Molecular Characterization of *Escherichia coli* isolated from estuarine environment by multiplex PCR and RAPD analysis

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Abstract

Five strains of Escherichia coli isolated from four different sampling stations of the estuarine environment were studied. These isolates were then subjected to Random Amplified Polymorphic (RAPD) finger printing by ten random primers (RAPD kit A1 to A10). The binding pattern generated by these ten random primers in different isolates was further analyzed by Nel's homology co-efficient and dendogram, which indicated that E. coli isolates 1 and 2 as well as 3 and 5 were more similar with each other respectively than E. coli isolate 4. The results clearly indicated the environmental dependent variation in genetic makeup of the studies of E. coli isolates.

Key words: *E. coli*, PCR; RAPD; DNA

Introduction

Maintenance of the microbial quality and safety of water systems is imperative as their fecal contamination may exert high risks to human health as well as result in significant economic loss. Human fecal material is generally perceived as constituting a greater human risk than animal fecal material, considering that it is livelier to contain human specific enteric pathogens [1]. Determining the source of fecal pollution enables as to apply appropriate management plans to remedy the problem and to prevent any further contamination. *E. coli* is now recognized as an important human pathogen of public health concern [2]. Infection with *E. coli* strains, especially of 01 57:47, which may not apparent, results in a spectrum of disease ranging from mild, non-blood diarrhoea to hemolytic – Uraemic syndrome. Being considered as a ubiquitous organism widely distributed in the environment, *E. coli* deserves in depth investigation. In the present study, the assessment of Randomly Amplified Polymorphic DNA (RAPD) analysis was performed through the genotypic source tracking method to identify the genetic variation of *E.coli* isolates derived from five different sampling stations of a minor estuarine environment in the southwest cost of India.

Materials and Methods

The present study was undertaken in four different sampling stations of Rajakkamangalam estuary, a minor estuary in the Southwest coast of India. Station 1 (S1) is the freshwater zone of the estuary near the Rajakkamangalam bridge, which is 3.0 km away from the sea front. Station 2 (S2) is the mixing zone of fresh water in the estuary and is 2.0 km away from the barmouth. Station 3 (S3) brackish water zone near Panniyor bridge and is 1.0 km away from bar mouth. Station 4 (S4) is the barmouth. In all the four sampling stations of the estuary, water samples were collected aseptically and transported to the laboratory in appropriate cold (4^0C) condition. In the laboratory, the bacterial strains present in the water samples were identified based on Holt *et al.* [3]. Among the organisms identified, the dominant *E .coli* strains from individual water samples were selected and named as *E. coli* 1 to 5 for further analysis.

DNA isolation

The *E. coli* cell pellets were ground individually in a glass homogenizer with 300µl of CTAB (cetyltrimethyl ammonium bromide) DNA extraction buffer (1% W/V CTAB; 1.4M NaCl; 10mM EDTA (pH

8.0); 100m Tris-HCl (pH 8.0); 0.2% V/V meracaptoethanol). The individual mixture was emulsified with equal volume of phenol: chloroform (1:1) and centrifuged at 10,000 X g for 5min at room temperature. The aqueous phase was collected and mixed with equal volume of chloroform: isoamyl alcohol (24:1). The mixture was then centrifuged at 10,000 X g for 5min and the ethanol was air-dried. The pellet was dissolved in 50µl of TE buffer (Tris 10mM- pH 8.0 and EDTA 1mM- pH8.0).The isolated DNA of the strains was quantified by spectrophotometer (260nm) and quality was tested by agarose gel electrophoresis.

