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DETECTION OF O ANTIGENS IN *ESCHERICHIA COLI* USING MOLECULAR METHODS

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ABSTRACT

Escherichia coli, a food-borne pathogen, causes gastroenteric diseases in both humans and animals. Cattle are the reservoirs for the pathogenic strains, therefore, a common mode of infection in humans is through the ingestion of contaminated beef or other food and water laced with *E. coli*.[3] Pathogenic strains of *E. coli* belonging to serogroups O157, O26, O45, O103, O111, O121, and O145 that produce Shiga toxins, cause diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in humans. While *E. coli* O157:H7 was considered an adulterant in meat in 1996, the Food Safety and Inspection Services of the United States Department of Agriculture recently declared the other six O groups as adulterants in meat and mandated that these seven serogroups be routinely screened for in ground beef. Therefore, a rapid method for detecting O groups are urgently needed.

E. coli are classified based on O antigens that are part of the lipopolysaccharide present on the surface of the bacteria. There are 187 different O antigens currently known. The traditional method of detection of the serogroups is by serotyping, utilizing antisera raised in rabbits against the 187 O antigens that are allowed to react with antigens for agglutination. This method is laborious and can exhibit equivocal results.

Real-Time Polymerase Chain Reaction (RT-PCR) assays were developed for the detection the *Escherichia coli* serogroups O116, O133, and O134 targeting the unique *wzx* (O-antigen flippase) gene, found in the O-antigen gene cluster for each serogroup. The RT-PCR assays developed were found to be specific for detecting the respective serogroups. The assays were validated using cultures belonging to the respective serogroups as well as those of other serogroups and other non *E. coli* bacteria. These assays may be used for determining *E. coli* O serogroups rapidly and accurately as an alternative method to serotyping commonly used for detecting O antigen of *E. coli*.

TABLE OF CONTENTS

List of Tables iii

Acknowledgements iv

Chapter 1 Introduction 1

Chapter 2 Materials and Methods 3

Chapter 3 Results and Discussion 5

Chapter 4 Conclusion 8

REFERENCES 9

LIST OF TABLES

TABLE 1. Oligonucleotide primers used for amplification of the *E. coli* O116, O133, and O134 *wzx* genes……..……4

TABLE 2. PCR assays tested against *E. coli* of different O groups and other bacterial genera….5

TABLE 3: Re-Serotyping of 16 clinical Isolates that Failed DNA Amplification……..6

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Chapter 1

Introduction

Since the outbreak of disease that occurred due to ingestion of hamburgers contaminated with the pathogen *Escherichia coli* O157:H7 in 1982, *E. coli* has been considered a major foodborne pathogen worldwide. *E coli* accounts for up to 120,000 gastroenteric illnesses, 2,000 hospitalizations, and 60 deaths just in the United States annually.[1] *E. coli* O157:H7, and other pathogenic serogroups of *E. coli*, that produce Shiga toxins, cause diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in humans that may lead to renal failure and long-term effects like hypertension and cardiovascular disease.[2]

Cattle's intestines are the largest reservoir for *E. coli* O157:H7, with 38.5% of dairy farms testing positive for the pathogen.[3] A large percent of the *E. coli* infections in humans are due to the ingestion of contaminated beef or other food and water laced with *E. coli*.[3] Outbreaks of diseases due to *E. coli* cause enormous economic losses to food producers that have to recall their products and to those suffering from the associated diseases. Food Safety and Inspection Services of the USDA has recently (June 2012) mandated that six more *E. coli* serogroups, in addition to O157, be tested as they are declared as adulterants in meat. Therefore, highly accurate and fast detection methods are needed to ensure food safety.

E. coli are classified based on O antigens that are on the surface of the bacteria. These O antigens are part of the lipopolysaccharide present in the outer membrane of gram-negative bacteria and are thus the contributors to antigenic variability of the strains. There are 187 different O antigens (O serogroups) that are currently known. It is important to distinguish the serogroups of *E. coli*, as strains belonging to some of the O serogroups are more pathogenic than others. The

conventional and traditional method of detecting the serogroups is by serotyping, involving antigen agglutination using antisera raised in rabbits against the 187 different O surface antigens. Therefore, in addition to using rabbits, the detection method itself is laborious and sometimes exhibits equivocal results due to cross-reactions. More rapid, sensitive, and specific molecular methods such as Real-Time Polymerase Chain Reaction (RT-PCR) are needed for the detection of each of the O serogroups. The objectives of the current investigations were to develop RT-PCR methods targeting the *wzx* gene unique for O116, O133, and O134 O serogroups, and validating the test using cultures belonging to these O groups from the *E. coli* Reference Center collection. The sensitivity and the specificity of the assays were also determined.

