variant of *S. cerevisiae* harbors the *STA1* gene, giving it the ability to produce extracellular glucoamylase, resulting in the breakdown and consumption of complex sugars such as residual starches and dextrins that typically remain unmetabolized by non-diastatic strains (7,13). Contamination of beer by diastatic strains can lead to hyper-attenuation and, in turn, increased carbon dioxide and ethanol production, resulting in an off-spec product, gushing, or possibly exploding containers, creating a liability for the brewer.

While the mechanism and repercussions of a diastatic contamination are well understood, implementing stringent quality control procedures is difficult since there are few cost-effective, rapid, and reliable detection methods. The current industry gold standard is polymerase chain reaction (PCR), which involves amplifying and visualizing the *STA1* gene to denote a positive (*STA1*+) diastatic yeast strain (14). The value of this method has been proven by its expansive integration into fermentation labs and breweries across the industry; however, it is important to recognize the inherent limitations of this method. PCR is extremely sensitive and therefore is susceptible to extrinsic factors and user errors that may result in false positives or false negatives. The method is also accompanied by a high startup cost and the inability to differentiate between viable and non-viable cells. PCR is performed on a template DNA strand that is isolated from the sample in question, which poses challenges, especially with low-level contaminations. Most affordable and easy to use PCR equipment that is easily accessible to brewers is accompanied by a lower sensitivity and typically requires a pre-enrichment step to increase the concentration of diastatic yeast cells in a beer sample to a detectable limit. Growth in the enrichment broth can take several days, greatly increasing the readout time of the results. Ultrasensitive PCR methods, such as real-time PCR (qPCR), on the other hand, do not require an enrichment step but come with a start-up cost upward of US$10,000–$50,000 (CAN$12,000–$60,000) and may require a trained technician. The ability to isolate DNA and perform PCR directly from a beer sample in a rapid manner would be considerably beneficial to the brewing industry, but a simple, reliable, cost-effective, and open-source protocol has been lacking.

An alternative strategy of detecting a diastatic contamination involves culturing a sample on selective media, since not all breweries are equipped to perform PCR. Diastatic yeast has inherent traits, such as copper resistance, that can be harnessed for detection strategies. Copper-containing growth medium, such as commercially available Lin's cupric sulfate medium (LCSM), is commonly used for this purpose (1,5). While this approach is much more cost effective and user friendly than PCR, it has long turnaround times for results due to growth periods that can take 3–7 days. Additionally, copper-containing medium was formulated to select for non-*Saccharomyces* wild yeast, not *STA1*+ yeast specifically (6). Since there is no genetic correlation between *STA1*+ yeast and copper resistance, *STA1*-wild yeast will also grow on the medium—leading to false positive results.

Selective agar medium offers a wide variety of benefits, mostly due to the low cost and simplicity, but still has room for improvements. In 2020, Burns and colleagues at Omega Yeast Labs carried out a comprehensive study comparing commercially available selective media and further improving the media to enhance the sensitivity (1). By optimizing the cupric sulfate and dipotassium ortho-phosphate concentrations in LCSM to 0.06 and 0.1%, respectively, they were able to show an increase in recovery of diastatic strains compared with unmodi-

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fied LCSM and Farber Pham diastaticus medium. With the increase in sensitivity, an increase in background signal (false positives) from non-diastatic strains was also observed.

Another overall challenge associated with diagnosing a diastatic contamination is the influence of diastatic strength. A relationship between the *STA1* promoter region and the diastatic ability has previously been described, illuminating the complexity of this variant (4). Briefly, a deletion in the promoter region reduces the expression of the *STA1* gene, resulting in a diastatic strain with a poor ability to break down and consume dextrin. The diastatic strength is of great concern to the brewer, since an infection by diastatic yeast with reduced ability to excrete glucoamylase will have much less severe consequences than a yeast with full diastatic capacity. Agar medium designed to select for copper-tolerant yeast will not be able to differentiate between a diastatic strain with and without the deletion, as it does not influence resistance to copper (1). Additionally, PCR designed for detecting the *STA1* gene will yield a positive result regardless of this deletion, negating the influence of the diastatic ability.

The aim of this paper is to present solutions for more reliable, cost-effective, and rapid detection strategies for diastatic contaminations. With varying resources available to different breweries, we decided to focus on two of the most common detection methods used throughout the industry: molecular methods and growth-based methods. First, we integrated a commercially available DNA isolation kit into a basic endpoint PCR protocol that uses equipment many breweries have already purchased, or which are available at low cost (PCR thermocycler and benchtop centrifuge). While the idea of using an isolation kit for DNA extraction combined with endpoint PCR is not novel, it is one that we have not seen thoroughly validated for use in beer in recent literature. We also adapted copper-based selective media by testing additional selective hurdles whereby yeast that consumes starch is preferentially grown. These changes offer a simple and inexpensive method of distinguishing between diastatic and non-diastatic yeast with fewer false positive results than traditional selective media.

