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Genetic diversity and molecular characterization of *Escherichia coli* from soil, water and human saliva by RFLP

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ABSTRACT

Escherichia coli have been intensively studied under various aspects in general bacteriology. Soil and water is a common source of infectious agents and the human saliva contain excess amount of microbes especially *E.coli*. The genetic variation in different population of *E.coli* was studied using restriction fragment length polymorphism (RFLP) analysis. Samples such as soil, water and human saliva were collected and the *E. coli* strains were isolated and screened using the selective media and subjected to Restriction digestion with EcoRI and Agarose gel electrophoresis. Upon molecular characterization, bands digested from *E. coli* gave five bands of different morphology. Out of the five bands obtained three bands were monomorphic in and all the other bands were polymorphic in all the three samples. Based upon the patterns of *E. coli* recovery among the different sample types, there is 60% homology that indicates the presence of common genes coding for common protein functions and 40% polymorphism in genes may be due to the variations in the environmental conditions and these polymorphic differences may provide adaptability to a changing climate, or some other trait necessary for the species to survive in the ever-changing world.

KEYWORDS

E.coli;
Genetic diversity;
Soil;
Water;
Saliva.

INTRODUCTION

Escherichia coli is an important member of the gastrointestinal tract of humans and warm-blooded animals (primary habitat). In the external environment outside the host (secondary habitat), it is often considered to be only a transient member of the microbiota found in water and soil, although recent evidence suggests that some strains can persist in temperate soils and freshwater beaches[1]. *E. coli* is the dominant aerobe in the gastrointestinal tract (GIT) of humans and other mammals[2].

The majority of diversity studies involving commensal *E. coli* have relied on faecal isolations[3, 4, 5]. However, for some bacterial species such as *Streptococcus mitis*, faecal isolations may not accurately represent the population colonizing the gut mucosa[6]. Most strains of *E. coli* are harmless commensal of mammals[7]; how-

ever, some strains are capable of causing either intestinal or extra-intestinal disease[8]. Strains responsible for intestinal diseases are thought to cause much of their pathology in the small intestine[9]. This pathology is due, in part, to the ability of the strains to adhere to gut epithelial cells[10]. Some commensal *E. coli* isolates can also adhere to gut epithelial cells[11, 12].

Genetic structure of natural population of *E. coli* present in different wild species including birds and reptiles has been reported[13]. RAPD and protein biochemical markers were also studied to differentiate bacterial strains[14]. RAPD technique was applied for rapid identification and differentiation of *E. coli* strains in clinical isolates[15]. Comparative sequence analysis of plasmid of enteropathogenic E. coli strains was studied in human[16].

Most studies describing genetic relationships among strains have focused on *E. coli* taken from host animals[17, 18, 19] with a few exceptions[20]. *E. coli* strains found in contaminated surface waters may not be directly comparable to *E. coli* that is isolated directly from host sources, thereby complicating the utility of source tracking using data sets of host source characteristics. Differences in survivorship may impact comparisons of host and environmental isolates; for example, only a small subset of *E. coli* from a host may survive in the environment, but these *E. coli* comprise the majority of strains that are isolated from contaminated waters. Previous studies provide evidence that *E. coli* can persist in the benthos environment and subsequently be detected in overlying surface waters[21]. Residual populations were reported in one study, where fecal coliform levels in wastewater subjected to low temperatures decrease rapidly but then stabilize to 1 to 10% of the initial population size[22]. In addition, *E. coli* that has been isolated from septic tanks has been found to be less diverse and genetically distinct than strains of *E. coli* from the inhabitants of the households served by those systems[3].

Recently, genetic analysis techniques have been developed to identify sources of environmental bacteria and these techniques include analysis of repetitive-DNA fragment lengths[19] and genetic analysis using restriction enzymes, particularly focusing on sequences in or near 16S ribosomal DNA sequences (ribotype analysis)[17, 23]. Although these previous techniques depend on genetic differences between E. coli strains, none have been based on direct knowledge of the specific sequence differences between the strains.

In this study, we evaluated the genetic profiles of *E. coli* strains found in tap water, rhizosphere soil and human saliva. The objective of the present study was to determine the genetic relatedness of *E. coli* isolates obtained from water, soil and human saliva using RFLP. The underlying hypothesis was that *E. coli* isolates can exhibit significant genetic diversity in a natural environment, and that their RFLP patterns can change during their survival in such environments complicating source tracking. We tested the hypothesis using E. coli isolates from water, soil and human saliva.

MATERIALS AND METHODS

Bacterial isolates

Soil, water and human saliva was selected as specimen for isolation of *E.coli* for extensive genetic variation studies. Soil and water samples were collected aseptically in a sterile disposable container and stored at 37ºC until use. Human saliva was collected using a swab and taken to the laboratory condition for experimental procedures. The *E. coli* strains were recovered from the samples using normal serial dilution plating methods. From which 15 *E.coli* strains were isolated and identified (five strains from each sample) based on morphological, physiological and biochemical tests as described by Kannan[24]. Of these, 3 isolates were randomly selected and subjected to RFLP analysis.