Chapter 2

Materials and Methods

The *E. coli* strains utilized for this study were obtained from the bacterial collection of the *E. coli* Reference Center at The Pennsylvania State University. *E. coli* reference standard strains 28w (O116), N 282 (O133) and 4370-53 (O134) from the World Health Organization (WHO) were used to develop the assays.[4] The O reference standard strains belonging to different O serogroups (O1 to O30, O32 to O46, O48 to O71, O73 to O92, O95 to O121, O123 to O175, X6, X9, X10, X13, X18, X19, X21, X23, X25, X28, X38, and X43 from the WHO wereused for testing the specificities of the RT-PCR assays. In addition to the standard strains, other *E. coli* strains (n = 50) from the collection were also used to test the specificity of the RT-PCR assays. Clinical cultures belonging to O116 (n=50), O133 (n=35), and O134 (n=23) were also used to test the PCR assay specificity. All cultures were grown in Lauria-Bertani (LB) agar medium.

For bacterial DNA extraction, a colony, was picked from the agar plate and resuspended in sterile, distilled water (100µl), and heated to 100°C for 10 minutes on a heating block. The suspension was centrifuged for 5 minutes at 13,000 x *g* and the resulting supernatant containing the DNA was used for the RT-PCR.

For the RT-PCR assay, 3 µl of template DNA, 0.5 µM concentration of primer (Integrated DNA Technologies, Inc., Coralville, Iowa), 0.18 mM concentration of each of the four deoxynucleoside triphosphates, 2mM $MgCl_2$, 0.4 U of *Taq*DNA polymerase (PGC Scientific, Gaitherburg, MD.), 50mM Tris (pH 8.3), 250 µg of bovine serum albumin per ml, and 0.1 mM Cresol Red were combined. The primers, listed in TABLE 1, for the *wzx* gene, were derived using Primer3 software program and used in the PCR assays for amplifying *wzx* gene.

TABLE 1. Oligonucleotide primers used for amplification of the *wzx* genes E. coli O116, O133, and O134

Target gene	Sequence[a]
O116 *wzx*	F, TTGGGTTTGGTGGGAAATTA R, TAATCCCAATACCGGCCATA
O133 *wzx*	F, TTGTTTCGCAGTAGCAATCG R, GCCCTTGCGTCAAGTATAGG
O134 *wzx*	F, GTCGTTCCTTTGGCATTTGT R, TCGAAACCGGAGCCATATAC

[a] F, forward; R, reverse.

The RT-PCR method consisted of one cycle at 95^{o}C for 10 min, then 40 cycles, denaturation at 95^{o}C for 15 minutes followed by amplification at 60^{o}C for 1 minute. The quantity and quality of DNA was determined by optical density measurements at 260/280 nm in a spectrophotometer against a known positive control.

Chapter 3

Results and Discussion

The RT-PCR assays were developed using the primers derived from *wzx* genes of O antigen gene clusters for O116, O133, and O134 serogroups. The assay for all three groups were found to be highly specific for the targeted O group, as seen by the lack in DNA amplification for all the other 180 O serogroups, 50 random isolates of dissimilar O serogroups, and 16 different types of bacteria (TABLE 2).

However, the assays conducted on clinical isolates belonging to target O type, as determined by serotyping, did not yield 100% specificity. While 70% of the clinical isolate considered to be O116 by serotyping reacted positively to O116 assay, 17% of O133, and 52% of O134 isolates reacted positively to O133 and O134 assays, respectively.

The disparity between the results of serotyping and PCR was addressed by re-serotyping the clinical isolates that did not exhibit the presence of *wzx* gene of the target O group. As seen on TABLE 3, most of the clinical isolates belonging to O116, O133, and O134 serogroups did not react with O116, O133, or O134 antisera respectively on re-serotyping thus, confirming the validity of the PCR assays for O116, O133, and O134. Only 17% of the total isolates retested were positive for O116, O133, and O134 by re-serotyping, which did not exhibit positive results with RT-PCR. This disparity could be due to other factors such as mutations in part of the *wzx* gene in these clinical isolates.