Materials and Methods

Agar Media Formulations

For all media formulations, all reagents were mixed thoroughly in distilled water prior to autoclaving at 121°C for 15 min (liquid cycle), and agar plates were stored overnight before use unless otherwise stated. LCSM (Omega optimized) was produced as described in Burns et al. (1). YPSD 1.0 medium (yeast extract, peptone, starch, and dextrose) was prepared using yeast extract (10 g/L), peptone (20 g/L), glucose (0.5 g/L), potato starch (Alfa Aesar no. 213400, 20 g/L), and agar (20 g/L). YPSD 2.0 medium was prepared using yeast extract (10 g/L), peptone (20 g/L), maltose (0.5 g/L), potato starch (Alfa Aesar no. 213400, 20 g/L), and agar (20 g/L). CSSM (copper sulfate starch medium) was prepared as a modified version of the LCSM recipe from Burns et al. (1) using ammonium sulfate (0.5 g/L), potassium phosphate dibasic (0.5 g/L), copper sulfate (0.6 g/L), maltose (0.5 g/L), potato starch (Alfa Aesar no. 213400, 20 g/L), liquid malt extract (Briess Ultralight, 2 g/L), peptone (2 g/L), yeast extract (4 g/L), and agar (20 g/L).

Yeast Strains and Culture Conditions

A selection of *Saccharomyces* yeasts was obtained from an industrial culture collection (Escarpment Laboratories). The yeast strains included in this study are listed in Table 1. With the exception of Isar Lager, which is classified as *S. pastorianus*, all yeasts were classified as *S. cerevisiae*. Unless otherwise noted, agar plates were incubated at 25°C. Unless otherwise noted, liquid cultures were incubated at 22°C with shaking at 170 rpm.

DNA Isolation Using the Qiagen DNeasy UltraClean Microbial Kit

Isolation of DNA from the sample was performed following the instructions provided with the Qiagen DNeasy UltraClean Microbial Kit (Qiagen, Hilden, Germany) with one minor change. To ensure that a sufficient number of diastatic cells were captured, all steps that required centrifuging were performed at 11,500 rpm for 1 min, instead of 10,000 rpm for 30 s. The sample collected in the microcentrifuge tube was centrifuged and the supernatant decanted. The decanted samples were centrifuged again, and any remaining supernatant was removed using a pipette. The pellet was then resuspended in 300 µL of the provided PowerBead solution, vortexed to mix, and then transferred to a PowerBead tube. Solution SL (50 µL) was added to the tube, vortexed at maximum speed for 10 min, and then centrifuged. The supernatant was transferred to a provided 2 mL collection tube and was centrifuged again. The supernatant was once again transferred to a new 2 mL collection tube, and 100 µL of Solution IRS was added, vortexed, and incubated at 4°C for 5 min. The sample was then centrifuged, and the supernatant was transferred to a 2 mL collection tube and 900 µL of Solution SB was added and vortexed. The sample (700 µL) was loaded into a provided MB Spin Column, centrifuged, and the flow-through was discarded. This was repeated once more. Solution CB (300 µL) was added, centrifuged, and the flow through was discarded and centrifuged once more to ensure the removal of any residual Solution CB. Solution EB (50 µL) was carefully added to the center of the white filter membrane and centrifuged. The spin column was discarded, and the eluted solution was stored at –20°C when not in use.

Table 1. Development of modified agar CSSM with respect to *STA1* and *STA1*-UAS (upstream activation sequence) presence in a selection of brewing yeasts[a]

Yeast strain	*STA1*	UAS	LCSM (Omega)	YPSD 1.0 (anaerobic)	YPSD 2.0 (anaerobic)	CSSM (aerobic)	CSSM (anaerobic)
French Saison	+	+	+	+	+	+	+
Dry Belgian Ale	+	+	+	+	+	+	+
Cerberus	+	–	+	+	+	+	+
Trappist	–	–	+	+	+	+	–
Vermont Ale	–	–	–	–	–	–	–
Isar Lager	–	–	–	–	–	–	–
			Colonies observed after 3 days incubation	Very small colonies observed after 5 days incubation	Small colonies observed after 3 days incubation	Small colonies observed after 3 days incubation	Small colonies observed after 3 days incubation

[a] + indicates presence of yeast colony formation, and – indicates a lack of yeast colony formation.

