



Risk-based Identification of Spoilage Bacteria for Quality Professionals

Empowering proactive decision-making for your brewery



THE CHALLENGE

Increasing competition and complexity require a new approach

The brewing industry has grown exponentially over the past decade with new entrants crowding the market. Today more than ever, due to the variety of beer styles and brewing complexity, quality management has become a critical differentiator. The need for accurate, actionable information during production and post-packaging is essential to maintain quality and protect brand image.

Traditional quality testing methods, if implemented properly, can identify issues, but they fail to do so quickly and efficiently. Delayed information leads to reactive quality decisions made after the damage has been done. To address this paradigm, several rapid microbial detection technologies have recently emerged. These rapid methods can provide same or next day information regarding contamination events, empowering quality personnel to make proactive and preventive decisions. However, these technologies typically require cumbersome workflows that are costly and complicated to use. Also, test results can be ambiguous with regard to the spoilage risk of a particular micro-organism.



THE PRODUCT

Delivering functional genetic analysis for the most critical beer spoilers

Our passion for innovation, our successful development of the Veripro platform and the need for a rapid, multiplexed, high-throughput assay for risk-based identification of beer spoilers, led us to develop brewPRO™. The first of the brewPRO assays, brewPRO for *Lactobacillus* and *Pediococcus*, provides risk-based detection and characterisation while maintaining the ease of use and cost effectiveness required by progressive breweries. This information enables an assessment of spoilage risk based on the abundance of genes that confer resistance to inhibitory hops compounds.

The multiplex assay simultaneously detects validated Veripro gene targets in a single assay:

- Presence of *Lactobacillus* and *Pediococcus* species on the FAM channel
- Presence of hop resistance plasmids horA and horC that enable persistence and beer spoilage on the ROX channel
- Internal amplification control (IAC) to demonstrate successful amplification on the HEX channel

THE TECHNOLOGY

Synergising DNA Signature Capturing Technology™ and RT-PCR

Invisible Sentinel's continued focus on innovation led to the development of a new Real-Time (RT) PCR technology platform, Veripro®. This new platform incorporates reagents from our core system, Veriflow®, into an assay compatible with RT-PCR instruments for the ultimate user experience. Veripro maintains the same robustness, speed to results, and ease of use of the Veriflow platform, but leverages RT-PCR's ability to multiplex and takes advantage of the platform's capacity for high throughput analyses.

Veripro features a multiplexed configuration that simultaneously detects multiple genetic targets including an internal amplification control, in a single, pre-aliquotted tube using three optical channels: FAM, HEX, and ROX. Results can be monitored in real-time to detect the presence and relative abundance of contaminants. A simplified data analysis approach enables clear interpretation of results, including spoilage potential, to empower rapid and effective quality decisions.

brewPRO <i>Lactobacillus</i> and <i>Pediococcus</i> Performance Specifications	
Sensitivity (LOD)	≤ 10 cells/ml or ≤ 1 cell per 100ml with enrichment
Time to Results	< 3 hours or 18-24 hours with enrichment
Matrix Compatibility	Beer, colony PCR, environmental, fermentation, yeast slurry
Assay Configuration	Multiplex, qualitative and risk assessment
Target Selection	<i>Lactobacillus</i> and <i>Pediococcus</i> -specific genomic target (FAM) <i>Lactobacillus</i> and <i>Pediococcus</i> -specific hop resistance genes horA and horC (ROX) Internal Amplification Control (HEX)
Specificity	<i>Lactobacillus</i> species Including: <i>L. brevis</i> , <i>L. buchneri</i> , <i>L. rhamnusus</i> , <i>L. jensenii</i> , <i>L. backii</i> , <i>L. paracollinoides</i> , <i>L. lindneri</i> , <i>L. delbrueckii</i> , <i>L. fructivorans</i> , <i>L. plantarum</i> , <i>L. pentosus</i>
	<i>Pediococcus</i> species Including: <i>P. damnosus</i> , <i>P. inopinatus</i> , <i>P. parvulus</i> , <i>P. pentosaceus</i> , <i>P. acidilactici</i> , <i>P. claussenii</i>