RAPD-PCR analysis

The DNA (20mg) was dissolved in 20µl PCR reaction buffer containing 10mM Tris-HCl (pH 9.0), 1.5mM $MgCl_2$, 50mM KCl, 0.01% gelatin, 0.2mM dNTPs, 21 pM of primer and 0.5U of DNA polymerase. Ten primers (RAPD Kit A1 to A10) obtained from IDT were used for RAPD - PCR studies. PCR was conducted according to the method described by Williams *et al*. [4]: initial heat step (94^oC for 5 min.), 40 cycles of denaturation (94^oC for 1 min.), annealing (36^oC for 1min.) and extension (72^oC for 2min.) and a finial extension step (72^oC for 7 min.). Amplification was performed using a programmable thermal Cycler PTC-150 (MJ Research, USA). The products of PCR and DNA size markers [λ DNA digested with *E. coli* and Hind III (Bangalore Genei, India)] were loaded onto a 1.6% tris-borate-EDTA agrose gel and run for 4 h, at 50V [5]. The gels were stained with ethidium bromide and photographed. Each lane of RAPD profiles was subjected to gel documentation system (Vilbert-Lourmat, France). The dendrogram analysis and similarity index were carried out using Bioprofile 1D software (Vilbert-Lourmat, France). For comparison of molecular variation a 6^{th} isolate namely *Pseudomonas* Sp. was also simultaneously analyzed with RAPD kit A10.

Sequence of the Primers:

RAPD Kit A1 5'CAGGCCCTTC3'
RAPD Kit A2 5'TGCCGAGCTG3'
RAPD Kit A3 5'AGTCAGCCAC3'
RAPD Kit A4 5'AATCGGGCTG3'
RAPD Kit A5 5'AGGGGTCTTG3'
RAPD Kit A6 5'GGTCCCTGAC3'
RAPD Kit A7 5'GAAACGGGTG3'
RAPD Kit A8 5'GTGACGTAGG3'
RAPD Kit A9 5'GGGTAACGCC3'
RAPD Kit A10 5' GTGATCGCAG3'

Results

Molecular markers offer many options to differentiate intra and inter specific populations. Molecular identification of microbes has been frequently conducted on the basis of existence of demonstrative polymorphic DNA fragments amplified by PCR [1]. It has been suggested that the outcome of a RAPD reaction was in part determined by a competition for priming sites in the Genome [6]. Amplification is probably initiated at many sites, but only a subset of all possible products is detected as visible bands after amplification [7, 8]. In the present study, the RAPD analysis was performed for five *E. coli* isolates with RAPD kit A10 for their ability to differentiate intra species variation. The primer RAPD kit A10 amplified two fragments of 1480 and 1870 base pairs (bp) in *E. coli* isolate 1. *E. coli* isolate 2 amplified single fragment of 1480 bp with magnified size. The RAPD product of 1870 bp fragment was present in *E.coli* isolate 3. In *E. coli* strain 4, the RAPD product 3900 bp to 913 bp were amplified with low intensity. The *E. coli* strain 5 amplified five fragments with base pair ranged from 3090 to 1200 bp. For comparison of molecular variation a 6^{th} isolate namely *Pseudomonas* Sp. was also simultaneously analyzed with RAPD kit A10. In this isolate five fragments with base pair ranging from3530 to 831 bp were amplified (Fig.)

The RAPD profiles of *E. coli* strains and also *Pseudomonas* sp. obtaining the present study were used for the calculation of similarity index. The RAPD fragments of *E. coli* strain 1 has the similarity index of 0.29 with isolate 2 and 0.22 with isolate 4.

In *E. coli* strain 2, the RAPD fragments had the similarity index of 0.20 with isolate 4. The *E. coli* strain 3 had the similarity index of 0.22 and 0.25 with *E. coli* strain 4 and 5 respectively. The *E. coli* strain 5 had the

