New Fast and Innovative Detection of Beer Spoilage Organisms

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HYBRISCAN® AN INNOVATIVE SCREENING METHOD FOR BEER SPOILAGE ORGANISMS BASED ON THE DETECTION OF rRNA

The popularity of beer remains high but the quality of beer has to be very high to survive in a competitive market. Beer spoilage organisms are either lactic acid bacteria belonging to the genera *Lactobacillus* and *Pediococcus* or they are obligate anaerobes of the species *Pectinatus* and *Megasphaera* (**Table 1**). Within the species of *lactobacilli* known to cause spoilage of beer, only certain strains can grow in the beer and are responsible for spoiling (exception: *Lactobacillus lindneri* all strains cause spoilage). *L. brevis* is the most common beer spoilage bacterium followed by *L. lindneri.*[1] Additionally many wild yeasts are responsible for beer spoilage such as *Saccharomyces cerevisiae* and *Candida pelliculosa.*[2] One of the biggest problems is biofilm formation in beer plants, which makes it very difficult to remove spoilage organisms.

Table 1.Species of beer-spoilage microorganisms that can be detected with the HybriScan®D Beer–Kit (Cat. No. 62533 , 96 assays)

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 parabuchneri (frigidus)
	Lactobacillus paracasei
	Lactobacillus paraplantarum
	Lactobacillus plantarum
	Lactobacillus rhamnosus
Genus *Pediococcus:*	*Pediococcus acidilactici*
	Pediococcus claussenii
	Pediococcus damnosus
	Pediococcus inopinatus
	Pediococcus parvulus
	Pediococcus pentosaceus
Genus *Pectinatus:*	*Pectinatus cerevisiiphilus*

	Pectinatus fringensis
Genus *Megasphaera:*	*Megasphaera cerevisiae*

Highly skilled lab staff perform microbiological analysis in specific quality control laboratories. Most of the laboratories still use conventional standard based cultivation methods, which are very time consuming and take 3 to 5 days for beer to be released to the market.

HybriScan® Beer kit, a rapid test system developed for a faster and more reliable product release of beer and could act as an alternative for the detection of beer spoilage contaminants. After as little as two hours (pre-enrichment for 24 h, if necessary) the brewery could have the first reliable results.

A variety of applications have been developed for HybriScan® including the detection of bacteria and yeast in non-alcoholic beverages. The robustness of the HybriScan® assay enables it, in contrast to other rapid test systems, to detect bacterial contamination in brewer's yeast and leads to efficient use of this valuable resource. Furthermore HybriScan® test system is a perfect tool for microbiological control of dispensing equipment. The legal standard for sterility control of dispensing equipment is 100,000 cfu/mL; a fast, direct determination of beer spoiling bacteria is possible without pre-enrichment-procedure delivering results within two hours.

Comparison of HybriScan® and other rapid test systems

Performing quality control by using the standard cultivation based method takes a long time. In recent years many companies have developed rapid test systems to hasten this procedure. For quality control of beer and beverages three main technologies are available:

- HybriScan® (sandwich hybridization)
- PCR (Polymerase Chain Reaction)
- VIT (Vermicon Identification Technology)

A comparison of these different technologies is given in **Table 2**. Comparing HybriScan® to PCR or VIT –technology the benefits of this rapid test system are:

- Fast and cost efficient analysis
- Inexpensive read-out technology
- High sensitivity and specificity

Using two different probes for detection of microbial RNA, false-positive results are almost impossible. In **Figure 1** results of quantification of *Lactobacillus buchneri* within a starter culture (silage) of three different samples are presented. Comparison of the HybriScan® test with a cultivation based analytical method (MRS agar) displays the equivalent results within the limits of microbiological sample variability.

Table 2. Comparison of different technologies for detection of beer spoilage bacteria.

	Cultivation based method	PCR	VIT	HybriScan®
Method	cultivation based method with optical or microscopic read out	PCR/real-timePCR	fluorescence microscopy	sandwich hybridization and photometrical signal read out
Detection spectrum	detection and identification of all beer spoilage microorganisms	identification of all relevant Beer spoilage microorganisms possible	*Lactobacillus* sp. and *Pediococcus damnosus*, *Lactobacillus* sp. + *L. brevis*, *Pectinatus* + *Megasphaera cerevisiae*	identification of all relevant Beer spoilage microorganisms possible
Sample preparation	selective pre-enrichment	enrichment and lysis of bacteria, if necessary pre-enrichment	selective pre-enrichment	enrichment and lysis of bacteria, if necessary pre-enrichment
Time	3 to 7 days	3 hours to 2 days	2 days	3 hours to 2 days
Costs per test	ca. 1 €	12 €	15 €	3 €
Detection limit (cfu)	1	1-5 x 10^5	1 x 10^3	1-5 x 10^3
Devices	None	PCR cycler	fluorescence microscopy	microplate reader
Advantages	high sensitivity, relatively cheap	high sensitivity, quantitative analysis	simple detection technology set up, detects only living cells (RNA)	rapid and sensitive, qualitative and quantitative detection of living cells, cost efficient analysis
Disadvantages	time consuming, no detection of non-culturable microbes, labor expensive	expensive devices needed, no discrimination between live and dead cells, not officially accepted	time consuming, low sample throughput, expensive, not automatable, difficult data analysis, not officially accepted	no differentiation of serotypes or subspecies, limited probe design (rRNA target), not officially accepted

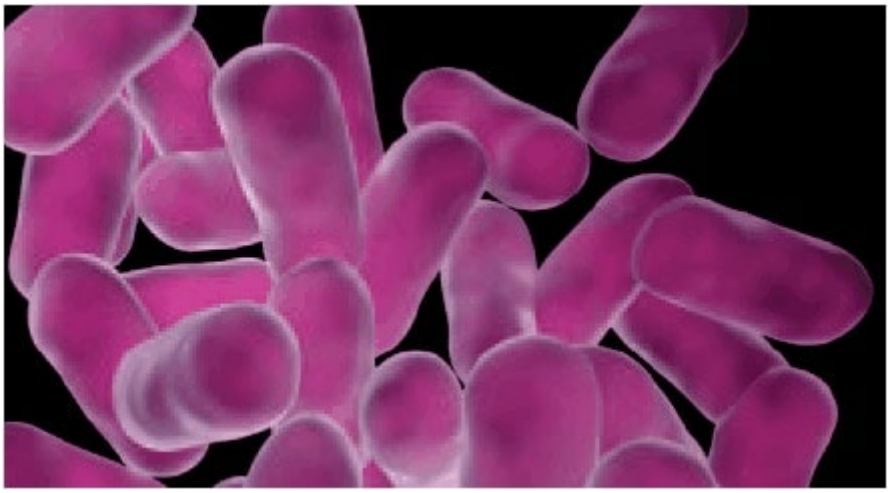

Figure 1. Lactobacilli (beer spoilage organisms)

References

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2. Timke M, Wang-Lieu NQ, Altendorf K, Lipski A. 2008. Identity, beer spoiling and biofilm forming potential of yeasts from beer bottling plant associated biofilms. *Antonie van Leeuwenhoek.* 93(1-2):151-161. **https://doi.org/10.1007/s10482-007-9189-8**