Rapid Test System for the Detection of Beer-Spoilage Bacteria

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Abstract

Beer-spoiling microorganisms cause an increase of turbidity and unpleasant sensory changes in beer. Since the improved process technology in modern breweries has resulted in significant reduction of oxygen content in the final product, the role of strictly anaerobic bacteria like *Pectinatus* and *Megasphaera* has increased. Detection of these organisms, which is traditionally done by incubation on culture medium, takes a week or even longer. A rapid molecular test system is desirable for the detection of all known beer-spoiling microorganisms in one test only. Results of this study on a rapid test kit demonstrate that all beer-spoiling microorganisms can be detected and identified in a shorter time and in one test only.

Introduction

Beer continues to be a popular drink. It is important to maintain quality and enhance stability of beer during the production process through early detection of beer-spoiling microbes. The known beer-spoiling species and genera listed in Table 1 can all be detected by HybriScan®***D*** Beer, a rapid molecular test system for the detection of these microorganisms in one test.

Table 1: The Beer Spoiling Species and Genera that can be Detected by the HybriScan®***D*** – Beer Test

Genus *Lactobacillus*
Lactobacillus acidophilus
Lactobacillus brevis
Lactobacillus brevisimilis
Lactobacillus buchneri
Lactobacillus casei
Lactobacillus collinoides
Lactobacillus coryniformis
Lactobacillus curvatus
Lactobacillus fermentum
Lactobacillus fructivorans
Lactobacillus lindneri
Lactobacillus malefermentans
Lactobacillus paracasei
Lactobacillus parabuchneri
Lactobacillus paraplantarum
Lactobacillus plantarum
Lactobacillus rhamnosus

Genus *Pediococcus*
Pediococcus acidilactici
Pediococcus damnosus
Pediococcus inopinatus
Pediococcus parvulus
Pediococcus pentosaceus

Genus *Pectinatus*
Pectinatus cerevisiiphilus
Pectinatus frisingensis
Pectinatus haikare
Pectinatus portalensis
Pectinatus spp.

Genus *Megasphaera*
Megasphaera cerevisiae

HybriScan®***D*** Beer test is based on the detection of target molecules from the microorganisms of interest by means of specific capture and detection probes in a so-called sandwich hybridization. The hybridization reaction of the target molecules with the Biotin-labeled capture and a DIG-labeled detection probe takes place in a streptavidin coated microtiter plate (Figure 1).

After coupling of the target molecule to the microtiter plate, an enzyme is attached in a subsequent incubation step. After several washing steps, reaction with a color substrate gives blue coloration that changes into yellow color after the addition of a stop solution. The yellow color enables highly sensitive photometric measurement at 450 nm (Figure 2). Comparison is made with the standard solutions contained in the test kit.

Experimental

In this study, beer samples with alcohol content between 2.5% and 6.7% were tested with HybriScan®***D*** beer test kit. The beer samples were spiked with *L. brevis*, *L. coryniformis*, *L. lindneri*, *Ped. damnosus*, *M. cerevisiae,* and *Pec. frisingensis* in a range between 10^3 and 10^6 cfu/sample. The bacteria were cultivated in media according to DSMZ. The cultures were harvested and washed with sterile saline solution. The bacterial counts were then determined by measuring OD_{600} and by counting colony forming units (cfu) on agar.

Afterwards cultures were inoculated into the beer.

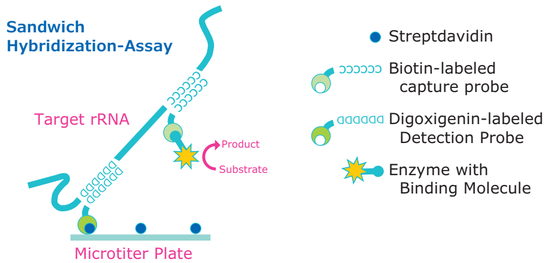

Figure 1: Sandwich Hybridization between Target rRNA Capture and Detection Probe

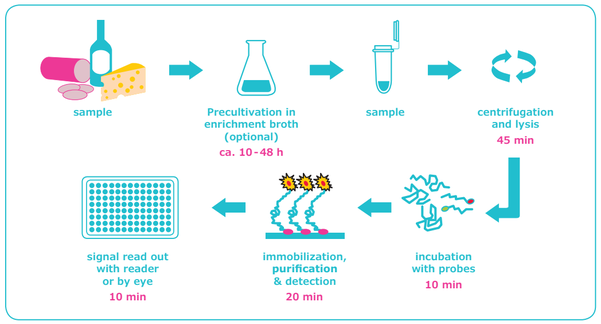

Figure 2: Test performance – HybriScan®***D*** Beer

Additional 30 brewery samples were examined with the HybriScan®***D*** beer test for beer-spoiling microorganisms. Up to 10 mL of the beer sample was processed according to the HybriScan®***D*** protocol published by Sigma-Aldrich®. The results were controlled by cultivation on NBB agar.

For the development of further genera and species, specific test solution experiments were performed with 11 additional pure bacterial cultures of most common beer-spoiling micro-organisms for detection by HybriScan®***D*** test experiments. These bacterial strains were also cultivated on media according to DSMZ and processed according to HybriScan®***D*** protocol.

