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Original Article

Natural Transformation of Plasmid DNA in *Escherichia coli* in Sterilized Soil Column

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The present study was carried out to assess transformability of natural and laboratory strains of *Escherichia coli* by plasmid DNA under different transformation conditions in sterilized soil column. Transformation experiments were carried out in laboratory conditions and in sterile soil columns with $CaCl_2$-treated competent cells and non-competent cells at log phase and stationary phase of growth using the broad host range plasmid pUC18. In soil column experiments, transformants were obtained after $CaCl_2$ induced competence in both *E. coli* K12 DH5α and strain BM09 in the frequency of 10^{-8} to 10^{-9}. In natural transformation assays, transformants appeared only in log phase cells of strain DH5α at a lower frequency (5.0×10^{-9}), and in $CaCl_2$-competent BM09 cells, but not in fresh cells. Thus the major limiting factor for natural transformation in environmental *E. coli* in soil column is probably the absence of a competent state. The significance of this finding has been discussed with respect to generally observed lower antibiotic resistance in environmental *E. coli* isolates from aquatic sources.

Keywords: Natural transformation, Plasmid DNA, *Escherichia coli*, Competent state

Introduction

Horizontal gene transfer allows rapid spread of antibiotic resistance genes[1], which in bacteria inhabiting natural aquatic systems occurs by conjugation, transduction, and transformation[2]. Natural genetic transformation is characterized by the uptake of free DNA by a recipient bacterium, its chromosomal integration or extra-chromosomal stabilization, and its expression, which leads to a new phenotype[2]. Natural transformation is distinct from induced transformation where cells are made competent (the physiologic state, which allows cells to take up DNA by physical, chemical, or enzymatic manipulation.

Transformation is thought to play major role in the spread of antibiotic resistance resulting from the extensive use of antibiotic in human medicine, and in livestock and poultry industries. As transfer of plasmid-encoded resistance to antimicrobial agents and spread of virulence genes from one bacterial strain to another is a significant public health concern. The possibility of transfer of resistance and virulence genes between bacteria in natural habitats has attracted much attention for scientific studies in recent years[4].

Natural transformation in *Escherichia coli* is of practical significance particularly because a significant proportion of diarrhoeal cases in developing countries is caused by this organism, which may thus spread genes in the environment through the natural transformation. The sediment samples in alluvial flood plain with high population density and poor hygiene and sanitation conditions can provide suitable condition for natural transformation. Pond and river sediments in our country may contain high concentrations of DNA because of very high microbial load in the sediment. Though there are many reports on the presence of highly pathogenic and drug resistant *E. coli* in our environment[5], the possibility that in these habitats there may be natural transformation has not yet been studied well. In the present study the transformability of natural isolates and laboratory strains of *E. coli* by plasmid DNA under different transformation conditions in sterilized soil column was assessed.

Materials and Methods

Bacterial strains

The ampicillin-sensitive environmental isolate of *Escherichia coli* BM09 and the laboratory strain of *E. coli* K12 DH5α were used in this study. Environmental strain of *E. coli* was isolated from a pond of Jahangirnagar University, Bangladesh by dilution plating and repeated streaking on MacConkey and nutrient agar (NA) plates. Isolated strain suspected to be *E. coli* was confirmed by Gram-staining, cultural and biochemical characteristics according to standard procedure[6]. Bacterial susceptibility to antibiotics was determined by the disc diffusion method[7] using three different commercially available antibiotic disc (Oxoid, England) such as tetracycline (30 µg/disc), ampicillin (10 µg/disc), and streptomycin (10 µg/disc).

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Plasmid DNA

DNA analyses were done after Maniatis *et al*.[8]. Two broad host range plasmid pGEM®-3Z (Promega, Madison, USA) and the plasmid pUC18 (obtained from Mr. Hasibur Rahman at ICDDR, B, Dhaka) encoding resistance to ampicillin were used as transforming DNA in this study. Plasmid DNA extraction was carried out using the alkaline lysis method of Birnboim and Doly[9] and analyzed by horizontal electrophoresis on 0.7% agarose gel and staining the gel with ethidium bromide in a UV-transilluminator. The gel was photographed using a digital camera.

Plasmid transformation assay

Standard $CaCl_2$-$MgCl_2$ induced transformation protocol was used for transformation of plasmid DNA in *E. coli* as described by Sambrook *et al*.[10]. Quantitation of plasmid DNA was made by an arbitrary method[11]. In the present study, the quantity of plasmid pUC18 was determined by comparing the intensity of this plasmid DNA band with the band found known quantity of pure plasmid pGEM.