THE ANALYSIS

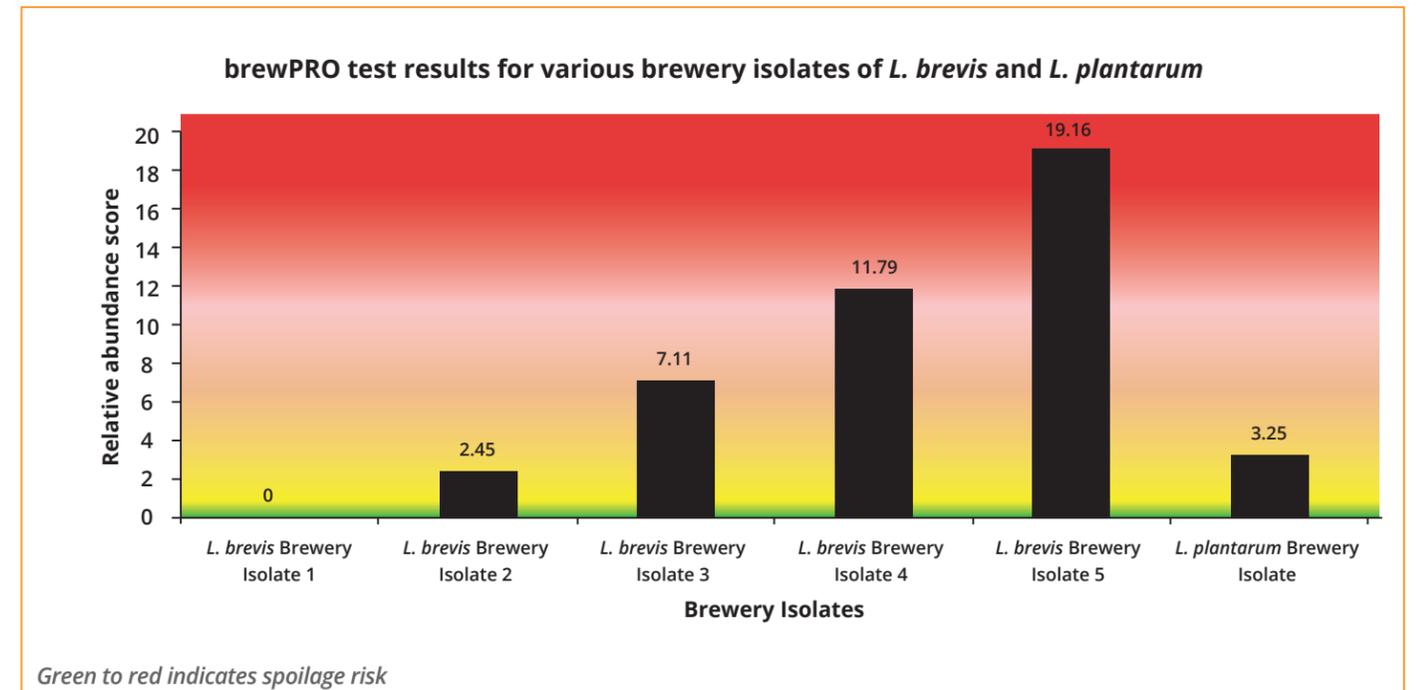
Scientific approach provides unique information to assess spoilage risk

The discovery and characterisation of hop resistance genes demonstrated that members of a single *Lactobacillus* or *Pediococcus* species vary widely in their ability to cause beer spoilage. Two hop resistant related genes known as horA and horC have been shown to enable lactic acid bacteria, such as *Lactobacillus* spp and *Pediococcus* spp, to grow in beer. The function of horA and horC is to encode transporters on the surface of cells that expel toxic hops compounds to enable survival in the presence of hops. These two genes were found to have nearly 100% correlation with species of beer spoiling lactic acid bacteria in several studies.

However, mere detection of hop resistance genes is insufficient to fully understand the risk and potential for spoilage these organisms present. brewPRO reports BOTH the presence of horA and/or horC AND the relative abundance of these gene targets. This information serves as an indicator of bacterial persistence in beer and spoilage potential. The relative abundance is calculated by comparing the cycle threshold value (Ct/Cp value) of hop resistance gene targets to the Ct/Cp value of ribosomal gene

targets in order to assess spoilage risk. The higher the relative abundance, the more likely the organism can thrive and spoil even high IBU & ABV beers.

