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Keerthy V. Chandrasekar
University of Southern Mississippi

R.D. Ellender
University of Southern Mississippi

Shiao Y. Wang
University of Southern Mississippi

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Keerthy V. Chandrasekar*, R. D. Ellender and Shiao Y. Wang, The University of Southern Mississippi

*keerthycv@gmail.com
601-266-5797

Abstract

The U.S. Environmental Protection Agency published guidelines in 1986 on recommended water quality criteria for bacteria to protect bathers from gastrointestinal illness in recreational waters. The criteria, based on *Escherichia coli* and enterococci counts as indicators of the presence of fecal pollution, are still in use today. With the availability of PCR-based methods for the detection and quantification of specific pathogens, it might be possible in the future to base recreational water quality standards on the level of specific pathogens instead of indicator counts. Because most pathogen detection methods were developed for clinical samples, research is needed to adopt such methods to environmental samples where the presence of PCR-inhibitors is a common problem. The purpose of the current project was to determine the best method to process beach water samples for the detection and quantification of *Salmonella* spp. by qPCR. Although *Salmonella* is considered a low-grade pathogen that does not persist in the environment, it is one of the most common causes of enteric diseases and several PCR-based methods for its detection have already been developed. Our findings are that filtration through a coarse filter to remove debris followed by centrifugation (5 min at 5,000 x g) was an efficient method to concentrate samples. Detection limit can be lowered to 10 cfu/dL using sample enrichment in either a nonselective medium such as Brain Heart Infusion or a selective medium such as Rappaport Vassiliadis Soya Peptone. DNA purification prior to PCR increases the frequency of false negatives probably as a result of co-precipitation of PCR inhibitors with bacterial DNA. A simple boiling lysis procedure was found to be the most efficient method to prepare samples for PCR. Our conclusion is that it is now feasible to use a PCR-based method to detect and semi-quantitate *Salmonella* in environmental water samples.

Research Goal

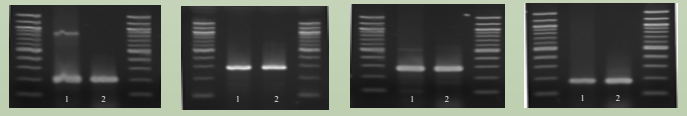
Develop a PCR-based procedure to detect *Salmonella* in environmental samples.

Specific objectives include:

- Determine which PCR primers to use based on specificity and suitability for real-time PCR
- Determine which sample processing procedure to use to obtain DNA suitable for PCR
- Develop a sample enrichment procedure that enhances assay sensitivity

SEN primers were selected as PCR primers of choice

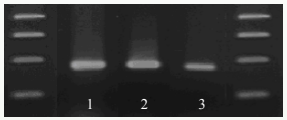
Salm Primers (*prgK* gene) | INVA Primers (*invA* gene) | Sal Primers (*invA* gene) | SEN Primers (*invA* gene)



1 *Salmonella choleraesuis*
2 *Salmonella typhimurium*

Primer		Sequence (5' → 3')	Target Gene	Product Size	Ref
Salm	F	CCT TTC TTA TTG CGG GCA	*prgK*	193 bp	1
	R	GCC GAT GTG GAT TAT GAC			
INVA	F	GTG AAA TTA TCG CCA CGT TCG GGC AA	*invA*	284 bp	2
	R	TCA TCG CAC CGT CAA AGG AAC C			
Sal	F	TAT CGC CAC GTT CGG GCA A	*invA*	275 bp	3
	R	TCG CAC CGT CAA AGG AAC C			
SEN	F	TTT CAA TGG GAA CTC TGC	*invA*	172 bp	4
	R	AAC GAC GAC CCT TCT TTT			

A rapid cell lysis procedure was effective for preparing samples for PCR

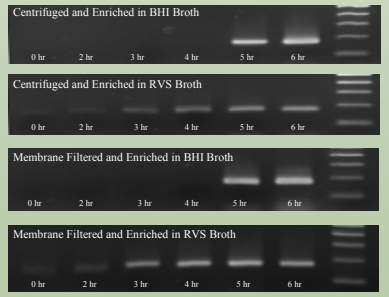


1. Boiled lysis
2. Watermaster™ DNA purification kit
3. ExtractMaster™ Fecal DNA extraction kit

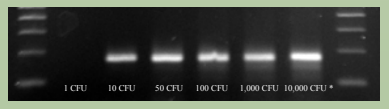
The expected PCR product was amplified from *Salmonella* DNA only

Bacterial Strains Tested	PCR
Salmonella typhimurium ATCC14028	+
S. choleraesuis ATCC14028	+
Salmonella bovis	+
Streptococcus Pneumoniae ATCC49619	–
Escherichia coli ATCC 25922	–
Lactococcus lactis ATCC11454	–
Proteus vulgaris ATCC13315	–
Pseudomonas aeruginosa ATCC27853	–
Serratia marcescens ATCC13880	–
Shigella flexneri ATCC12022	–
Staphylococcus aureus ATCC49476	–
Enterococcus faecalis ATCC29212	–

Sample enrichment improves assay sensitivity



The detection limit was 10 CFU per dL



* CFU per dL

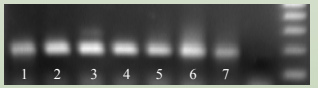
Assay Procedure

Filter water sample (500 mL) through coarse filter to remove debris
↓
Concentrate by centrifugation or membrane filtration (4,000 x g, 10 min or 0.45 um filter)
↓
Enrich samples using either BHI (non-selective) or RVS broth (selective) (6 hr, 37°C)
↓
Concentrate cells by centrifugation and lyse cell to obtain template DNA for PCR
(300 uLTE, 100°C, 10 min)
↓
Perform PCR assay

Salmonella was detected in coastal water samples

Date of Sample Collection	Sample Type	No. of Samples Tested	No. of Positives
09-24-05	Beach Water	6	5
11-20-05	Beach Water	6	5
12-13-05	Beach Water	3	1

Salmonella was found in the sediment along beaches



1 Centrifuged & Enriched in BHI broth
2 Centrifuged & Enriched in RVS broth
4 Membrane Filtered & Enriched in BHI broth
5 Membrane Filtered & Enriched in RVS broth
3,6,7 Positive Control

Summary

- A PCR assay to detect *Salmonella* spp. in environmental samples was optimized using the SEN primers of Csordas *et al* (2004).
- The procedure developed to process water samples is simple, rapid and can also be used with sediment samples.
- A sample enrichment procedure was necessary to increase the sensitivity of the assay.
- Research is currently in progress to develop a real-time PCR procedure to increase the sensitivity of the assay.

References

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