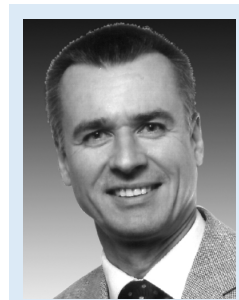




Screening of Beer-Spoilage Bacteria Using the LightCycler PCR Workflow System

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Introduction

The new LightCycler **foodproof** Beer Screening Kit (dedicated to the detection of beer-spoilage bacteria on the LightCycler Instrument) in combination with the MagNA Pure LC DNA III Kit (for the automated isolation of bacterial DNA on the MagNA Pure LC Instrument) offers breweries an innovative and automated solution for monitoring contamination of beer. This PCR Workflow System enables breweries to test their product consistency in less than two hours.

The LightCycler **foodproof** Beer Screening Kit includes all reagents necessary for the amplification and the detection of beer-spoilage bacteria in a single reaction. The kit detects 22 of the most relevant species that belong to the genera *Lactobacillus*, *Pediococcus*, *Pectinatus*, and *Megasphaera*. In addition, the LightCycler **foodproof** Beer Screening Kit allows the identification of the most important beer-spoilage bacteria via melting-curve analysis.

The Bitburger Brewery checked the suitability of the PCR Workflow System using different routine brewery samples. Additionally, Bitburger assessed the sensitivity and reliability of the melting-curve analysis in identifying beer-spoilage bacteria.

Materials and Methods

Sample preparation

Sample preparation (nucleic acid isolation) and post-elution steps (PCR setup) were carried out on the MagNA Pure LC Instrument using the modified MagNA Pure DNA Isolation Kit III (Proteinase K dissolved in 6 ml Elution Buffer). Advantages compared with a manual workflow are:

- ➔ Significantly less hands-on-time
- ➔ Reduced risk of cross-contamination
- ➔ Highest purification quality by “Magnetic Bead Technology”
- ➔ Improved reproducibility by precise pipetting steps

Sample names were directly imported with a bar-code reader and transferred via network from the MagNA Pure Instrument to the LightCycler Instrument. This minimized the risk of wrong entries and further reduced the hands-on time. Utilizing the MagNA Pure “Total NA Large Volume” protocol meant that the enriched samples could be directly used for nucleic acid purification without any additional centrifugation steps.

Table 1: Different dilutions of beer-spoilage bacteria enrichment cultures were used to assess the LightCycler **foodproof Beer Screening Kit**

| Tested species | Number of cells (colony forming units cfu/ml) | | | |
|--|---|-----------------|-----------------|-----------------|
| | 10 ⁶ | 10 ⁴ | 10 ² | 10 ¹ |
| <i>Lactobacillus brevis</i> | + | + | + | + |
| <i>Pectinatus frisingensis</i> | + | + | + | - |
| <i>Megasphaera cerevisiae</i> | + | + | + | + |
| <i>Pectinatus frisingensis</i> / <i>P. cerevisiophilus</i> | + | + | + | - |
| <i>Lactobacillus lindneri</i> | + | + | + | + |
| <i>Lactobacillus collinoides</i> | + | + | + | + |
| <i>Pediococcus</i> | - | (+) | - | - |
| <i>Lactobacillus parabuchneri</i> | + | + | + | - |
| <i>L. parabuchneri</i> / <i>Pediococcus</i> | + | + | + | - |

For sample preparation, 1 ml of enrichment culture was predispensed into the wells of the MagNA Pure Sample Cartridge. Following fully automated nucleic acid purification, DNA was eluted in 50 µl final volume. The complete PCR mixes were then set up automatically. The MagNA Pure LC Instrument compiled and loaded the PCR mixes directly into LightCycler Capillaries in the LightCycler Carousel.

PCR setup

For one reaction of 20 µl final volume, 13 µl LightCycler **foodproof** Beer Screening Mastermix, 1 µl LightCycler **foodproof** Beer Screening Enzyme Solution and 1 µl LightCycler **foodproof** Beer Screening Internal Control were automatically mixed and pipetted together with 5 µl sample DNA into each LightCycler Capillary.

