Multiplex PCR Protocol for selective amplification of a new pathogenic lineage of *Escherichia* which includes *Escherichia albertii* and *Shigella boydii* serotype 13

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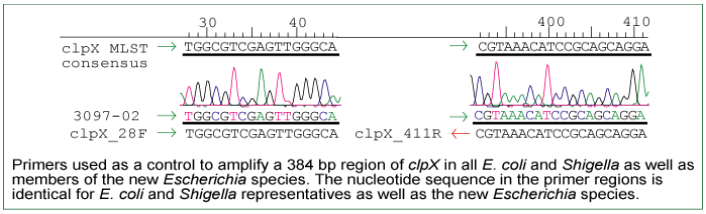
Comparative analysis of sequences of 14 housekeeping genes in *Escherichia albertii* (1, 3) to those of strains representing the major groups of pathogenic *Escherichia coli* and *Shigella* revealed that *E. albertii* strains differ, on average, at approximately 7.4% of the nucleotide sites from pathogenic *E. coli* strains and at 15.7% from *Salmonella enterica* serotype Typhimurium (4). The *E. albertii* strains were closely related to strains of *Shigella boydii* serotype 13 (2), a distant relative of *E. coli* representing a divergent lineage in the genus *Escherichia*. Members of the *E. albertii/Shigella boydii* 13 lineage carry a homologue of the intimin gene (*eae*) that is distinct from *eae* variants reported for pathogenic *E. coli*. Bacteria of this lineage also have a cytolethal distending toxin gene cluster (*cdt*) that has diverged into three allelic groups corresponding to *E. albertii, Shigella* 13, and a nontypeable isolate serologically related to *S. boydii* serotype 7.

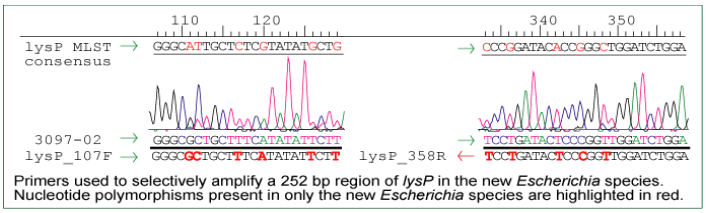
We developed a simple PCR assay based on nucleotide sequences of multiple conserved housekeeping genes to identify or confirm member of the *E. albertii/Shigella boydii* lineage. Two sets of primers were designed to include nucleotide polymorphisms unique to the new lineage, and amplify two different gene segments exclusively in the new lineage. A set of primers was designed to amplify a gene segment in the new lineage as well as in *E. coli* and *Shigella,* and serves as a control.

Multiplex PCR Primers

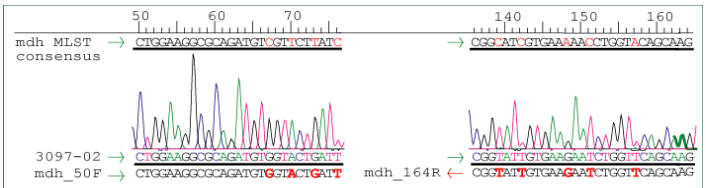
Name	Sequence	Product	Comment
clpX_28F	5' TGG CGT CGA GTT GGG CA 3'	384 bp	control
clpX_411R	5' TCC TGC TFC GGA TGT TTA CG 3'		
lysP_107F	5' GGG CGC TGC TTT CAT ATA TTC TT 3'	252 bp	specific
lysP_358R	5' TCC AGA TCC AAC CGG GAG TAT CAG GA 3'		
mdh_50F	5' CTG GAA GGC GCA GAT GTG GTA CTG ATT 3'	115 bp	specific
mdh_164R	5' CTT GCT GAA CCA GAT TCT TCA CAA TAC CG 3'		



Primers used as a control to amplify a 384 bp region of *clpX* in all *E. coli* and *Shigella* as well as members of the new *Escherichia* species. The nucleotide sequence in the primer regions is identical for *E. coli* and *Shigella* representatives as well as the new *Escherichia* species.