Results

In this study, a total of 178 samples, with alcohol content between 2.5% and 6.7%, were tested with HybriScan®***D*** beer test. In order to confirm that it is possible to detect different beer-spoiling species with the test solution beer, the samples were spiked with *L. brevis*, *L. coryniformis*, *L. lindneri*, *Ped. damnosus*, *Pec. frisingensis* and *M. cerevisiae*. Results were compared with inoculated amount of bacteria. Results of 18 samples are presented in Figure 3. The bacterial counts in the beer samples which were determined by the HybriScan®***D*** test were in the same range as the inoculated values.

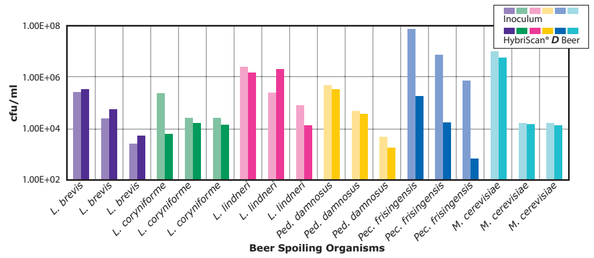

Figure 3: Bacterial Counts of 18 Spiked Beer Samples Determined by the HybriScan®***D*** Beer Compared to the Counts of Inoculated Amounts.

Pec. frisingensis was detected only in beer sample with 2.5% alcohol. Results of the HybriScan®***D*** test were below the counts of the inoculum. The number of microbes in beer is possibly diminished because the strain used in the study was not adapted to beer. *Pec. frisingensis* were not detected in beer with a higher alcohol content (data not shown). It was observed that beer with a low alcohol content is more prone to spoilage with *Pectinatus* and *Megasphaera* species (1). *Pectinatus* species are more resistant and can survive in acidic condition. The pH tolerance of these anaerobic bacteria is influenced by the presence of ethanol (2).

Real beer samples from the brewery were also tested with the HybriScan®***D*** beer test for beer-contaminating bacteria. These samples were also simultaneously checked with NBB agar. The HybriScan®***D*** test gave a positive signal for each sample. These results were confirmed on agar (Figure 4).

For a more sophisticated analysis, seven other test solutions "*Pectinatus spp./Megasphaera spp.*", "*Pediococcus spp.*", "*Lactobacillus brevis*", "*L. lindneri*", "*L. collinoides*", "*L. rossiae*" and "*L. backii*" were developed and optimized. Experiments were performed with 17 beer-spoiling bacteria strains. Eight different test solutions were tested with 10 beer-spoiling bacteria species. The experiment was performed with bacterial cultures which contained bacterial amounts ≥10^6 cfu/sample.

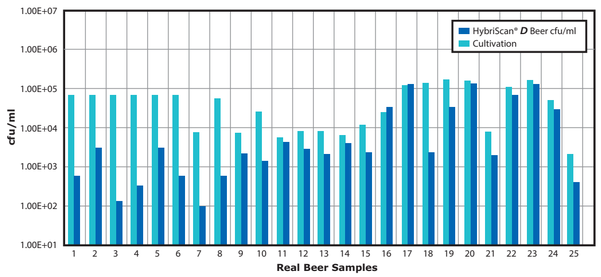

Figure 4: Counts of Real Beer Samples Determinated by HybriScan®***D*** Compared to the Counts of NBB Agar.

The test solution beer gave a positive signal with all used bacteria. The yellow signals at the microtiter plate confirm that the optimized test solutions react specifically and show no cross-reactivity (Figure 5). The experiments for the optimization of the test solutions indicated that the detection limit of those test solutions differs for *Lactobacillus* spp. and the other mentioned beer spoiling genera between 10^4 and 10^5 cfu/sample.

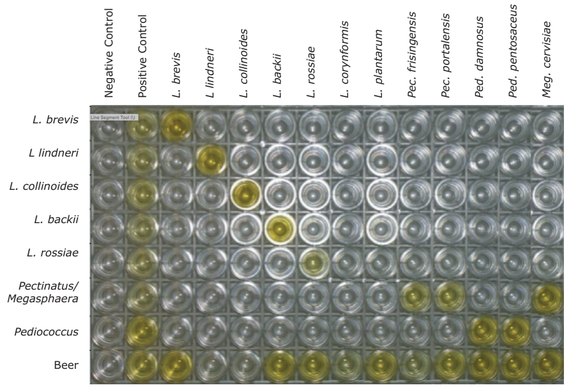

Figure 5: Result of a HybriScan®***D*** Test with 8 Different Test Solutions and 10 Different Beer Contaminating Bacteria after Addition of the Stop Solution following HybriScan®***D*** Test Protocol

Conclusions

- It is a rapid and sensitive molecular biological test system that is comparable to the classical cultivation method.
- It offers qualitative and quantitative detection of the most well-known beer-spoiling organisms in one test only.
- It is highly flexible as it allows combination with different read out technologies, and detection of rRNA and mRNA is possible.
- It is a robust test with easily predictable specificity of the probes, and easy synthesis of the oligonucleotide probes.
- It is a faster and cost-effective test with inexpensive read out device technology.

References

1. A. D. Paradh, W. J. Mitchell and A. E. Hill J. Inst. Brew. 117(4), 498 - 506 (2011).
2. Suzuki, K., 125th Anniversary review: Microbiological instability of beer caused by spoilage bacteria. J. Inst. Brew., 2011, 117, 131-155 (2011).