Suitability testing of routine samples

58 routine samples including control, different kinds of beer, fermenter, rinsing water and other yeast-containing samples were tested. To assess the efficiency of the MagNA Pure LC System in removing PCR inhibitors, the purified DNA was analyzed with the LightCycler **foodproof** Beer Screening Kit.

Sensitivity and specificity testing of dilution series

To gain information about sensitivity and specificity of the LightCycler **foodproof** Beer Screening Kit, eight different beer-spoilage bacteria species and one mixture of two different species of Bitburger reference stock were tested:

- *L. brevis*
- *P. frisingensis*

- *Megasphaera*
- *P. cerevisiophilus*
- *L. lindneri*
- *L. collinoides*
- *Pediococcus*
- *L. parabuchneri*
- mix of *Pediococcus* and *L. parabuchneri*

The samples were tested in four dilution steps: 10⁶ colony forming units (cfu)/ml, 10⁴ cfu/ml, 10² cfu/ml, and 10¹ cfu/ml. The samples were prepared as duplicates.

Melting-curve analysis

20 different DNA samples of single and mixed beer-spoilage bacteria were analyzed. Sample preparation was not necessary in this case. DNA concentrations were different for each sample, ranging between 5 copies and 3,000 copies. The different beer-spoilage bacteria were analyzed by melting-curve analysis.

Results and Discussion

Suitability testing of routine samples

Ten of 58 samples showed positive results that were clearly identified as beer-spoilage bacteria via melting-curve analysis. The remaining 48 samples tested negative, and the internal control (IC) was amplified with a similar PCR efficiency. These results indicate that the MagNA Pure LC efficiently removed potential PCR inhibitors originating either from the beer routine sample or from the enrichment culture (data not shown). This clearly demonstrates the suitability and the reliability of the PCR Workflow System for analyzing beer routine samples.

Sensitivity and specificity

Table 1 shows that the LightCycler **foodproof** Beer Screening Kit detects all beer-spoilage bacteria species.

The kit allows the detection of beer-spoilage bacteria from as little as 10² cfu/ml. In some cases, such as *L. brevis* and *Megasphaera*, even the highest dilution with 10¹ cfu/ml can be detected. Except for *Pediococcus*, all tested spoilage bacteria were detectable in the 10² cfu/ml dilution and in higher concentrations. In the case of *Pediococcus*, a *Pediococcus* species was identified that is not a beer-spoilage organism.

Melting-curve analysis

Figure 1 gives an example for the most frequent beer-spoilage bacteria: *L. brevis*. For all four different cell concentrations the typical melting peak for *L. brevis* at 59.5°C is clearly visible and allows the easy identification of this very troublesome bacterium. These results show that the

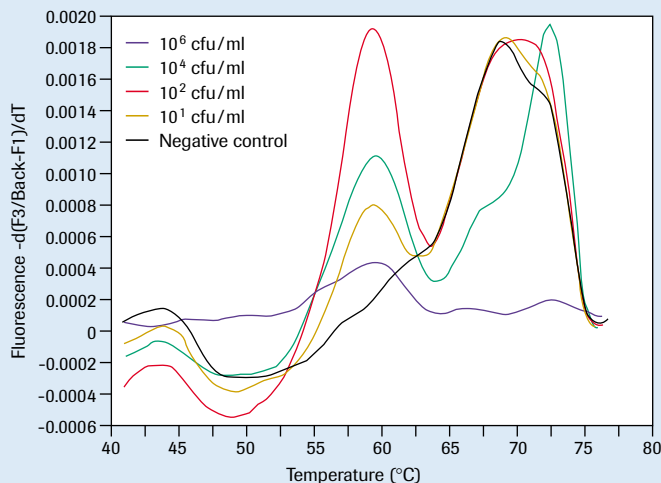


Figure 1: Typical *L. brevis* melting curve with a melting temperature at 59.5°C (+/-1°C) for all four dilutions (10⁶–10¹ cfu/ml) and the typical internal control peaks at 69°C and 72°C (+/-1°C).