PCR Protocol

The presence of the nuclear internal transcribed spacer (ITS) and *STA1* was tested with end-point PCR using previously published ITS primers, and SD-5A and SD 6-B, respectively (10, 14). The ITS sequence encompasses two slightly variable non-coding regions surrounding a highly conserved gene (2). Since this region is present in all *S. cerevisiae* strains, it was used as a control to ensure the PCR protocol was capable of amplifying DNA. The PCR reaction mixture was prepared with miniPCR EZ-PCR Master Mix (miniPCR bio, Cambridge, MA, U.S.A.) and 0.3 µM of the primers (Integrated DNA Technologies, Coralville, IA, U.S.A.). The PCR was carried out in a thermocycler (ThermoFisher Scientific, Waltham, MA, U.S.A.). The following program was used: initial denaturation step at 94°C for 15 min, followed by the amplification cycle repeated 30 times consisting of denaturation at 94°C for 1 min, annealing at 49°C for 2 min, and extension at 72°C for 2 min, and finally a final extension step of 72°C for 10 min. The samples were then held at 4°C until use. The PCR products were separated and visualized on a 1% agarose gel, run consistently at 48 V for 20 min.

Preparation and Evaluation of Proof-of-Concept Beer Samples

A single colony of Escarpment Labs French Saison strain (*STA1*+, UAS+) was inoculated into 500 µL of a filtered and pasteurized beer sample and diluted serially 10,000-fold using nuclease-free water to a final concentration of 1.6×10^3 CFU/mL. The Qiagen DNeasy Ultra Clean Microbial kit was used to extract DNA directly from the inoculated sample following the previously described DNA isolation using the Qiagen DNeasy UltraClean Microbial Kit procedure, without enrichment or sample preparation. PCR was subsequently performed following the previously described PCR protocol.

Preparation and Evaluation of Mixed-Culture Beer Samples

A filtered and pasteurized beer sample (500 µL) was inoculated with both diastatic (Escarpment Labs French Saison) and non-diastatic yeast (Escarpment Labs Anchorman) in a 1:5, 1:50, and 1:100 (diastatic to non-diastatic) ratio. The contaminated samples were then diluted five times using nuclease-free water. The Qiagen DNeasy Ultra Clean Microbial kit was used to extract DNA directly from the inoculated sample following the previously described DNA isolation using the Qiagen DNeasy UltraClean Microbial Kit procedure, without enrichment or sample preparation. PCR was subsequently performed following the previously described PCR protocol.

Preparation and Evaluation of Commercial Beer Samples

A 1 mL sample was aseptically taken from unfiltered, unpasteurized canned beer samples of different varieties with a known diastatic contamination. The presence of diastatic *S. cerevisiae* was first confirmed using selective medium plating and colony PCR (data not shown). DNA was isolated from the sample following the previously described DNA isolation using the Qiagen DNeasy UltraClean Microbial Kit procedure. The DNA was then amplified and visualized using the previously described PCR protocol.

Results and Discussion

Qiagen DNeasy UltraClean Microbial Kit Proof of Concept

A proof-of-concept experiment was first performed to determine if DNA could be isolated and amplified from beer containing diastatic yeast using the commercially available Qiagen DNeasy UltraClean Microbial kit and subsequent PCR. Figure 1 shows the resulting gel after DNA was isolated and amplified from a beer sample inoculated with diastatic yeast. It can