Transformation assay in soil column was done with soil sediment collected from pond at Jahangirnagar University. The wet soil was dried, ground and sieved through a fine wire mesh. Ten-ml screw cap tubes were filled with 2 g of soil powder in a packed column. The tubes were then autoclaved three times for an hour each time. Both the stationary phase cells and log phase cells of bacterial strains were subjected to test for natural transformation without making them competent. First, approximately 1 µg of plasmid DNA was mixed with 500 µl of sterile 50 m*M* Tris. The solution was added to sterile soil column and allowed to adsorb in the soil until the liquid meniscus just touched the soil surface. The loaded tubes were held on the bench for 30 min followed by the addition of 1.0 ml of bacterial suspension to the soil column and allowed to absorb to the soil until the meniscus touched the soil surface. After overnight (O/N) incubation of the tubes at 37°C, 2.0 ml of sterilized water was added to each column. The contents were mixed by vortexing and then centrifuged at low speed (500 rpm) for 1.0 min. Supernatant was taken immediately into an Eppendorf tube and the cells in the supernatant were collected by centrifugation at 10,000 rpm for 5 min in an Eppendorf centrifuge. Cells were then resuspended into 100 µl Tris buffer that was plated on ampicillin (50 µg/ml) containing NA plates. Ampicillin resistant (Amp^r) colonies were selected on the plates after O/N incubation. Control tubes containing only the cells to which only sterile water was added in place of plasmid DNA was included in all transformation assays.

Putative transformant colonies, that is, amp^r colonies surrounded by microcolonies around after prolonged incubation were picked randomly from each selection plate and subcultured on NA containing ampicillin (50 µg/ml). Presence of the transforming plasmid in the putative transformants was checked by isolating plasmid DNA and electrophoresing isolated extracts in 0.7% agarose gel along with suitable marker.

Results

Results of the antibiotic sensitivity patterns showed that selected *Escherichia coli* BM09 pond isolate was sensitive to all three tested antibiotics ampicillin, streptomycin and tetracycline. Plasmid pGEM and pUC18 were transformed in *E. coli* K12 DH5α. Plasmids were then extracted from the transformants and electrophoresed in 0.7% agarose gel. The yield of plasmid pUC18 was better than plasmid pGEM for which we decided to use plasmid pUC18 for our experiments.

As purified plasmid pGEM (purchased from Promega, USA, in concentration: 1 mg/ml) was available to us we used this plasmid for quantitation of pUC18 plasmid DNA. Plasmid pGEM DNA solution corresponding to 2 µg and 0.2 µg DNA were electrophoresed along with 20 µl of pUC18 plasmid stock solution in an agarose gel and were observed under UV transilluminator (Figure 1). The intensity of pGEM band in left lane and that of pUC18 plasmid band in right lane suggests that the pUC18 bands would probably correspond to approximately 2 µg DNA.

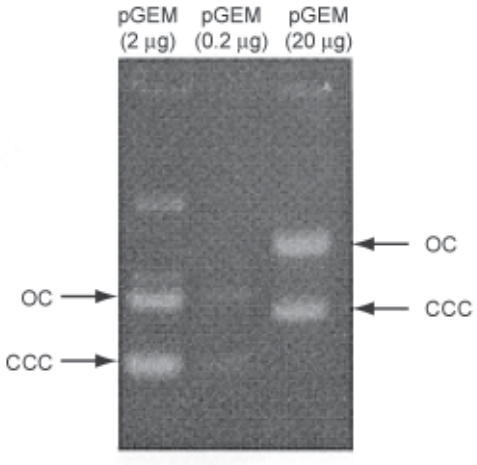

Figure 1. *Agarose gel electrophoresis of pUC18 DNA along with known quantities of plasmid pGEM. An aliquot of 20 µg of plasmid pUC18 DNA was electrophoresed along with 2.0 µg and 0.2 µg of pGEM. CCC = Covalently closed circle; OC = Open circle.*

The *E. coli* pond isolate BM09 and the laboratory strain DH5α were tested for transformation in sterile soil column by pUC18 DNA after $CaCl_2$ induced competence of cells and in non-competent cells. In case of BM09 strain, transformation was found when log phase cells were used, but in DH5α both competent log phase cells and freshly cultured stationary phase cells showed transformation in sterile soil columns in low frequency (Table 1). Presence of plasmid DNA in the transformants were confirmed by plasmid isolation and electrophoresis of purified pUC18 DNA in adjacent lanes. Figure 2 presents plasmid profile in transformants recovered from sterile soil column using competent cells. Plasmid in transformants of non-competent cells in similar soil column experiment is shown in Figure 3.

Table 1. *Transformation of $CaCl_2$-induced competent and natural non-competent cells of Escherichia coli strains in sterile soil column*[*]

Strain	Culture condition	Cell used in transformation assay	Number of transformant colonies	Transformation frequency
E. coli BM09	Stationary phase, Competent	2.5×10^{10}	None	0
E. coli BM09	Log phase, Competent	2.0×10^{9}	18	9.0×10^{-9}
E. coli K12 DH5α	Stationary phase, Natural	5.0×10^{9}	63	1.3×10^{-8}
E. coli K12 DH5α	Log phase, Natural	1.0×10^{9}	70	7.0×10^{-8}
E. coli BM09	Stationary phase, Competent	3.5×10^{9}	None	0
E. coli BM09	Log phase, Competent	1.2×10^{9}	None	0
E. coli K12 DH5α	Stationary phase, Natural	1.0×10^{9}	None	0
E. coli K12 DH5α	Log phase, Natural	5.7×10^{9}	29	5.1×10^{-9}

[*]Approximately 1 µg plasmid pUC18 DNA was used in each transformation assay.