The graph below highlights the value of this information by comparing the relative abundance of several brewery isolates of *Lactobacillus* species. *L. brevis* is the predominant beer spoiler among *Lactobacillus* species, and its detection can strike immediate fear into brewers. However, not all *L. brevis* isolates are created equally. Several *L. brevis* isolates were tested with brewPRO and test results demonstrate that each isolate poses a significantly different spoilage risk in beer. An isolate of *L. plantarum*, a subspecies not typically associated with beer spoilage, was also tested and results showed hop resistance capability and a relative abundance score that would make it a cause for concern in certain beer styles. With this comprehensive information on spoilage potential, brewers can make more accurate decisions, and remediation activities can be tailored – improving overall beer quality and operational efficiencies. This unique information empowers quality teams to make real-time decisions about their processes by providing data on the specific nature of the organism in their facility from pitch through packaging.

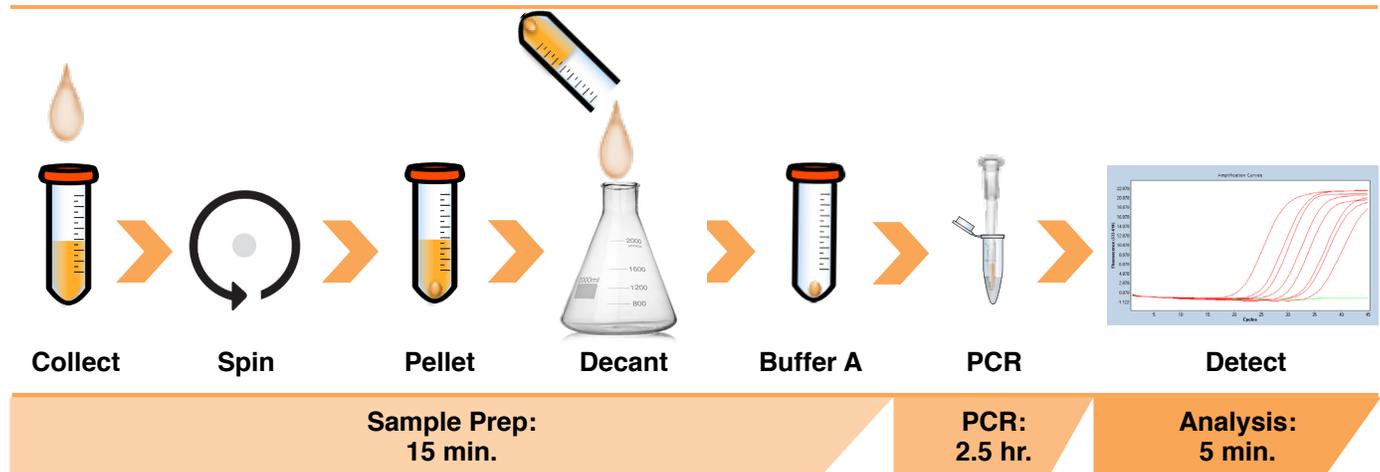


The higher the relative abundance score the higher the risk of spoilage.
Assessment of spoilage risk should also take into consideration beer style.

Finally, a system that provides the information you need to make the right decision at the right time, improving beer quality and production efficiencies – all of which protects brand integrity.

Unparalleled Analysis	Unrivalled Ease of Use
<ul style="list-style-type: none"> • Superior, risk-based identification of the presence of <i>Lactobacillus</i> and <i>Pediococcus</i> in samples ranging from environmental to raw materials to in-process beer through finished products • Proprietary analysis that quantifies the relative abundance of hop resistance genes • Results in under 3 hours for wide variety of sample types • Automated data collection and analysis 	<ul style="list-style-type: none"> • Simplified workflow with easy sample preparation - no DNA purification • Multiplexed format • High throughput with fast time to results • Easy to train personnel • Compatible with multiple instruments

brewPRO Same Day Workflow



Adaptive workflow will accommodate colony pick, fermentation and yeast slurry as well as enrichment if desired.

ITEM #	DESCRIPTION	SIZE
IS0701	brewPRO for <i>Lactobacillus</i> and <i>Pediococcus</i>	1 Kit, 24 tests

brewPRO has been validated for use on the following instruments: Roche Lightcycler 480 Instrument II and Applied Biosystems QuantStudio 3 & QuantStudio 5.

For more information or to order, please contact us on **(02) 9882 3666** or at orders@amsl.com.au

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