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What is Really in Your Beer?

Microbial Testing in a Brewery

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How Beer is Made

Beer is one of the most beloved tipples in the world, with many beer festivals being held across continents. The World Health Organisation (WHO)'s 2010 Global Alcohol Report shows Singaporeans displayed an overwhelming preference for beers to other alcoholic beverages, imbibing 70 per cent beer of total alcohol consumption.

Beer is primarily made from four basic ingredients, namely barley, water, hops and yeast. The science behind brewing is to extract the sugars from grains via a multi-step process, including malting, mashing, boiling and fermentation. Once alcoholic beer has been created, it is still flat and uncarbonated. The beer is then bottled and either artificially carbonated like soda, or naturally carbonated with the CO2 produced by the yeast.

Beer Spoilage Caused by Microorganisms

Although beer has been recognised as a beverage with high microbiological stability, many microorganism species have been reported to spoil beer. The bitter compounds in beer derived from the hop extracts are a primary defence against microorganisms. However, several species of bacteria and yeast are tolerant to these compounds. If contaminated beer is delivered to commercial markets, even small numbers of hop-tolerant bacteria will continue to grow and eventually infuse a strange taste and flavour to the beer.

Gram-positive lactic acid 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 some of the most reported bacterial spoilers in beers. Wild yeasts, either coming from the environment or contributing to the contamination of brewing yeast after multiple cycles of fermentation, are often very difficult to identify when their population is small. To avoid serious situations, such as product recall, a sensitive and accurate method for detection of low level of microorganism contaminants is urgently needed.

Although conventional microbial culture is the most commonly used technique in identifying bacteria and yeast contaminants in breweries, dye terminator sequencing has also been used in these labs to identify both beer spoiling bacteria and yeast by characterising the genus and species of such bacteria or yeast from culture when morphology and biochemical analyses are inconclusive. Conventional microbial culture techniques have several limitations compared to molecular techniques when characterising unculturable or slow-growing bacteria. Specifically, conventional microbial techniques cannot detect hop-resistant genes that allow these microbes to grow in beer.

Molecular methods are also used to detect different types of microbes in tank cleaning, brewing process and final product quality check (QC).

Separation Technology for Bacteria and Yeast Detection

In recent years, a novel method has emerged utilising SCIEX capillary electrophoresis multiplex PCR (XP-PCR) that enables simultaneous identification of six major genera of beer spoilage bacteria and yeast, as well as their potential to spoil beer by detecting five hop-resistant markers within approximately 24 hours of sampling.

Capillary electrophoresis (CE) is an analytical technique that separates molecules based on their electrophoretic mobility within a matrix in the presence of an applied voltage. CE is as advantageous as it delivers fast results and provides high resolution separation, and its ability to couple to a large range of detection methods makes it diverse for a number of different applications.

In this article, we will explore how microbial testing is done by using CE technology and instruments in a single sample experiment, as well as the benefits it offers to lab scientists.

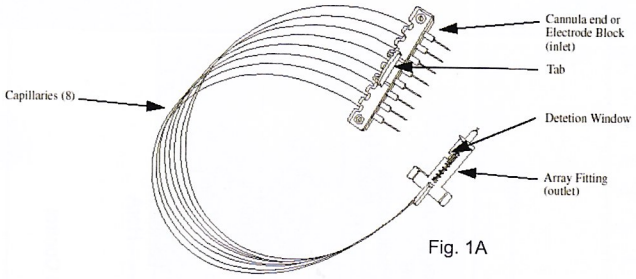

Fig. 1A

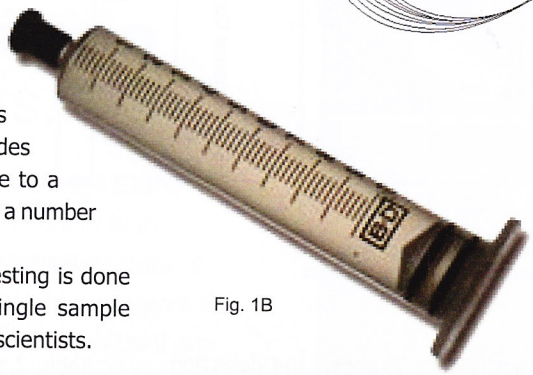

Fig. 1B

Materials and Methods

The objective of this experiment is to determine the specificity of the multiplex microbial detection test. The first step is to prepare several types of bacteria including *Lactobacillus, Pediococcus, Pectinatus, Megasphaera, Acetobacter* and *Gluconobacter*, all of them purchased from American Type Culture Collection (ATCC). Most of these wild yeast strains were isolated from spoiled beers, except *Candida mesenterica*. DNA of the microorganisms is then isolated.

