PCR DETECTION OF BEER SPOILING BACTERIA

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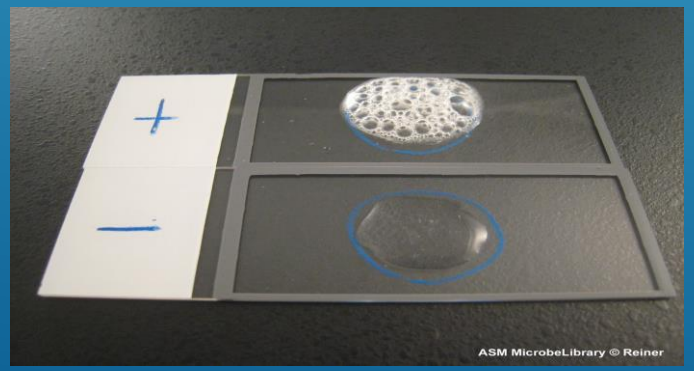
In partnership with Invisible Sentinel Inc.

HOW DO YOU TEST FOR BEER SPOILERS?

- Aseptic plating
- Gram staining
- Microscopy
- Specialty medias
- PCR



WHAT IS PCR?

First step the two strands of the DNA double helix are physically separated at a high temperature in a process called DNA melting.

Second step the temperature is lowered and the two DNA strands become templates for DNA polymerase to selectively amplify the target DNA.

POLYMERASE CHAIN REACTION

Initializing- If polymerase need activation time this is a high heat activation.

Denaturing- It causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

Annealing- annealing of the primers to the single-stranded DNA template. The polymerase binds to the primer-template hybrid and begins DNA formation.

Elongation-DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs *(Deoxynucleoside triphosphates)* that are complementary to the template

Final hold -This step at 4–15 °C for an indefinite time may be employed for short-term storage of the reaction.

INVISIBLE SENTINEL PCR

- **Sample to result in under 3 hours**
- **Accurate, sensitive, and specific to beer spoilers**
- **Simple and cost efficient deployment**
- **Quantitative and actionable results**
- **Reliable detection at any stage of brewing**
- **Robust assay compatible with all styles of beer**

Veriflow DNA Signature Capturing Technology	
DNA Amplification	Proprietary reagents eliminate need for sample purification
DNA Identification	Proprietary DNA signature detection specifically targeting beer spoilers
Visualization of Results	Proprietary vertical flow mediated visualization of results for easy interpretation
Sample Preparation	No enrichment or purification steps required

brewPAL Performance Specifications	
Sensitivity (LOD)	10 cells/ml
Time to Results	< 3 hours
Matrix Compatibility	Beer, yeast slurry, colony PCR, environmental
Assay Configuration	Qualitative and quantitative
Target Selection	*Pediococcus and Lactobacillus-specific hops resistance genes horA and horC*
Specificity	*Pediococcus Species: P. damnosus, P. inopinatus, P. parvulus, P. pentosaceus, P. acidilactici, P. claussenii*
	Lactobacillus Species: L. buchneri, L. brevis, L. rhamnosus, L. jensenii, L. backii, L. paracollinoides, L. lindneri, L. delbrueckii, L. fructivorans

HOW DOES STYLE AFFECT GROWTH OF CERTAIN BEER SPOILERS?

Common Culprits

IBU

ABV

Gravity

Gene resistance

Malt Profile

HYPOTHESIS

If you inoculate different styles of beer with known spoilers found in the brewing process, dilute up to 100,000 dilution and run on PCR, than you will find certain spoilers can survive in brands that you may not have suspected they could based on hop resistance and malt profile.

STYLES INVOLVED

Key: High IBU > 60
High ABV > 7%
High Terminal Gravity > 3 Plato

METHODS

1. Discover beer spoiler from sample tested on media

2. Preliminary Identification and Isolation

3. Allocate 25 mL into 6 sterile conical tubes for each brand

4. Inoculate one tube of each brand with organism in question

5. Incubate for 5 days

6.Vortex and serial dilute spiked sample into 4 other vials leaving one untouched as a control

Results	CFU	Score
+	42	2
+	60	3
-		
+	86	3
+	34	2
+	10	1
-		

8. Interpret results

7. Run PCR methods developed by Invisible Sentinel Inc

PILOT RESULTS ORGANISM 1

Brewpal	American Strong Ale	-			**CONTROL**
Brewpal	American Strong Ale	+	113	4	**1:1 DILUTION**
Brewpal	American Strong Ale	-			**1:10 DILUTION**
Brewpal	American Strong Ale	-			**1:100 DILUTION**
Brewpal	American Strong Ale	-			**1:10,000 DILUTION**
Brewpal	American Strong Ale	-			**1:100,000 DILUTION**
Brewpal	Milk Stout	-			**CONTROL**
Brewpal	Milk Stout	+	86	3	**1:1 DILUTION**
Brewpal	Milk Stout	+	5	1	**1:10 DILUTION**
Brewpal	Milk Stout	-			**1:100 DILUTION**
Brewpal	Milk Stout	-			**1:10,000 DILUTION**
Brewpal	Milk Stout	-			**1:100,000 DILUTION**
Brewpal	Session	-			**CONTROL**
Brewpal	Session	+	5	1	**1:1 DILUTION**
Brewpal	Session	-			**1:10 DILUTION**
Brewpal	Session	-			**1:100 DILUTION**
Brewpal	Session	-			**1:10,000 DILUTION**
Brewpal	Session	-			**1:100,000 DILUTION**