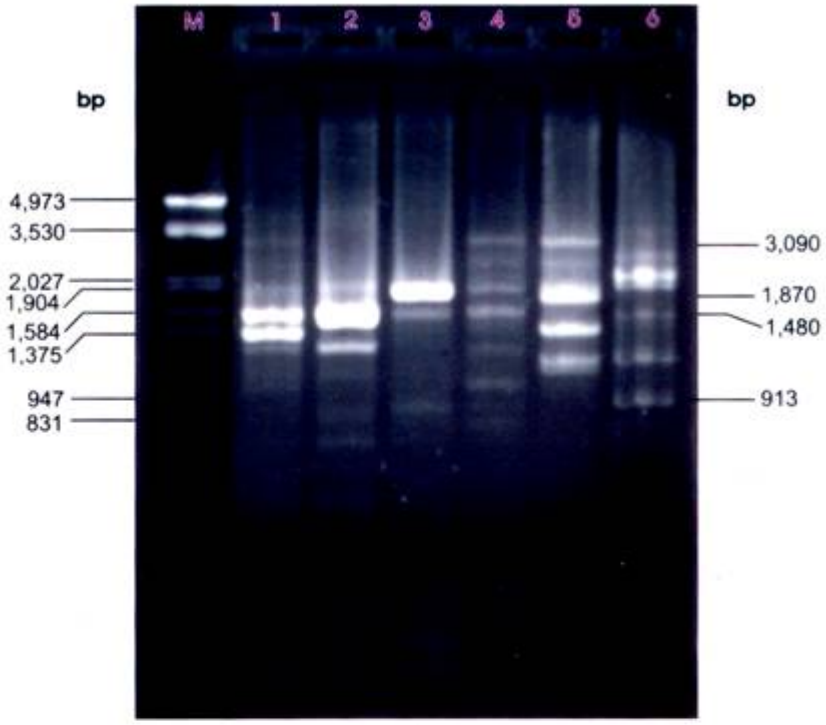
similarity index of 0.18 with isolate 4. However the isolate 6 (*Pseudomonas* Sp) has no similarity index with the RAPD fragment of any of the other tested strains.

Dendograms in the phylogram form depicting the phylogenetic relatedness between various isolates and Nels' genetic homology coefficients generated by the unweighed pair group method using arithmetic averages (UPGMA) is presented in Figure. Examination of dendogram indicated that the selected *E. coli* isolates were grouped into three major clusters excluding the *Pseudomonas* sp, which formed a separate cluster. *E. coli* isolates 1 and 2 with 29% homology coefficient were clustered together as the same species. With 25% homology co-efficient of *E. coli* strains 3 and 5 were clustered together as the same species. Isolate 4 was grouped with a third cluster forming solitary cluster with isolate 1 and 2.

Discussion

The variations noted in the Nel's genetic homology co-efficient of *E. coli* isolates in the present study may be attributed to environmental conditions prevailed in the sampling sites. Though the *E. coli* isolates 1 and 2 were obtained from two different stations, due to environmental similarity in those stations, these isolates were clustered together as the same species. *E. coli* isolates 3 and 5 were clustered together as the same species because these strains were isolated from the same station and they also indicated from the influence of unlike environment variables in that station. *E. coli* strain 4 isolated from 4^{th} station was groups as a separate cluster also indicated the different environmental variables influence on the genetic homology coefficient. Test results showed the influence of variation in environmental variables on genetic variation of *E. c*oli strains isolated from the estuarine environment.

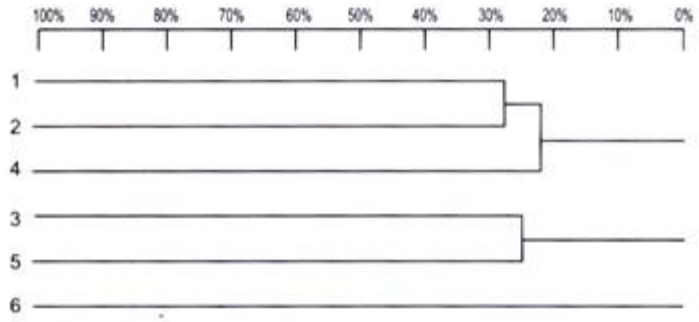
Random amplified polymorphic DNAs generated by the Primer **RAPD Kit A10** using genomic DNA of different strains of ***E. coli*** .