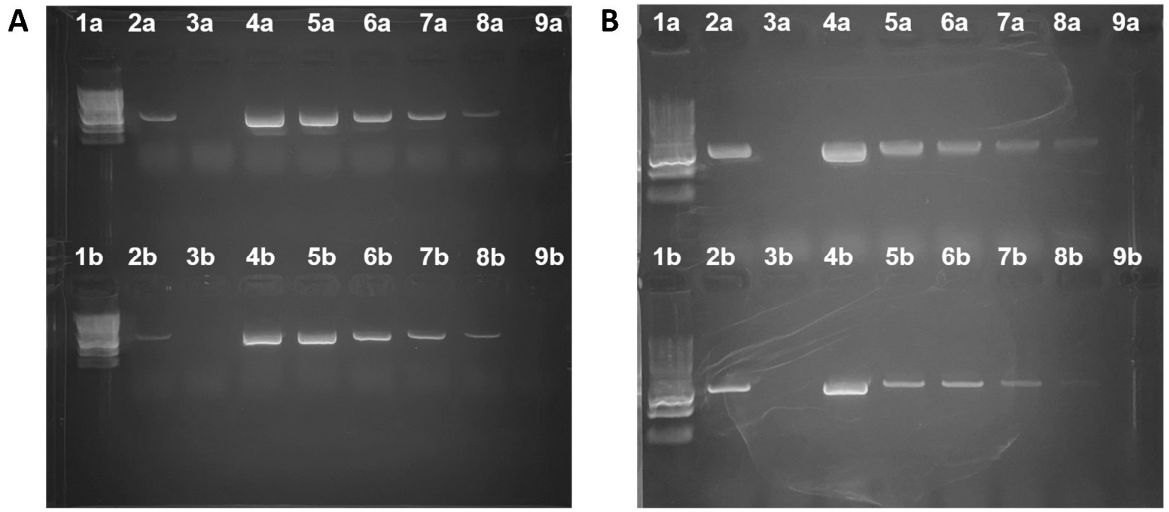

Figure 1. Direct PCR sensitivity of *STA1* up to 1.6×10^3 CFU/mL in filtered and pasteurized beer. Image of the gel electrophoresis of the sensitivity assay. French Saison inoculated into a pasteurized beer sample was serially diluted and DNA was extracted using the Qiagen DNeasy UltraClean Microbial Kit. In both images, lanes 2a–9a show the results of ITS region amplification and lanes 2b–9b show the results of *STA1* amplification. **A,** Lane 1 contained a 1 kb DNA ladder; lane 2 contained French Saison DNA extracted via colony PCR; lane 3 contained uninoculated pasteurized beer; lane 4 contained the undiluted sample (1.6×10^7 CFU/mL); lane 5 contained the 1/5 dilution; lane 6 contained the 1/25 dilution; lane 7 contained the 1/100 dilution; lane 8 contained the 1/125 dilution; and lane 9 contained nuclease-free water. **B,** Lane 1 contained a 1 kb DNA ladder; lane 2 contained French Saison DNA extracted via colony PCR; lane 3 contained uninoculated pasteurized beer; lane 4 contained the $1/10^2$ dilution; lane 5 contained the 1/125 dilution; lane 6 contained the 1/625 dilution; lane 7 contained the $1/10^3$ dilution; lane 8 contained the $1/10^4$ dilution (1.6×10^3 CFU/mL); and lane 9 contained nuclease-free water.

be seen that all dilutions of the original inoculated beer sample produced bands for both ITS (lanes 2a–9a) and *STA1* (lanes 2b–9b), confirming that the kit was able to successfully isolate DNA from yeast in beer in a concentration range of 1.6×10^7 to 1.6×10^3 CFU/mL. We recognize that a detection limit of 1.6×10^3 CFU/mL is too high to be considered a sensitive detection method for low-level contaminations. This experiment was not designed to replicate a low-level contamination that may occur in a brewery but rather to validate the ability of the kit to isolate DNA from diastatic yeast in a beer. Our intention of integrating a commercially available isolation kit was to provide a method of DNA isolation that is accessible to brewers in terms of cost and result turnaround time. As discussed previously, the startup cost of PCR combined with the many required reagents can deter breweries from using it in their quality control programs. The Qiagen DNeasy UltraClean Microbial Kit designed for microbial DNA isolation, which costs approximately US$4 (CAN$5) per sample, concentrates and purifies DNA, allowing for template DNA to be extracted from samples, and eliminates any PCR interferents that may be present in the sample medium. In addition to the low cost, minimal equipment—which includes a centrifuge that can reach 11,500 rpm, a refrigerator, a vortex, and a water bath—is required to carry out the isolation protocol using this kit.

Next, the PCR method was tested on a mixture of diastatic and non-diastatic yeast. When detecting a diastatic contamination in a practical setting, the sample in question will likely be a complex mixture with both diastatic and non-diastatic yeast present. To evaluate whether or not this method could be used in this capacity, a mixed culture in beer was tested. The mixed-culture or "contaminated" sample was also diluted and then tested in order to challenge the sensitivity. The results can be seen in Figure 2A. The results show that the ITS (lanes 2a–9a) region could be detected in all of the contaminated samples, whereas the *STA1* gene (lanes 2b–9b) could be detected in the undiluted contaminated samples and in the diluted 1:5 contamination ratio sample. Since the ITS region is present in both diastatic and non-diastatic yeast, the concentration of template DNA extracted from the contaminated samples should be higher than that of *STA1*, which will only be extracted from diastatic strains. For this reason, the amplification of ITS in all samples, but not *STA1*, was expected and demonstrates sensitivity limitations of the method.

Until this point, the samples from which DNA was successfully isolated have been model samples in light beer that has been filtered and pasteurized, effectively limiting the impact of adjuncts found in beer that could potentially interfere with PCR. In order to further evaluate the applicability of this method, commercial canned craft beer samples with a known diastatic contamination were tested. The results are shown in Figure 2B, where a clear band for both ITS (lanes 2a–9a) and the *STA1* gene (lanes 2b–9b) can be seen for all of the contaminated samples. Samples in which we confirmed the absence of a diastatic contamination were also included to demonstrate the ability to differentiate between contaminated and non-contaminated samples. This performance evaluation also acted as an exemplar for comparing classic colony PCR and our proposed method of direct isolation. Having first used colony PCR to validate the diastatic contamination in the beer samples, we were able to demonstrate the significant improvement in turnaround time attributed to the PCR method. Prior to direct isolation of DNA from beer, the sample was plated onto LCSM and incubated until colony formation could be detected. This was achieved after 3 days of incubation at 25°C. After the growth period, a colony

