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Abstract

Although beer has been recognized as a beverage with high microbiological stability, many microorganism species have been reported to spoil beer. To improve the quality control of beer products, it is important to rapidly detect the presence of microorganisms and determine their beer spoilage potential. The most commonly used method is to culture the samples under either aerobic or anaerobic conditions. However, this method generally takes a few days for detecting aerobic bacteria and up to a few weeks for detecting anaerobic bacteria. Another drawback of this method is that it cannot determine the beer spoilage ability of these microorganisms. Therefore, protein or nucleic acid based approach that can rapidly and reliably detect the microorganisms and determine their beer spoilage ability has long been desired in the brewing industry. Here we present a multiplexed capillary electrophoresis (MP-CE) method that can simultaneously identify six major genera of beer-spoilage bacteria and their potential to spoil beer by detecting five hop-resistant markers within 24 hours of sampling. This multiplexed approach also detects more than 10 wild yeast species that are commonly discovered during beer brewing. The capacity to detect all major beer-spoilage bacterial genera and wild yeast, along with the information to determine their beer-spoilage ability in a timely manner, will greatly help brewers to improve the quality control procedure.

Introduction

In the brewing industry, beer-spoilage microorganisms have been problematic for centuries. The two major types of beer-spoilage microorganisms are bacteria and wild yeast. Gram-positive bacteria such as *Lactobacillus brevis*, *Lactobacillus lindneri* and *Pediococcus damnosus*, and some Gram-negative bacteria such as *Pectinatus cerevisiiphilus*, *Pectinatus frisingensis* and *Megasphaera cerevisiae* are the most frequently reported bacteria associated with beer spoilage. Wild yeasts, either coming from environment or due to the mutation of brewery yeast after multi cycles of fermentation, are often very difficult to identify when their population is small. Bacteria and wild yeast can spoil beer by turbidity, acidity and the production of an unfavorable smell such as diacetyl or hydrogen sulfide. Hop compounds, mainly iso-alpha-acids in beer, have antibacterial activity against Gram-positive bacteria. However, the presence of hop-resistant genes in *Lactobacillus* and *Pediococcus* will enable them to grow in the beer even though the hop is added. Since many different microorganisms have been found as beer spoilers, identifying the genus or species of the spoilers and quantifying their population are necessary for the breweries to improve their quality control.

Materials and Methods

Bacteria and Wild Yeasts: All bacteria including *Lactobacillus*, *Pediococcus*, *Pectinatus*, *Megasphaera*, *Acetobacter* and *gluconobacter* were purchased from American Type Culture Collection (ATCC). Most of the wild yeast strains were isolated from spoiled beers except *Candida mesenterica*, which was purchased from ATCC.

Primer Design: Primers for bacterial genera and wild yeast groups were designed on the conserved yet unique region of 16s rRNA of a particular bacterial genus or 18s rRNA of wild yeast group based on multi alignment information. Primers for hop-resistant genes were designed based on sequence information from the genbank. Representative accession number and fragment size for each target was listed in Table 1.

DNA Isolation: DNA of the microorganisms used in this study was isolated using the Beckman Coulter Genfind V2 Kit.

Target Amplification: Specific fragments for the targets were amplified using the GenomeLab® GeXP Start Kit (PN A21019, Beckman Coulter, Inc). This kit incorporates a universal PCR priming strategy that ensures equal amplification of all DNAs in the sample to achieve quantitative results.

Capillary electrophoresis: Dye-labeled PCR fragments were separated by size using a GeXP capillary electrophoresis system. The 8-channel capillary array (Figure 1A) was coated with polyvinylpyrrolidone (PVP) to minimize electroosmotic flow that enhances run to run, capillary to capillary, and array to array reproducibility. An advanced linear polyacrylamide gel (LPA) was used as the separation medium (Figure 1B). The exceptional sieving properties of LPA allows short-fast reads, long-fast reads and fragment sizing to be performed from the same polymer formulation. Separation method: 6kV for 35 minutes at 50°C.

Data Analysis: The peak area of each fragment was quantified and compared to the internal control by the GeXP software.

† The PCR process is covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffman-LaRoche, Ltd.

Table 1: The multiple targets of the beer QC panel. The target names and their fragment sizes are shown in the table. All these targets can be detected simultaneously in a single reaction.