PILOT RESULTS ORGANISM 1

Brewpal	IPA	-			**CONTROL**
Brewpal	IPA	+	70	3	**1:1 DILUTION**
Brewpal	IPA	+	13	1	**1:10 DILUTION**
Brewpal	IPA	-			**1:100 DILUTION**
Brewpal	IPA	-			**1:10,000 DILUTION**
Brewpal	IPA	-			**1:100,000 DILUTION**
Brewpal	PLS	-			**CONTROL**
Brewpal	PLS	+	13	1	**1:1 DILUTION**
Brewpal	PLS	-			**1:10 DILUTION**
Brewpal	PLS	-			**1:100 DILUTION**
Brewpal	PLS	-			**1:10,000 DILUTION**
Brewpal	PLS	-			**1:100,000 DILUTION**
Brewpal	PALE	-			**CONTROL**
Brewpal	PALE	+	1000	5	**1:1 DILUTION**
Brewpal	PALE	+	1000	5	**1:10 DILUTION**
Brewpal	PALE	+	1000	5	**1:100 DILUTION**
Brewpal	PALE	+	22	2	**1:10,000 DILUTION**
Brewpal	PALE	-			**1:100,000 DILUTION**

PILOT RESULTS ORGANISM 2

Brewpal	IPA	-			**CONTROL**
Brewpal	IPA	+	33	2	**1:1 DILUTION**
Brewpal	IPA	+	51	3	**1:10 DILUTION**
Brewpal	IPA	-			**1:100 DILUTION**
Brewpal	IPA	-			**1:10,000 DILUTION**
Brewpal	IPA	-			**1:100,000 DILUTION**
Brewpal	American strong ale	-			**CONTROL**
Brewpal	American strong ale	+	34	2	**1:1 DILUTION**
Brewpal	American strong ale	+	16	1	**1:10 DILUTION**
Brewpal	American strong ale	-			**1:100 DILUTION**
Brewpal	American strong ale	-			**1:10,000 DILUTION**
Brewpal	American strong ale	-			**1:100,000 DILUTION**
Brewpal	PALE	-			**CONTROL**
Brewpal	PALE	+	92	3	**1:1 DILUTION**
Brewpal	PALE	+	43	3	**1:10 DILUTION**
Brewpal	PALE	-			**1:100 DILUTION**
Brewpal	PALE	+	7	1	**1:10,000 DILUTION**
Brewpal	PALE	-			**1:100,000 DILUTION**

PILOT RESULTS ORGANISM 2

Brewpal	Session	-			**CONTROL**
Brewpal	Session	+	174	4	**1:1 DILUTION**
Brewpal	Session	+	71	3	**1:10 DILUTION**
Brewpal	Session	+	6	1	**1:100 DILUTION**
Brewpal	Session	-			**1:10,000 DILUTION**
Brewpal	Session	-			**1:100,000 DILUTION**
Brewpal	Gluten red. IPA	-			**CONTROL**
Brewpal	Gluten red. IPA	+	57	3	**1:1 DILUTION**
Brewpal	Gluten red. IPA	+	35	2	**1:10 DILUTION**
Brewpal	Gluten red. IPA	-			**1:100 DILUTION**
Brewpal	Gluten red. IPA	-			**1:10,000 DILUTION**
Brewpal	Gluten red. IPA	-			**1:100,000 DILUTION**
Brewpal	Milk Stout	-			**CONTROL**
Brewpal	Milk Stout	+	82	3	**1:1 DILUTION**
Brewpal	Milk Stout	+	34	2	**1:10 DILUTION**
Brewpal	Milk Stout	+	19	2	**1:100 DILUTION**
Brewpal	Milk Stout	-			**1:10,000 DILUTION**
Brewpal	Milk Stout	-			**1:100,000 DILUTION**

GENETIC RESULTS

Organism 1: large colonies. The large colonies have both horA and horC and come up as lacto/pedio

Organism 2: large and small colonies. The small colonies do not come up as lacto/pedio. The large colonies have horA/horC and are lacto/pedio

CONCLUSIONS

- Both organisms have a strong preference for growth in Pale Ale
- For Organism 1, although the Pale Ale and PLS have very similar ABV, IBU, and TG, growth is significantly restricted in PLS compared to the Pale Ale
- For Organism 2, the extent of growth in Milk Stout and Session are very similar, but the beers have very different ABV, IBU, and TG
- These preliminary findings indicate that additional factors other than ABV, IBU, and TG play a major role in bacterial growth and spoilage (could these differences and similarities be due to malt?)

WHY BREWPAL?

- The Veriflow brewPAL system provides a means to rapidly detect spoilage organisms with high sensitivity and is a valuable tool to measure and quantify the presence of bacteria in beer samples
- Using brewPAL as a diagnostic tool in this study will enable the identification of factors that influence growth and will provide better insight into spoilage risk for different styles of beer

MORE TO COME

This trial has finished the pilot stage and is now being researched more in depth between myself and the Invisible Sentinel lab.

Keep an eye out for more data in the near future.

QUESTIONS?