Primers used to selectively amplify a 252 bp region of *lysP* in the new *Escherichia* species. Nucleotide polymorphisms present in only the new *Escherichia* species are highlighted in red.



Primers used to selectively amplify a 115 bp region of mdh in the new *Escherichia* species. nucleotide polymorphisms present in only the new *Escherichia* species are highlighted in red.

Protocol

Combine the following PCR reagents for 50 ul PCR reactions:

Taq = Amplitaq Gold (Applied Biosystems, Foster City, CA).

	uL per reaction
10X gold buffer	5.00
dNTP (2 mM)	5.00
$MgCl_2$ (25 mM)	4.00
lysP_107F (10 uM)	2.00
lysP_358R (10 uM)	2.00
mdh_50F (10 uM)	0.40
mdh_164R (10 uM)	0.40
clpX_28F (10 uM)	3.00
clpX_411R (10 uM)	3.00
Taq (3 U)	0.60
ddH_2O	to volume

2 ul of purified genomic DNA at a concentration of 100 ng/ul was used as a template. Genomic DNA was isolated from 2 mL of culture using the Purgene DNA isolation kit (Gentra Systems, Minneapolis, MN).

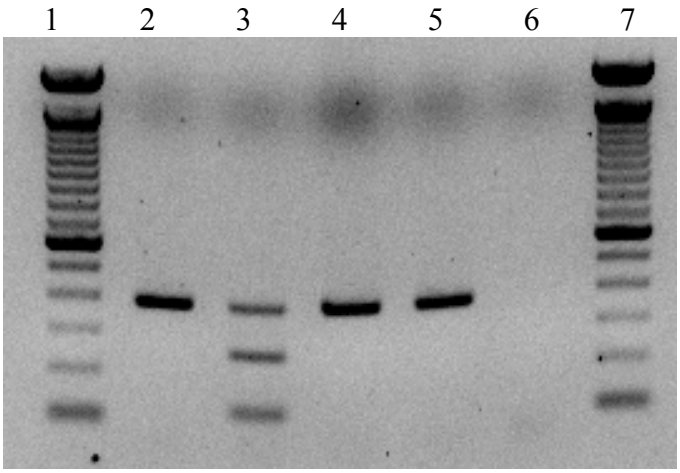
OR ALTERNATIVELY

5 ul of genomic DNA obtained from boiling a single colony pick in 50 ul of TE buffer at 95C for 10 minutes was used as a template

Amplify under the following conditions:

Thermocycle	Temp.	Time
Soak	94 C	10 min.
Denature	92 C	1 min.
Anneal	65 C	1 min.
Extend	72 C	30 sec.
Cycles	25	
Soak	72 C	5 min.
Hold	4 C	

Results



1.5% agarose gel. 15 ul PCR product.

Lanes 1 and 7: 100 bp Ladder
Lanes 2, 4, and 5: *E. coli* and *Shigella* representatives
Lane 3: A member of the new lineage - strain 3097-02
Lane 6: negative control – ddH_2O

References

1. **Abbott, S. L., J. O'Connor, T. Robin, B. L. Zimmer, and J. M. Janda.** 2003. Biochemical properties of a newly described *Escherichia* species, *Escherichia albertii*. J. Clin. Microbiol. **41:**4852-4.
2. **Brenner, D. J., A. G. Steigerwalt, H. G. Wathen, R. J. Gross, and B. Rowe.** 1982. Confirmation of aerogenic strains of *Shigella boydii* 13 and further study of *Shigella* serotypes by DNA relatedness. J. Clin. Microbiol. **16:**432-6.
3. **Huys, G., M. Cnockaert, J. M. Janda, and J. Swings.** 2003. *Escherichia albertii* sp. Nov., a diarrhoeagenic species isolated from stool specimens of Bangladeshi children. Int J Syst Evol Microbiol **53:**807-10.
4. **Hyma, K. E., D. W. Lacher, A. M. Nelson, A. C. Bumbaugh, J. M. Janda, N. A. Strockbine, V. B. Young, and T. S. Whittam.** 2005. Evolutionary genetics of a new pathogenic *Escherichia* species: *Escherichia albertii* and related *Shigella boydii* strains. J. Bacteriol. **187:**619-28.