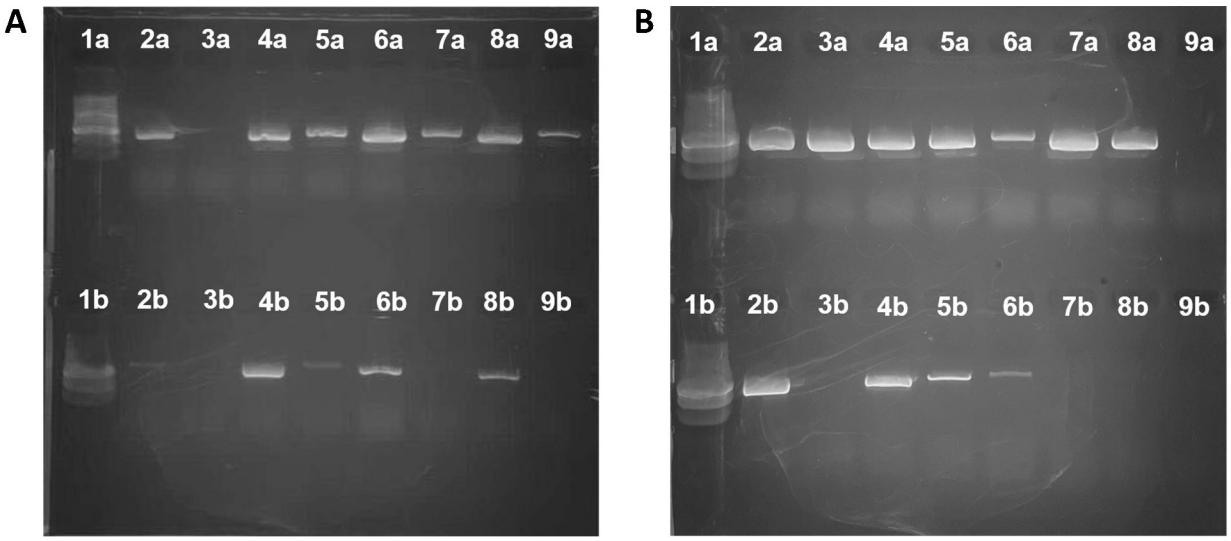

Figure 2. PCR sensitivity of *STA1* in contamination assays shows sensitivity in a ratio of 1:100 (diastatic to non-diastatic) and detection in a real-world sample. In both images lanes 2a–9a show the results of ITS region amplification and lanes 2b–9b show the results of *STA1* amplification. **A,** Image of the gel electrophoresis of the contamination assay. Both diastatic (French Saison) and non-diastatic (Anchorman) *S. cerevisiae* were inoculated into filtered and pasteurized beer in varying ratios. The contaminated samples were then diluted to a 1/5 dilution sample. Lane 1 contained a 1 kb DNA ladder; lane 2 contained French Saison DNA extracted via colony PCR; lane 3 contained uninoculated pasteurized beer; lane 4 contained the 1:5 ratio; lane 5 contained the 1/5 dilution of the 1:5 ratio; lane 6 contained the 1:50 ratio; lane 7 contained the 1/5 dilution of the 1:50 ratio; lane 8 contained the 1:100 ratio; and lane 9 contained the 1/5 dilution of the 1:100 ratio. **B,** Image of the gel electrophoresis of the real-world sample trial. DNA was extracted from canned beers with confirmed diastaticus contaminations. Lane 1 contained a 1 kb DNA ladder; lane 2 contained French Saison isolated from inoculated filtered and pasteurized pilsner; lane 3 contained Foggy London Ale isolated from inoculated filtered and pasteurized pilsner; lane 4 contained a contaminated bourbon-aged English ale; lane 5 contained a contaminated pilsner; lane 6 contained a contaminated India session ale; lane 7 and 8 contained uncontaminated India pale ales from different batches; and lane 9 contained uninoculated filtered and pasteurized pilsner.

was taken from the plate and template DNA isolated using an internally developed protocol, PCR was performed, and gel electrophoresis was used to visualize amplicons. In total, colony PCR took 4 days to yield results, while direct isolation produced results in 1 day.

Development of CSSM Agar

We aimed to develop a medium that builds upon the findings of Burns and colleagues, further optimizing diastatic selective media to minimize growth of non-diastatic strains (1). Most agar media used to detect diastatic yeast utilize either 2% glucose or 2% starch as a primary carbon source. Glucose results in rapid growth of yeast colonies but is not itself selective. Starch results in extremely slow growth of yeast colonies of up to 3 weeks, but it is selective. By limiting preferred sugar content of the media (glucose and/or maltose), we hypothesized that it would be possible to create micro-colonies of yeast and then select for diastatic yeasts on the basis of starch consumption. Since a yeast micro-colony contains orders of magnitude more yeast cells than a single cell on a plate, we hypothesized that growth on starch would occur faster due to radically higher secretion of Sta1 by the micro-colony.