Target	Size (nt)
HorC homolog	135
Wild Yeast 1	142
Wild Yeast 3	147
ORF5	153
Megasphaera	161
HorB homolog	167
Pediococcus	176
Pectinatus	181
HitA homolog	185
Wild Yeast 2	188
Acetobacter & Gluconobacter	239
Lactobacillus & Pediococcus	244
HorA homolog	250
Internal Control	277

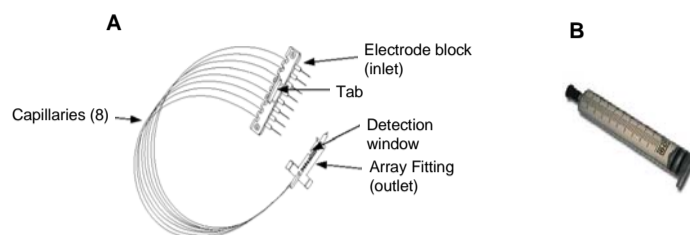
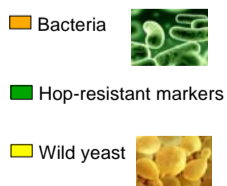


Figure 1: The 8-channel capillary array and replaceable gel cartridge. A: The 8-channel capillary array was PVP-coated to minimize electroosmotic flow that enhances run to run, capillary to capillary, and array to array reproducibility. B: A gel cartridge that contains an advanced polyacrylamide gel (LPA) which was used as separation medium. The exceptional sieving properties of LPA allows short-fast reads, long-fast reads and fragment sizing to be performed from the same polymer formulation.

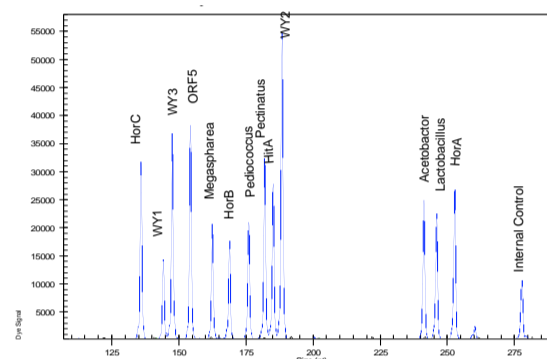


Figure 2: A representative electropherogram showing the combined identification of bacteria, wild yeast and hop-resistant genes. The multiplexed capillary electrophoresis (MP-CE) method is able to identify beer-spoilage microorganisms and determine their beer-spoilage potential from sample collection to data report within 24 hours.

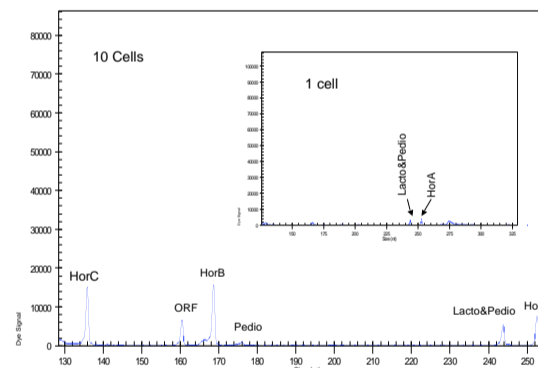


Figure 3: The detection sensitivity on *Pediococcus damnosus* (ATCC 29358). MP-CE can clearly detect the ribosomal RNA and hop-resistant genes at 10 cell level. At one cell level (from dilution series), weak signals from ribosomal RNA and HorA were detected.

Table 2: Comparison of detection results between GeXP and cultured method. As highlighted in red, GeXP was able to detect co-contamination and viable but nonculturable (VBNC) bacteria.

Sample ID	Source	Brewer's Culture Result	GeXP Detection Result		
			Bacterium	Hop-Res Markers	Wild Yeast
1	Brewery A	Lactobacillus	Lactobacillus	HorA, HitA	Neg
2	Brewery A	Lactobacillus	Lactobacillus, pectinatus	HorA, HorB, HorC, HitA, ORF	Neg
3	Brewery A	Neg	Pediococcus	HorA, HorB, HorC, HitA, ORF	Neg
4	Brewery A	Wild Yeast	Neg	Neg	Candida krusei like wild yeast
5	Brewery B	Neg	Neg	Neg	Neg
6	Brewery B	Neg	Acetobacter, Lactobacillus	HorB, HitA	Neg
7	Brewery B	Lactobacillus	Lactobacillus	HorC	Neg
8	Brewery C	Neg	Neg	Candida-like	Candida tropicalis
9	Brewery M	Bacterium	Lactobacillus	HorA, HorB, HorC, HitA, ORF	Neg
10	Brewery M	Bacterium	Lactobacillus	HorA, HorB, HitA, ORF	Neg
11	Brewery M	Bacterium	Pediococcus	HorA, HorB, HorC, HitA, ORF	Neg
12	Brewery M	Wild Yeast	Neg	Neg	Candida tropicalis
13	Brewery U	Lactobacillus	Lactobacillus	Neg	Neg
14	Brewery U	Pediococcus	Pediococcus	HorA, HorB, HorC, ORF	Neg
15	Brewery U	Lactobacillus	Lactobacillus	HitA	Wild Yeast 2 type

Conclusions

MP-CE, a PCR and capillary electrophoresis based approach, can

- detect the co-contamination of multiple microorganisms
- identify all five hop-resistant markers, HorA, HorB, HorC, HitA and ORF.
- sensitively detect beer spoilers to as low as less than 10 cells.