Table 2: Identification of different spoilage bacteria DNA samples by melting-curve analysis

| Sample type | Identification by melting-curve analysis |
|---|--|
| <i>L. brevis</i> 5–10 GE | <i>L. brevis</i> |
| <i>Megasphaera</i> 3,000 GE | <i>Megasphaera</i> |
| <i>L. lindneri</i> 3,000 GE | <i>L. lindneri</i> |
| <i>P. spec.</i> 5–10 GE | <i>P. frisingensis</i> or <i>P. spec.</i> |
| H ₂ O | none |
| <i>L. casei</i> 3,000 GE | <i>L. parabuchneri</i> or <i>L. buchneri</i> or <i>L. casei</i> or <i>L. paracasei</i> |
| <i>L. parabuchneri</i> 5–10 GE | <i>L. parabuchneri</i> or <i>L. buchneri</i> or <i>L. casei</i> or <i>L. paracasei</i> |
| <i>P. spec.</i> 3,000 GE | <i>Pectinatus</i> , <i>P. cerevisiophilus</i> |
| <i>P. pentosaceus</i> | <i>P. pentosaceus</i> or <i>P. parvulus</i> or <i>P. acidilatici</i> |
| <i>P. claussenii</i> 5–10 GE | <i>L. plantarum</i> <i>L. coryniformis</i> |
| <i>L. lindneri</i> 5–10 GE | <i>L. lindneri</i> |
| <i>Megasphaera</i> + <i>P. spec.</i> 150 GE | <i>Pectinatus</i> + <i>Megasphaera</i> , <i>P. cerevisiophilus</i> |
| <i>P. damnosus</i> 3,000 GE | <i>P. damnosus</i> |
| <i>L. brevis</i> + <i>L. lindneri</i> 150 GE | <i>L. brevis</i> + <i>L. lindneri</i> , |
| <i>L. brevis</i> + <i>L. lindneri</i> + <i>P. damnosus</i> 100 GE | <i>L. brevis</i> + <i>L. lindneri</i> + <i>P. damnosus</i> |
| <i>L. lindneri</i> + <i>Megasphaera</i> 150 GE | <i>L. lindneri</i> + <i>Megasphaera</i> |
| <i>L. brevis</i> + <i>P. spec.</i> 15 GE | <i>L. brevis</i> + <i>P. frisingensis</i> , <i>P. cerevisiophilus</i> |
| <i>L. brevis</i> + <i>L. lindneri</i> + <i>P. damnosus</i> + <i>Megasphaera</i> 75 GE | <i>L. brevis</i> + <i>L. lindneri</i> + <i>Megasphaera</i> + <i>P. damnosus</i> |
| <i>P. inopinatus</i> 3,000 GE | <i>P. inopinatus</i> |

(GE = genome equivalent)

LightCycler **foodproof** Beer Screening Kit allows the detection of beer-spoilage bacteria with high sensitivity and specificity.

The results of the melting-curve analysis of 20 different DNA samples of the most important beer-spoilage bacteria are presented in Table 2. Out of the 20 samples tested, 19 were identified correctly. *Pediococcus claussenii* belongs to the group of *L. plantarum* and *L. coryniformis*; thus, this result also has to be regarded as correct. Mixed DNA samples containing up to four different species were also identified. The high conformity shows the wide specificity of the LightCycler **foodproof** Beer Screening Kit and confirms its suitability for the user.

Conclusion

The use of the PCR Work Flow System, in combination with the LightCycler **foodproof** Beer Screening Kit, provides a simple and quick system for testing beer-spoilage bacteria in less than two hours. The automated sample preparation with the MagNA Pure LC System saves time and provides DNA of a superior quality from any kind of brewery routine samples.

The LightCycler **foodproof** Beer Screening Kit enables the detection of 22 important beer-spoilage bacteria in a single PCR test with high sensitivity (down to 10¹ cfu/ml). Furthermore, the LightCycler **foodproof**

Beer Screening Kit allows the identification of the most important beer spoilage bacteria via melting-curve analysis.

The PCR Workflow System provides fast and sensitive results as well as detailed information about beer contaminants. This enables the quality assurance labs to better control the hygiene of the complete process from raw material to bottled products. ■

| Product | Pack Size | Cat. No. |
|---|----------------|----------------|
| LightCycler foodproof Beer Screening Kit | 96 reactions | 03 610 888 001 |
| Short Prep foodproof III Kit | 100 isolations | 03 755 304 001 |
| High Pure foodproof II Kit | 100 isolations | 03 358 054 001 |
| MagNA Pure LC DNA Isolation Kit III | 96 reactions | 03 264 785 001 |