To test novel agar formulations, we chose a selection of industrial brewing yeasts. Three strains contained the *STA1* gene, with two containing the upstream activation sequence conferring a strongly diastatic phenotype (Table 1). One non-diastatic yeast that shows growth on LCSM (Trappist Ale) was chosen because it is desirable to differentiate between this yeast and true *STA1*-positive yeasts. Additionally, a lager yeast (Isar Lager; *S. pastorianus*) was also included.

Several iterations of media formulations were tested (see Materials and Methods). First, a medium containing 2% starch and 0.05% glucose (YPSD 1.0) was tested by streak plating the yeasts and incubating the plates under aerobic and anaerobic conditions. Previously, it was shown that anaerobic incubation is necessary to avoid false positives on starch agar plates (8). On this medium, slow growth was observed in all strains under aerobic conditions and by the *STA1*+ strains as well as Trappist Ale under anaerobic conditions. All yeasts likely showed growth under aerobic conditions because yeast grown on glucose under aerobic conditions is capable of obtaining energy through the aerobic consumption of ethanol produced as a result of yeast growth, as part of the make-accumulate-consume strategy or Crabtree effect (11,12).

To further optimize growth rate and selection of the medium, the glucose was exchanged for maltose, resulting in a medium containing 2% starch and 0.05% maltose (YPSD 2.0). Maltose was chosen as the expression of the *STA1* gene is glucose repressed, and therefore growth on glucose followed by growth on starch may result in a delay as glucose de-repression must occur prior to expression of *STA1* (3,9). The agar containing limited maltose and abundant starch resulted in visible colony observation after 3 days of anaerobic incubation. However, the non-*STA1* Trappist Ale strain was still capable of growth on this medium.

Prior to the final iteration of the agar medium, other mechanisms to obtain useful information based on the YPSD 2.0 formulation were attempted. This includes testing an iodine overlay for starch consumption. However, there was not enough starch degradation in the medium to show any meaningful results. The bromophenol blue pH indicator was also tested, but color change was not observed after 7 days of incubation. Reducing the starch content of the medium, to reduce ethanol production as part of the Crabtree effect, was also tested but resulted in slower growth.

The final iteration of the medium involved modifying the Omega optimized LCSM to replace glucose with 2% starch and 0.05% maltose. It should be noted that LCSM also contains a small amount of malt extract, which will also contribute some malt sugars to the medium. LCSM contains copper sulfate as a selective agent, which is effective in preventing growth of copper-sensitive yeasts. Thus far, there has not been any instance of a copper-sensitive but *STA1*-positive yeast, so we consider LCSM to be a viable platform for further developing selection for Sta1 glucoamylase enzyme function. Supporting the use of copper as a selective agent, the publicly available long-read genome sequence of the *STA1*-positive strain revealed that this yeast contains a duplication of the *CUP1-1* locus conferring copper resistance, resulting in four copies on the diploid genome (4).

The modified LCSM, which we call CSSM, presented good selectivity and growth rate for *STA1*+ yeasts (Fig. 3). Small colonies were observed after 3 days of anaerobic incubation, with larger colonies observed after another 2 days (5 days in total). When incubated anaerobically, the Trappist Ale strain was restricted, indicating that this formulation is effective in selecting against non-diastatic copper-resistant yeasts. Interestingly, the agar formulations worked well for diastatic yeasts both with the wild-type UAS as well as the strains with a deletion in the UAS. As a result, this medium may be used as an effective component of a quality control program to screen for yeasts containing the *STA1* gene, which require secretion of Sta1 glucoamylase enzyme in order to form visible colonies under anaerobic conditions. Colonies may be further screened using colony PCR methods for presence of the UAS gene.

Comparison of the PCR and Plating Methods

The aim of this report was to offer affordable and open-source methods to diagnose diastatic yeast contaminations for the brewing community. We have done this by presenting two common techniques with improvements made to address and alleviate the main challenges that prevent breweries from utilizing these tools. It is important to recognize that a method suitable for one brewery may not be applicable to another. For this reason, the two methods we have proposed differ quite significantly in both sensitivity and turnaround time, to satisfy the most common concerns.

In terms of turnaround time, the PCR method outperforms the improved selective agar. The PCR method, performed directly from beer, yielded same-day results, compared with 3–5 days for CSSM. The limitation of the direct DNA isolation and PCR procedure, however, was the sensitivity. When evaluating the limit of detection (Fig. 1), we found that using the kit without any optimization we were able to detect both ITS and the *STA1* gene in an inoculated beer sample with a detection limit of 1.6×10^3 CFU/mL. In contrast, the detection limit of the novel CSSM was approximately 1.6×10^1 CFU/mL (Fig. 4).