Primers from bacterial genera and wild yeast groups are designed on the conserved 16s rRNA regions for bacterial genus, while 18s rRNA are chosen for the wild yeast group.

Specific fragments for the targets are amplified using the GenomeLab GeXP Start Kit, which incorporates a universal polymerase chain reaction (PCR) priming strategy (XP-PCR) that makes sure all amplification of DNAs in the sample are equal in order to achieve quantitative results.

The samples are then put through a separation CE system (GenomeLab GeXP™ Genetic Analysis System) to separate the dye-labelled PCR fragments. The eight-channel capillary array (see Figure 1A) and an advanced linear polyacrylamide gel (LPA) are 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. The separation method is calibrated at 6kV for 35 minutes at 50 degrees.

The GeXP software is used in conjunction with the system to quantify and normalise the peak area of each fragment. A table of multiple targets of the beer QC panel will be generated, together with their fragment sizes. All targets are 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

Bacteria
Hop-resistant markers
Wild yeast

Table 1: The multiple targets of the beer QC panel

A representative electropherogram (Figure 2) shows 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.

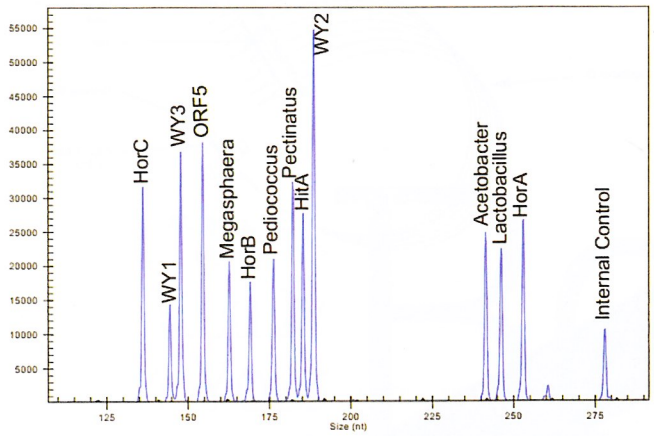

Figure 2. Sample electropherogram of multiple bacteria, wild yeast, and hop-resistant genes

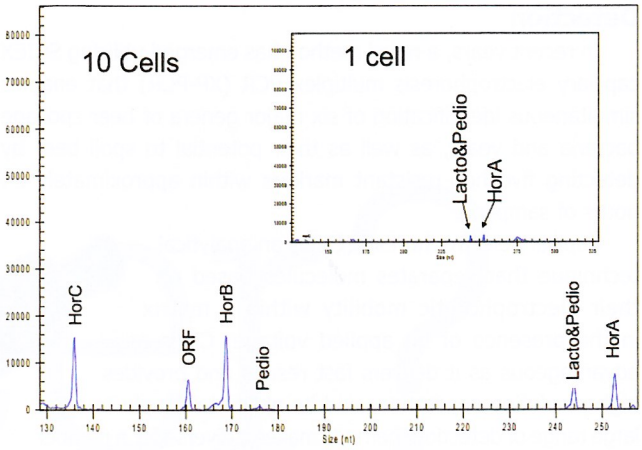

Figure 3. Detection of *Pediococcus damnosus* with high sensitivity

A second electropherogram (Figure 3) shows the detection sensitivity on *Pediococcus damnosus*. Multiplexed CE can clearly detect the ribosomal RNA and hop-resistant genes at 10 cell level. At one cell level, weak signals from ribosomal RNA and HorA were detected. This is to showcase the high sensitivity of CE technology.

Table 2 shows a comparison of detection results between the GeXP and cultured methods. Highlighted in red are the co-contamination and beer-spoilage bacteria from samples that either have bad flavour or turbidity yet could not be cultured by the conventional method.

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 tropicallis*
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 tropicallis*
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

Table 2. Comparison of GeXP and cultured method results

Conclusion

Apart from detecting the microorganisms playing tricks in the beer we drink, there are still many other food safety-related concerns in a brewery that need addressing. The beer brewing industry is moving toward miniaturisation, automation, and making things simpler for brewers and equipment operators. But beyond all those, what a consumer would really appreciate boils down to a fresh pint of icy cold beer, and that is the brewer's responsibility to produce. FBA