Overall, the RT-PCR assays for the serogroups O116, O133, and O134 are acceptable for detecting the respective O groups that were highly sensitive and specific. These assays were able to identify strains that were previously thought to belong to serogroups O116, O133, and O134.

TABLE 2: Specificities of PCR assays developed for *E.* coli O groups[a]

Strain (no.)	Source	Assays tested		
		O116 *wzx*	O133 *wzx*	O134 *wzx*
E. coli O116 (n=50)	ECRC	+(70%)*	ND	ND
E. coli O133 (n=35)	ECRC	ND	+(17%)*	ND
E. coli O134 (n=23)	ECRC	ND	ND	+(52%)*
E. coli different serogroups[b] (n=50)	ECRC	−	−	−
Staphylococcus aureus (n=1)	ATCC13709	−	−	−
S. aureus (n=1)	ATCC29213	−	−	−
Klebsiella pneumoniae (n=1)	ATCC27736	−	−	−
Serratia marcescens (n=1)	Unknown	−	−	−
Shigella boydii (n=1)	Unknown	−	−	−
Salmonella typhi (n=1)	Unknown	−	−	−
Enterobacter coleae (n=1)	Unknown	−	−	−
Salmonella Arizona (n=1)	Unknown	−	−	−
Salmonella choleraesus (n=1)	ATCC14028	−	−	−
Salmonella choleraesus (n=1)	ATCC51741	−	−	−
Salmonella anatum (n=1)	ATCC9270	−	−	−
Citrobacter frundii (n=1)	ATCC8090	−	−	−
Hafnia alvei (n=1)	ATCC29926	−	−	−
Shigella flexneri (n=1)	Unknown	−	−	−
Yarsinia enterocolitica (n=1)	Unknown	−	−	−
Listeria inocua (n=1)	ATCC51742	−	−	−

[a] +, positive; −, negative; ND, not done.

[b] Other than O116, O133, and O134

*Because of the equivocal results that typical serotyping provides, these numbers are further discussed

TABLE 3: Re-Serotyping of 16 Clinical Isolates that Failed DNA Amplification

Accession	Original O Group in Database	RT-PCR	Re-serotyping
71.0043	134	NEGATIVE	101
80.0184	134	NEGATIVE	X43
82.1227	116	NEGATIVE	116
83.1459	116	NEGATIVE	7
85.1955	133	NEGATIVE	19
87.1437	133	NEGATIVE	19
87.1449	134	NEGATIVE	N[a]
88.1390	116	NEGATIVE	N
89.0431	133	NEGAIVE	19
94.0635	116	NEGATIVE	116
94.0757	133	NEGATIVE	133
95.0975	134	NEGATIVE	134
1.4514	133	NEGATIVE	133
3.2104	134	NEGATIVE	134
4.2198	134	NEGATIVE	134
10.0593	116	NEGATIVE	N

[a] N = no one serotype could be matched

Chapter 4

Conclusion

Serotyping is a conventional method of determining the O type of an *E. coli* sample that is laborious and may sometimes exhibit equivocal results. State-of-the-art RT-PCR assays were developed for detecting serogroups O116, O133, and O134 utilizing the unique *wzx* gene of the O antigen gene cluster for each of these O groups. Each of the RT-PCR assays for the serogroups were found to be specific against the other O serogroups and common bacteria and accurate among clinical isolates of the same serogroup. Because of this development, diagnostic laboratories using serotyping will be able to switch to the faster, more accurate method of RT-PCR for O group determination. This will improve diagnostic capabilities, thus improving prevention and treatment of patients and food safety requirements.

REFERENCES

1. Brooks, J. T., Sowers, E. G., Wells J. G., Greene, K. D., Griffin, P. M., Hoekstra, R. M., and N. A. Strockbine. 2005. Non-O157 Shiga toxin producing *E coli* infections in the United States, 1983-2002. J. Infect Dis. **192:** 190-198.

2. Clark CF, Sontrop JM, Macnab JJ, Moist L, Suri R, Garg A, 2010. Long term risk for hypertension, renal impairment, and cardiovascular disease after gastroenteritis from drinking water contaminated with Escherichia coli. *BMJ*. Res. 341

3. Doyle ME, Archer J, Kaspar CW, Weiss R, 2006. Human Illness Caused by E. coli O157:H7 from Food and Non-food Sources.

4. Ørskov, I., Ørskov, F., Jann, B., Jann, K. 1977. Serology, Chemistry and genetics of O and K antigens of Escherichia coli. Bacteriol. Rev. 41: 667-710.

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