We saw the effect of this sensitivity limitation when testing a beer sample where colony PCR combined with selective plating on LCSM produced a positive result, but the *STA1* gene could not be isolated and amplified directly from beer. It is important to highlight the fact that this was an initial investigation into whether or not a commercially available kit could be used to isolate DNA directly from beer without enrichment. The kit that was used was not designed specifically for this purpose and was used without optimization. Further optimizations such as increasing the sample size in the initial centrifugation step or adding a filtering step or a step to remove PCR inhibitors could potentially capture more diastatic cells and increase the likeli-

hood of successful DNA amplification. Further optimization studies should be performed as a follow-up to this report, as the sensitivity needs to be enhanced before it can be used to detect low-level contamination in a quality control program.

Conclusion

Herein, we presented two methods of detecting diastatic strains of *S. cerevisiae*. The first method proposed enhances an endpoint PCR method by incorporating a DNA isolation step directly from the beer sample into the full PCR protocol, using a commercially available kit. The Qiagen DNeasy UltraClean Microbial Kit that was tested demonstrated the ability to detect a high-level diastatic contamination in a variety of unfiltered, unpasteurized beer samples, within 1 day and utilizing simple, robust, and low-cost endpoint PCR equipment. Use of this kit was limited to beer samples with high level of contamination ($\geq 1.6 \times 10^3$ CFU/mL), but it allowed DNA isolation to be performed directly from a beer sample following a simple protocol with no pre-enrichment step, greatly decreasing the result readout time.

The second approach resulted in a selective medium called CSSM designed to enhance sensitivity and selectivity toward diastatic strains. The resulting medium utilizes both copper resistance and starch consumption to increase the reliability of detection of *STA1*-positive yeasts and to decrease growth of