Similarity index of ***E. coli*** strains based on RAPD profiles

	1	2	3	4	5	6
1	1.00					
2	0.29	1.00				
3	0.00	0.00	1.00			
4	0.22	0.20	0.22	1.00		
5	0.00	0.00	0.25	0.18	1.00	
6	0.00	0.00	0.00	0.00	0.00	1.00

Dendrogram with Homology Coefficient % : 0.0 (UPGMA)



Our findings are consistent with Naveen Kumar *et al*. [9], who isolated 5 strains of septicemia and diarrheic *E.coli* 078. from sheep and calves and reported that there was much polymorphism between isolates and the sheep isolates were more similar with each other than isolates from calves by RAPD method with seven random Primers viz. OPB1, OPB2, OPB5, OPB6, OBP7, OBP8 and OPB9. They further inferred that, high homogeneity among isolates from sheep and lower homogeneity among isolates from cleave may probably be

due to the fact that the former isolates were cultured from the same farm, having endemic infection; whereas, the later isolates were cultured from different locations though with similar disease condition. Akopyanz *et al*. [10] also reported that, the 60 strains of *Helicobacter* p*ylori* isolated from the patients at one hospital were found to be distinguishable from each other by RAPD methods with each of the two arbitrary primers tested.

References

1. VENIERI, D., VANTARAKI, A., KOMNINOU, G., PAPAPETROPOULOU, M.: Development and evaluation of Random Amplified Polymorphic DNA-PCR (RAPD-PCR) as a microbial source tracking method. Water microbiology*,* 1 – 3: (//E:\RAPD\WaterMicro.htm) (2005)
2. COIA, J.E.: Clinical, microbiological and epidemiological aspects of *Escherichia coli* 0157 infection. FEMS Immunology and Medical Microbiology, 20: 1 – 9 (1998)
3. HOLT, F.J.G., KREG, N.R., SNEATH, P.H.A., STANLEY, J.T., WILLIAMS, S.T.: Bergey's Manual of Determinative Bacteriology, Vol. 1-4, 9th edn., Wavely Press Inc., Baltimore, USA (1996).
4. WILLIAMS, J.G.K., KUBELIK, A.R., LIVAK, K.J., RAFALSKI, J.A., TINGEY, S.V.: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research, 18: 6531 – 6535 (1990).
5. SAMBROOK, J., FRITSCH, E.F., MANIATIS, T.: Molecular cloning: a laboratory manual, 2nd ed. Cold spring harbor laboratory press, cold spring harbor, New York (1989).
6. WILLIAMS, J.G.K., HARAFEY, M.K., RAFELSKI, J.A., TRIGEY, S.V.: Genetic analysis using random amplified polymorphic DNA markers methods. Enzymology, 218: 704-740 (1993).
7. HALLDEN, C., HANSEN, M., NIELSON, N., HJERDIN, A.: Competition as a source of errors in RAPD analysis. Theoretical and Applied Genetics, 93: 1185-1192 (1996).
8. KIM, J., KIM, S.H., KWON, N.H., BAE, W.K., LIM, J.Y., KOO, H.C., KIM, K.M., NOH, K.M., JUNG, W.K., PARK, K.T., PARK, Y.H.: Isolation and identification of *Escherichia coli* 0157:H7 using different detection methods and molecular determination by multiplex PCR and RAPD. Journal of Veterinary Science, 6 (1): 7-19 (2005).
9. NAVEEN KUMAR, R.A., SHARMA, S., MAHER CHANDARI, S.K., KASHYAP, M., SHARMA, M.: DNA finger printing of septicemic and diarrhoeic *Escherchia coli* 078. Indian Journal of Microbiology, 44 (1): 63-65 (2004).
10. AKOPYANZ, N., BUKANOV, N.O., WESTBLUM, T.U., BERG, D.E.: DNA diversity among clinical isolates of *Halicobacter pyroli* detected by PCR bases RAPD finger printing. Nucleic acid Research, 20: 5137 – 5142 (1992).