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Concentration of *Lactobacillus brevis* from Experimentally Infected American Lager Beer and Wine by Innovaprep's Concentrating Pipette and Be Flat Degassing Jar

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INTRODUCTION

Spoilage organisms and contamination present a major risk for the brewing industry. As such, microbiological testing for these organisms is necessary throughout the brewing process. However, most laboratories still use conventional cultivation methods, which are time-consuming – requiring 3 to 5 days for beer to be released to the market. Rapid microbiological analytical methods offer great potential for increasing the reliability of spoilage detection in beer while reducing labor costs and product hold times; however, small analysis volumes limit the usefulness of these methods. In this study, InnovaPrep's Concentrating Pipette was investigated as a bridge to concentrate 12 ounces (355 mL) of beer and bottle of wine (750 mL) into volumes more appropriate for rapid detection methods.

BACKGROUND

The InnovaPrep Concentrating Pipette is an automated and rapid bioconcentrator. The system uses special consumable pipette tips with an internal filter of either flat membrane filter or hollow fiber membrane filters in various pore sizes to capture microorganisms from large liquid volumes. Following filtration, rapid recovery of the microorganisms is performed by a process termed 'Wet Foam Elution™' in which carbonated elution fluid is released from a canister, to tangentially flush the trapped particles into a final volume of a few hundred microliters. Following elution, the foam breaks down immediately to allow for rapid analysis. The process eliminates the need to use time-intensive enrichment steps prior to molecular detection.

METHOD

A special degassing glassware container and a method were developed and patented by InnovaPrep for more efficient decarbonation. The glassware's interior is specially sandblasted to create a large surface area of nucleation points to help in the degassing process. Below is the method used for beer concentration

Be Flat™ Degassing Jar

- Room temperature beer was poured into a degassing jar from a height of 8-12 inches above the jar to achieve maximum foaming. For wine, an entire bottle was poured into a sterile jar and then concentrated as below.
- Beer was then incubated for 10 minutes at 4ºC
- Sample was concentrated using the Concentrating Pipette Select with a 0.45 µm hollow fiber Concentrating Pipette Tip and eluted with PBS with 0.075% Tween 20 elution fluid.
- The elutions were captured and weighed to determine elution volumes.

Concentrating Pipette Select

For concentration of infected beer and wine, the same protocol was used as above except:

- 1 mL of 100 CFU/mL *Lactobacillus brevis* (WLP 672, White Labs) was dispensed into beer prior to 4ºC, 10 minute incubation. No incubation was required for wine.
- Elution volumes were plated onto MRS agar plates and incubated at 30ºC for 48 hours, at which time colonies were enumerated.
- Quantitative PCR results were obtained by using primers designed to amplify a 168 bp region within the *L. brevis hsp60* gene.

RESULTS

CONCENTRATION OF *L. BREVIS* EXPERIMENTALLY INFECTED AMERICAN LAGER BEER

American Lager from Brewery 1									
	1	2	3	4	5	6	7	average	st. dev.
Concentrate									
Recovery Efficiency	68.23%	74.73%	68.23%	73.48%	81.57%	53.88%	71.18%	70.19%	7.89%
Elution volume (mL)	0.1646	0.1271	0.1526	0.3288	0.3102	0.1661	0.1509	0.2	0.0766
run time (mins)	5.06	5.14	5.44	4.52	4.76	6.00	5.56	5.21	0.46
Second elution									
Recovery Efficiency	17.33%	13.00%	23.83%	4.04%	2.02%	6.65%	15.30%	11.74%	7.28%
Total Recovery									
Recovery Efficiency	85.56%	87.73%	92.06%	77.53%	83.60%	60.53%	86.47%	81.92%	9.64%
Concentration factor	1471.57	2087.24	1587.29	793.39	933.54	1151.56	1674.43	1385.58	419.80

American Lager from Brewery 2					
	1	2	3	average	st. dev.
Concentrate					
Recovery Efficiency	67.81%	74.45%	72.97%	71.74%	2.84%
Elution volume (mL)	0.2408	0.2175	0.2187	0.2257	0.0637
run time (mins)	5.40	5.96	6.32	5.80	0.36
Second elution					
Recovery Efficiency	2.21%	0.74%	5.16%	2.70%	1.84%
Total Recovery					
Recovery Efficiency	70.02%	75.18%	78.13%	74.45%	3.35%
Concentration factor	999.74	1215.11	1184.52	1133.12	95.14

Samples were concentrated using the HOLLOW setting on the CP select, using a 0.45 µm hollow fiber tip and phosphate buffered saline with 0.075% Tween 20 as the elution fluid. After concentration, the samples were weighed and plated onto MRS agar plates, incubated at 30C for 48 hours, at which time colony forming units were counted.

DETECTION OF L. BREVIS BY QPCR IN BEER

	C_t value	
	concentrate	2nd elution
sample A	34.97	38.01
sample B	34.1	37.04
sample C	31.35	35.22
sample D	31.86	35.36

Four additional experimentally infected beer samples from Brewery 1 were concentrated as described previously, except eluted with 25 mM TRIS with 0.075% Tween 20. The samples where then bead beat for 1 minute and subjected to qPCR using *L. brevis hsp60* primers on a Life Technologies StepOne thermocycler. Cycle thresholds were determined by comparison of standard curve on serial dilutions of bead beat *L. brevis*.

CONCENTRATION OF *L. BREVIS* EXPERIMENTALLY INFECTED RED AND WHITE WINE

	Blended Red Wine					Chardonnay				
	1	2	3	average	st. dev.	1	2	3	average	st. dev.
Concentrate										
Recovery Efficiency	60.48%	69.52%	71.90%	67.30%	4.92%	66.19%	70.00%	48.10%	61.43%	9.56%
Elution volume (mL)	0.4673	0.4301	0.3889	0.4288	0.0320	0.2345	0.2252	0.2221	0.2273	0.0053
run time (mins)	5.48	5.54	5.49	5.5039	0.0275	7.00	7.03	8.15	7.3943	0.5349
Second elution										
Recovery Efficiency	2.38%	5.71%	1.43%	3.17%	1.84%	21.90%	22.38%	20.95%	21.75%	0.59%
Total Recovery										
Recovery Efficiency	62.86%	75.24%	73.33%	70.48%	5.44%	88.10%	92.38%	69.05%	83.17%	10.14%
Concentration factor	970.62	1212.34	1386.70	1189.89	170.60	2116.97	2331.26	1624.11	2024.11	296.07

For each sample, an entire 750 mL bottle of wine was poured into a sterile Be Flat jar and 1 mL of 100 CFU/mL of *L. brevis* was pipetted into the wine. Wine samples were concentrated using a 0.45 µm hollow fiber Concentrating Pipette tip and eluted using PBS with 0.075% Tween 20 elution fluid. Samples were wieghed and plated onto MRS agar plates, incubated and enumerated 48 hours later.

CONCLUSION

- Up to 80% of spoilage organisms in a 355 mL American lager beer sample can be concentrated into under 250 µL quickly using the Be Flat degassing jar and the CP select in tandem
- The beer concentrate can be used for detection using molecular methods, such as qPCR
- The CP select efficiently concentrates *L. brevis* spiked in 750 mL of both blended red wine and chardonnay, resulting in concentration factors of 1000-2000X

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