Preparation of chromosomal DNA

E.coli was grown in 5ml of EMB (Eosin methylene blue) broth at 37°C for 18-24h. The *E. coli* culture in EMB broth (1.5ml) was centrifuged at 3,000 rpm for 3 minutes. The pelleted colonies were transferred to a micro centrifuge and 0.5 ml of TEN9 buffer and 30µl of 20% SDS was added. The contents in the tubes were mixed vigorously and to this 400 µl of phenol and chloroform were added. Again the tubes were mixed vigorously and centrifuged at 15000rpm for 15 min at 4ºC. The aqueous phase was collected and 100 µl of 0.3M sodium acetate and 0.8 ml of ethanol were added. The tubes were inverted gently and kept at -20ºC overnight for DNA separation from *E. coli*.[25]. Amount of DNA

present in each sample was determined using UV-spectrophotometer.

Restriction digestion

To 1 µl of separated DNA, 0.5ml of double distilled water, 0.5ml of 10X assay buffer,1 µl of EcoR1 restriction enzyme was added and transferred to a micro centrifuge tube to prepare the reaction for restriction digestion. The contents were mixed gently and incubated at 37ºC for 1-3 hrs. The enzymes were inactivated by heating at 70ºC-100ºC for 10 minutes.

RFLP analysis

The RFLP reaction product was loaded onto a 1.0% agarose gel containing 0.5µgml^{-1} ethidium bromide and electrophoresed in TBE buffer at 50 - 100 V for one and half hours. The DNA fragments were visualized by placing the gel on a UV (300nm) transilluminator and the gel recorded using 'Statistica trial version 8 software'.

RESULTS AND DISCUSSION

Molecular characterization techniques are now widely used both for ecological and epidemiological analyses of a wide range of bacterial species[14]. Studies of genetic diversity by RFLP are highly reliable, repeatable and unique finger print of that particular organism. It helps in identification of gene diversity in organisms. Analysis of RFLP variation in genomes is a vital tool in genome mapping and genetic analysis. Since its introduction over seventy years ago, Wright's metaphor of the adaptive landscape has become one of the most influential concepts in evolutionary biology, yet empirical understanding of the structures of actual landscapes remains elusive.

In this study, we monitored the evolution of three experimental populations of *E. coli* under three habitats such as water, soil and saliva in order to investigate the effects of environmental complexity on their dynamics. We were interested, in particular, whether heterogeneous resource environments would influence the repeatability of evolution by impacting the ruggedness of the adaptive landscape. The present study concerns with the characterization of *E.coli* strains isolated from soil, water and human saliva by RFLP analysis. There were five isolates belonging to *E.coli* were isolated and identified from each sample (15 isolates), of which three isolates (one from each) were randomly selected and subjected to RFLP analysis.

The DNA content of each isolates and the corresponding OD values were given in TABLE 1. The ge-

TABLE 1 : Genomic size of E.coli from different samples

Sample	OD Value	DNA content (µg/mg)
Soil	0.072	3.60
Water	0.069	3.45
Saliva	0.076	3.80

