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Molecular differentiation between *Shigella* and *Escherichia coli* using PCR Technique

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Abstract : Objectives: To Show molecular differential between two bacteria.
Methods: Multiplex polymerase chain reaction (PCR) detection of target four genes were used to differentiate *E.coli* from *Shigella* depends on: *uid*A, *lacZ, lacY* (coding for lactose permease), and cyd (coding for cytochrome bd complex) genes.
Result: PCR fragments of the predicted size (147,264,393,463bp respectively) were observed only for *E. coli* strains, but not for relatives as close as *Shigella* sp.
Conclusions: Lactose permease is found in only in *E.coli* but not in *Shigella* species that are so related to *Escherichia.*
Keywords : Multiplex PCR, *Shigella, E coli, uid A, lac Z,lac Y, cyd.*

Introduction:

Shigella causes bacillary dysentery and is classified into four species based on their antigen characteristics. This classification does not reflect genetic relatedness; in fact, *Shigella* species are so related to *Escherichia coli*, they should be classified as one distinctive species in the genus Escherichia[1]

Molecular analysis of selected genes of enteroinvasive *E. coli* strains and *Shigella* strains revealed very close evolutionary relationship between these species[2,3]. Interestingly[4].Recently suggested to consider *Shigella* strains as pathovars of *E. coli* on the base of sequence similarity, among housekeeping and plasmid genes of several Shigella and E. coli strains. The close relationship between E. coli and Shigella species hampers their differentiation. Therefore,it is obvious that many E. coli could be called Shigella and vice versa. Using multiplex PCR targeting four genes: uidA, lacZ, lacY (coding for lactose permease), and cyd (coding for cytochrome bd complex) genes.[5]

Products of these genes could be considered as biochemical hallmarks of *E. coli* sp. Indeed, enzymatic products of lacY and lacZ genes are necessary for lactose fermentation; lactose permease is essential for lactose transport across cytoplasmatic membrane and cytochrome b-d-galactosidasec leaves the disacharide lactose into glucose and galactose[6, 7]. Those four PCR fragments of the predicted size were observed only for *E. coli* strains, but not for relatives as close as *Shigella* sp.[8]

Materials and Methods

All strains examined by PCR were grown on MacConkey agar plates at 37°C. DNA was extracted from bacteria by resuspending one bacterial colony in 50 µl of deionized water, boiling the suspension for 5 min, and centrifuging it at 10,000 × *g* for 1 min. The supernatant was then used as the DNA template for PCR.

Primers selection

The primers specific for the lacZ gene have been previously described[9,10].The sequences oflacy and cyd genes were obtained from a public database (Entrez, GeneID: 946149 – uidA, 945341 – cyd). The primers were carefully designed to permit co-amplification. Primer sequences were as follows: lac Z, upper primer: 5-ATGAAAGCTGGCTACAGGAAGGCC-30, lowerprimer:

5-GGTTTATGCAG CAACGAGACGTCA-3 /uidA, upperprimer: 5-ATCGGC GAAATTCCATACCTG-3, lowerprimer: 5-GTTCTGCGACGCTCACACC-3
5-GCCGGCTGAGTAGTCGTGGAAG-3.
lacY were following:EClpma (-1): 5-ACCAGACCCAGCACCAGATAAG-3,
EClpma (+1): 5-GCACCTACGATGTTTTTGACCA-3[11].

Multiplex PCR amplification

Multiplex PCR amplification The mixture consisted of 1× PCR buffer (10 mmol$^{1^{-1}}$ Tris-HCl Ph 8.8, 1.5 mmol l)1 $MgCl_2$, 50 mmol $^{1^{-1}}$ KCl,0.1% Triton X-100), 1 U of Taq DNA polymerase (Finnzymes, Espoo, Finland), 0.5 µ m0l$^{1^{-1}}$ of each primer, 200 µ mol$^{1\ -1}$ of each dNTPs and 5 µ l of template DNA. PCR reaction was performed in total volume of 25 µ l. Conditions of PCR amplification were as follows: initial denaturation at 94℃ for 90 s, and 30 cycles with denaturation at 94℃ for 30 s, annealing at 58℃ for 25 s and extension at 72℃ for 30 s.