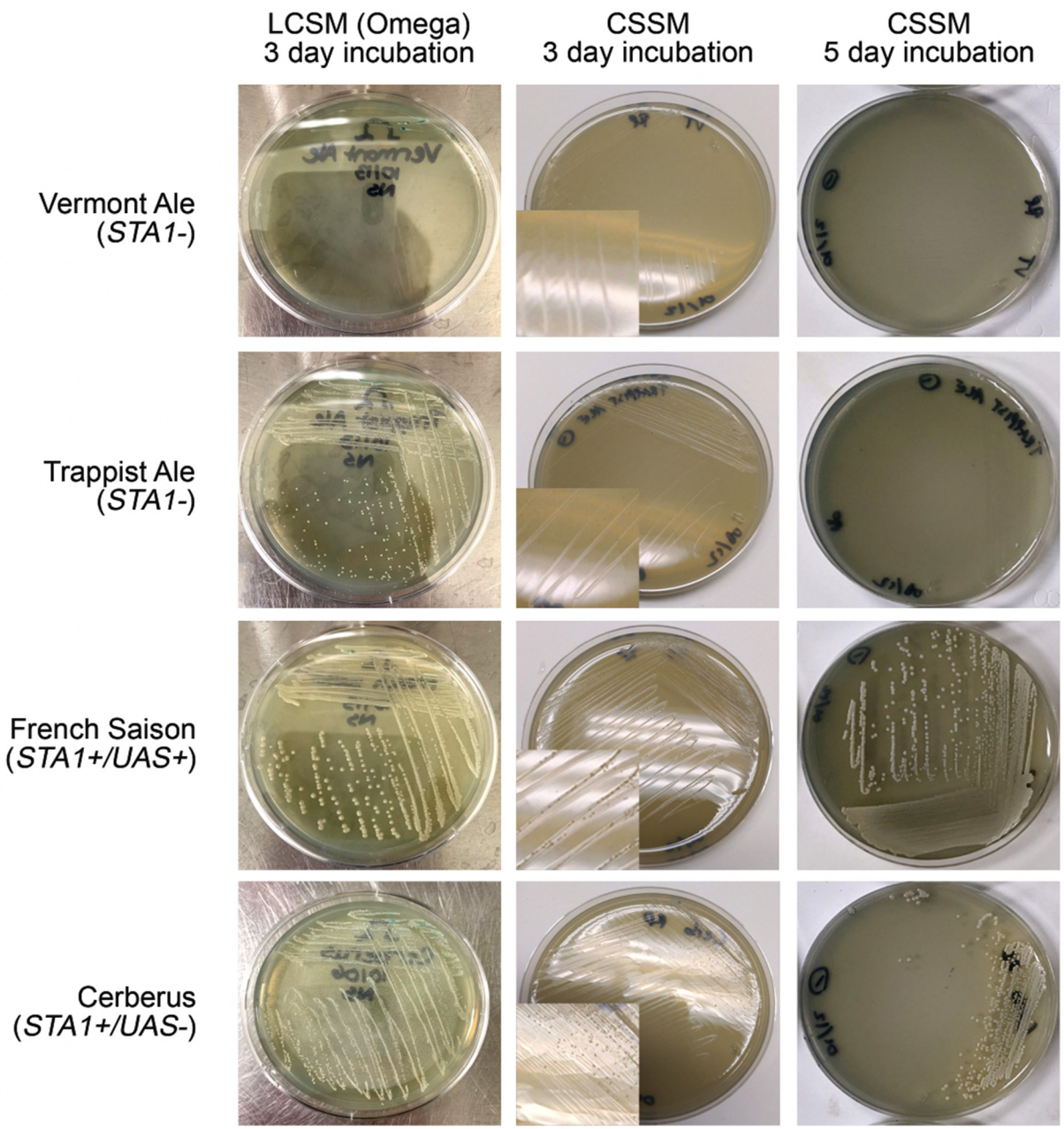

Figure 3. CSSM agar is selective for *STA1+* yeasts and restrictive to non-diastatic copper-tolerant yeasts due to modification in medium formulation from LCSM. For all agar plate images, yeasts were streak plated onto the agar 1 day after pouring the plates. The incubation time for each plate is specified in the figure. The center panels include an inset to better visualize the small colonies formed at this time point.

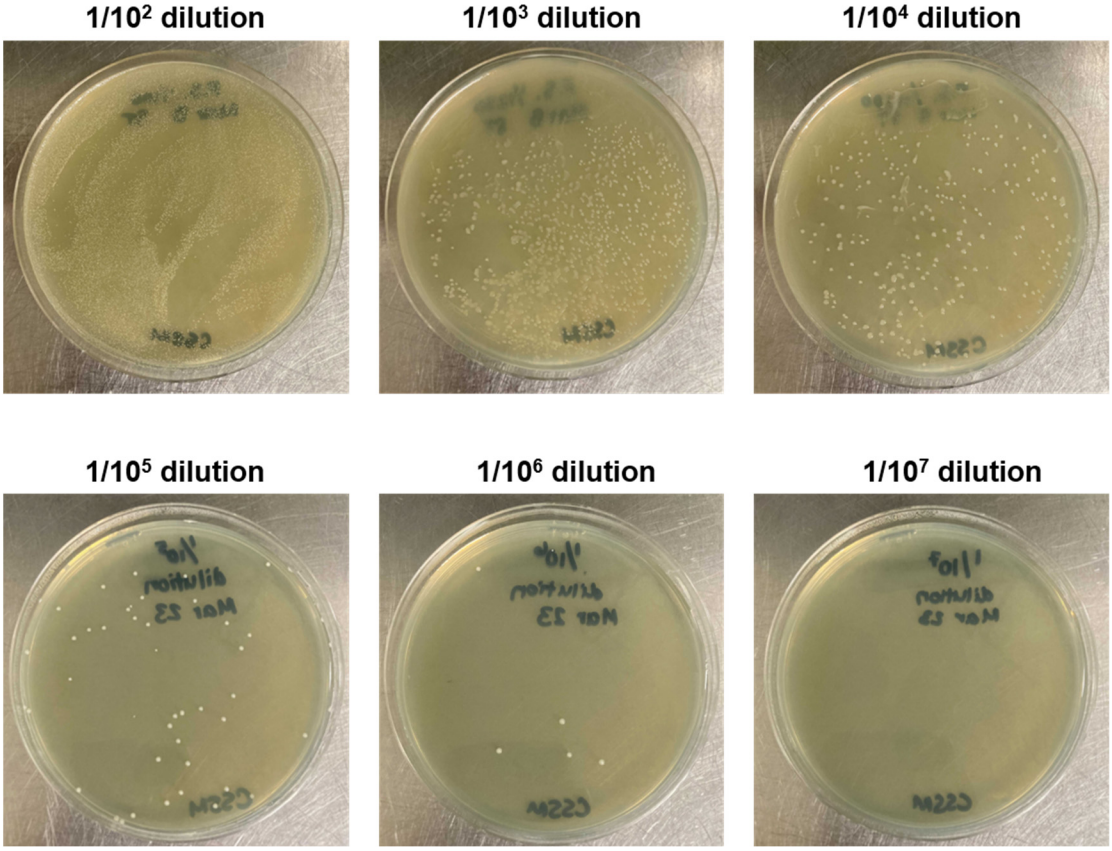

Figure 4. CSSM agar sensitivity of diastatic *S. cerevisiae* to a $1/10^6$ dilution in filtered and pasteurized beer. For all images, 250 µL of inoculated beer was spread plated onto the agar 1 day after pouring the plates. The plates were incubated anaerobically for 5 days at room temperature. The original, undiluted sample was a single colony of Escarpment Labs French Saison inoculated into 500 µL of filtered and pasteurized beer.

non-diastatic, copper-resistant strains. Taken together, our results indicate that a small brewery could begin testing for diastatic yeast using equipment costing less than US$1,500 (CAN$2,000), while avoiding high per-sample costs from proprietary, closed-source PCR and agar plate options.

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