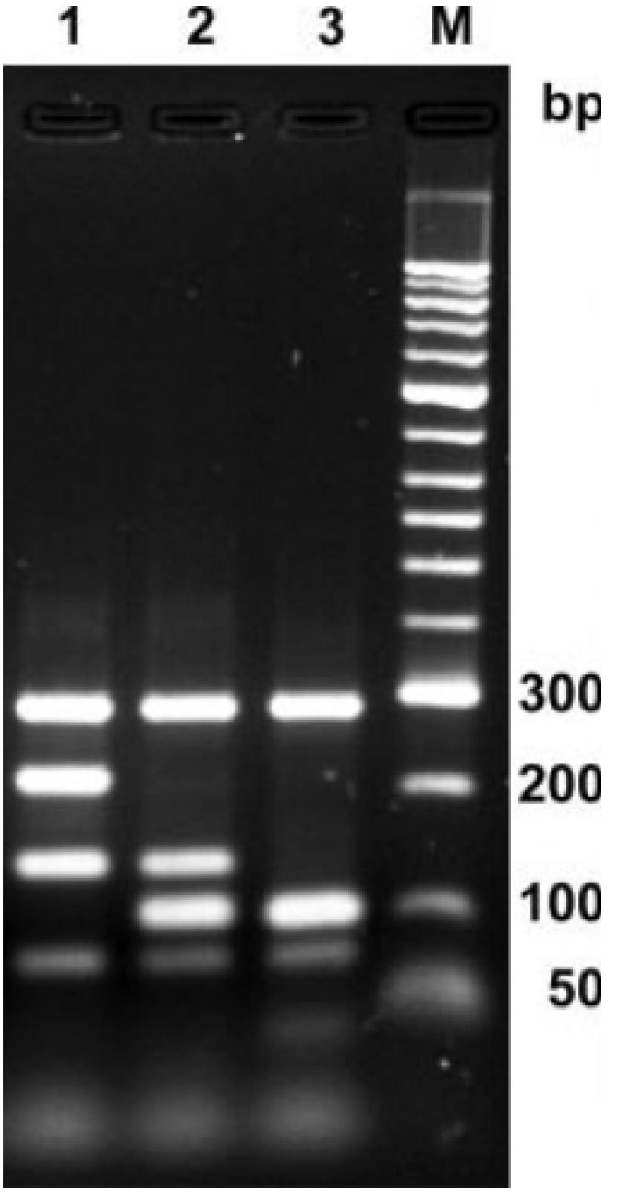

Figure 1 : RFLP profile of *E.coli* strains isolated from soil, water and saliva.
Lane1: soil, Lane 2: water Lane 3: Saliva Lane M: Marker

nomic size of *E.coli* ranged from 3.45 to 3.80µg/mg. The result of restriction analysis showed that there was clear separate DNA banding patterns in different isolates and the fragment migration were ranged from 10 to 300 bp (Figure 1.).

Upon molecular characterization, bands digested from *E. coli* gave five bands of different morphology. The first band of the lane 1, 2 and 3 were detected at approximately 300 bp, is monomorphic in nature. The second band of lane 1 (~200bp) and the fourth band of lane 3 (25bp) are unique to that sample and is not matched with any other sample that indicates some genes are more specific that help to withstand particular environment. The third band in lane 1 and the second band in lane 2 are detected at approximately 150bp and the third band in lane 2 and the second band in lane 3 are detected at approximately 100bp matched together and they are polymorphic in nature. The fourth band in lane1 and 2 is matched with the third band of lane 3, and the fifth band (~10bp) in all the three samples are monomophic in nature that indicates the presence of common gene in all the three samples. Out of the five bands obtained three bands were monomorphic in all the three samples and three bands were polymorphic in all the three samples. This shows that there is 40% polymorphism and 60% homology among the three samples.

Walk et al.[1] quantified the population genetic structure of *E. coli* from a longitudinal collection of environmental strains isolated from six freshwater beaches along Lake. Multilocus enzyme electrophoresis (MLEE) and multilocus sequence typing (MLST) revealed extensive genetic diversity among 185 *E. coli* isolates with an average of 40 alleles per locus. Repetitive element anchored PCR was used to evaluate the genetic profiles of *Escherichia coli* isolated from surface water contaminated with urban stormwater, sanitary sewage, and gull feces to determine if strains found in environmental samples reflect the strain composition of *E. coli* obtained from host sources. Overall, there was less diversity in isolates collected from river and beach sites than with isolates obtained from human and nonhuman sources[26].

In principle, replicate populations could diverge from one another not only by selection acting on different beneficial mutations but also by drift and hitchhiking. Divergence by drift could occur through the accumulation of mutations that are neutral in the selective environment, but which might have some fitness effects in other environments. Deleterious mutations might hitchhike to high frequency if they become linked with a beneficial mutation[27, 28], which could occur since the bacteria in our experiments are strictly asexual (i.e., they lack any mechanism for horizontal gene transfer). However, the *E. coli* strain we used has a very low total genomic mutation rate[29], which should limit the rates of substitution by drift and hitchhiking. Indeed, high-coverage whole-genome sequencing of another population founded from the same strain found that only three synonymous mutations achieved detectable frequencies in 20,000 generations[30]. Moreover, the patterns of correlated responses in 12 populations, again founded from the same ancestral strain, indicate that pleiotropic effects of beneficial mutations have been more important than mutation accumulation by drift or hitchhiking in explaining patterns of phenotypic evolution over 20,000 generations[31, 32, 33]. Therefore, it appears unlikely that drift or hitchhiking have contributed much, if at all, to the among-population divergence in our experiment, nor is it evident why any such effects would be stronger in the fluctuating environment treatments than in other treatments that experienced the same resources alone or in combination.

CONCLUSION

Depending upon the environmental conditions the diversity in the genetic material in each strain also varies. Genetic diversity among *E. coli* isolated from soil, water, and gut indicated high degree of variation among *E.coli* depending on habitat where they colonize. Therefore we can conclude that each collection as gene pools of *E.coli.* This variation in genetic diversity may be due to variation in habitat, variation in dietary conditions, genetic mutation and drifts, replication errors, induced mutations due to environmental stresses, variation in genetic constitutions etc. The result from the above experiment revealed that, there is 60% homology in the three strains isolated from three different habitats that indicates the presence of common genes coding for common protein functions and 40% polymorphism in genes may be due to the variations in the environmental conditions.

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