The amplified products were loaded onto a 1.8% agarose gel containing ethidium bromide (0.25 µ g ml$^{1\wedge(-1))}$ and run in 1× TBE buffer (tris-borate buffer) for 1.5 h at 80 V. PCR fragments were visualized with UV transilluminator. A 100-bp DNA ladder was loaded on each gel as a DNA size standard.

Results

DNA extracted from *E. coli* one of the fully characterized *E. coli* strains, served as a template. Then co-amplifications using different combination of two, three, and finally all four primer pairs specific for lacZ, uidA, cyd, and lacY genes were tested with the same template DNA to Amplification of PCR mixtures containing template DNA, which were extracted from control E. coli strains, resulted in appearance of four fragments of the predicted size as shown in figure 1. All four PCR products were also detected for amplification with DNA template extracted from, *Shigella flexneri*, or *Shigella sonnei*, which are relative to *E. coli*, gave two to three PCR fragments of the predicted size.

PCR protocols utilized four sets of primers, the first primer set derived from lacZ gene sequence served to detect all coliform bacteria, and the second primer set derived from uidA gene sequence was used for detection of *E. coli*. Unequivocal advantage of this approach was that *E. coli* strains with undetectable b-d-glucuronidase activity (i.e., GUR-negative) were detectable by PCR amplification targeting the uidA gene[12].However, it was demonstrated that the primer set derived from the uidA gene could also identify the non-*E. coli*coliforms[13]. In addition, theabove mentioned duplex PCR protocol does not allow distinguishing *Shigella* sp. from *E. coli*[11,14]. The third genes, cyd, coding for cytochrome bd complex,and lacY, coding for lactose permease, which could serveas *E. coli* hallmark genes. The cytochrome bd complex (i.e., cytochrome bdquinol oxidase) is one of two respiratory oxidases in *E. coli.* It oxidizes dihydroubiquinol or dihydromenaquinol while reducing dioxygen to water. The bd-type oxidases found in prokaryotes only are induced underconditions of very low aeration, either to generate aproton motive force by reducing O2 to water or byscavenging O2 to protect the cell (2:3). This gene was already successfully applied fordetection of *E. coli*[11]. However, cydis expressed also in *Shigella sonnei* and *Shigella flexneri* Lactose permease, which is product of the lacY gene,transduces free energy stored in the electrochemical H+gradient into a sugar concentration gradient by catalyzingthe coupled stoichiometric

translocation of galactosides and H+ (lactose⁄H+ symport, reviewed by[14].Lactose permease is found in only in *E.coli* but not in *Shigella* species that are so related to *Escherichia*.

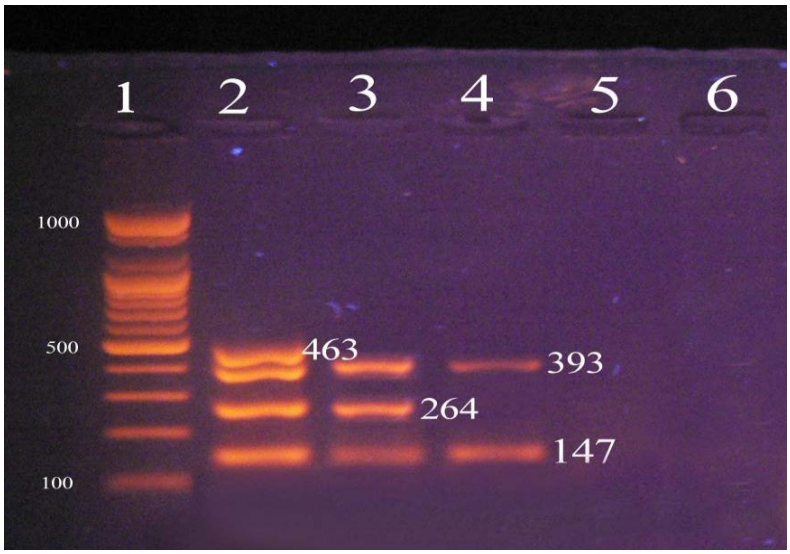

Figure 1: Multiplex amplification of DNA from control *E. coli, Shigella flexneri* and *Shigella sonnei* strains using lacZ, uidA, cyd, and lacy primers. Lane 1: DNA size marker (Fermentas); lane 2: *E. coli* lane 3: *Shigella sonnei* lane4: *Shigella flexneri*lane 5: negative control

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