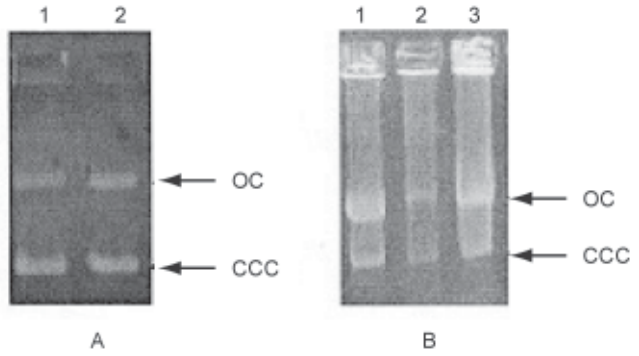

Figure 2. *Agarose gel electrophoresis of plasmid DNA obtained from transformed $CaCl_2$-treated competent cells. [A] Lane 1: Plasmid from DH5a transformant, and Lane 2: pUC18 marker plasmid. [B] Lane 1: pUC18 plasmid marker, Lane 2: BM09 transformant, and Lane 3: DH5α transformant. CCC = Covalently closed circle; OC = Open circle.*

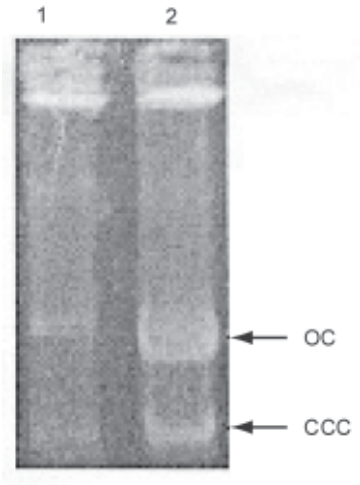

Figure 3. *Agarose gel electrophoresis of plasmid DNA of strain DH5α non-competent natural transformant obtained from sediment column (Lane 1) and pUC18 marker plasmid (Lane 2). CCC = Covalently closed circle; OC = Open circle.*

In soil transformation assay using non-competent *E. coli* BM09 and strain DH5α, transformants were found in a very low frequency (5.1×10^{-9}) in *E. coli* DH5α only when log phase cells were used, while no transformants appeared in the *E. coli* pond isolate BM09. All the putative transformants (amp^r colonies) that were tested showed plasmid DNA band in the gel having similar electrophoretic mobility as that of pUC18 DNA (Figure 3).

Discussion

The aim of this study was to examine whether transformation of *Escherichia coli* occurs in a natural environment. It is quite difficult to assay transformation in exact natural settings, so an alternative route is to create a situation close to the environment in physicochemical characteristics. Many investigators studied transformation under such conditions. Most studies, however, used sand or marine sediments[12]. To the best of our knowledge very few studies have been performed using clay from pond or river in tropical countries.

Sediment samples in alluvial flood plains with high-density human population living in poor sanitation conditions are rich in organic matter. Evidence is now emerging that water and sediment rich in organic matter can provide a suitable condition both for release of free DNA from bacteria in water and of natural transformation of bacteria inhabiting the sediment. Thus pond and river sediments of our country may contain high concentrations of DNA because of extremely high microbial load in our sediment. Though there are many reports on the presence of highly pathogenic and drug resistant *E. coli* in our environment in large numbers[5], the possibility that in these habitats there may be widespread natural transformation has not been extensively studied. Many antibiotic-resistance genes have been detected as free DNA pieces in water and sediment samples in areas directly affected by urban and farm activities.

We selected two different *E. coli* strains for our experiment – one is natural *E. coli* strain isolated from pond water and another is

laboratory strain of *E. coli* K12 DH5α to study whether cultures of these two strains in sterilized soil could be transformed with plasmid DNA pUC18 without artificial competence.

Results presented in Table 1 indicate a lower level of natural transformation in the *E. coli* strain BM09 compared to the laboratory strain DH5α. This could reflect in addition to intrinsic genetic differences, also differences in the cell surface lipopolysaccharide (LPS) structure. The wild type *E. coli* contains complex cell wall with LPS coating the outer membrane, which might be an effective barrier to DNA entry into the cell. On the other hand, very little of LPS is left with the laboratory strain *E. coli* K12 DH5α because of extensive genetic modifications that have been done in the strain over the past many decades.

Jahan *et al.*[13] studied natural transformation of some common enteric bacteria in sterile sediment. The authors reported natural transformation of plasmid DNA in *Salmonella typhimurium* and *Vibrio cholerae.* But natural transformation in environmental isolate of *E. coli* in such habitats has so far not been reported in Bangladesh. Our results thus appear to be the first demonstration of natural plasmid transformation in an environmental isolate of *E. coli* carried out in pond clay.

It is not known whether the extent of antibiotic resistance is lower in environmental *E. coli* strains relative to other enteric bacteria such *Salmonella, Shigella* and *Vibrio* species. It would be interesting to examine in carefully designed studies whether low transformability in environmental *E. coli* correlates with lower antibiotic resistance in these bacteria. This interesting and testable speculation merits further studies.

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