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Full Length Research Paper

Persistence of plasmid DNA in different soils

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Natural genetic transformation is believed to be the essential mechanism for the attainment of genetic plasticity in many species of bacteria. Dying cells are likely to release naked DNA that may survive for many hours. Although numerous studies have shown that horizontal gene transfer between distantly related genera, but how long that DNA persists in the environment is yet be reported. We present evidence from studying the plasmid DNA retaining capability of different sterilized/abiotic soils (red, black, river, silt and loose sand soils). The study also explains how long DNA molecules are in the active transformable form in the above soils. Plasmid DNA after purification was quantified and 2 µg DNA was mixed in 2 kg of sterile soils having less than 20% moisture and experimental set up was maintained in laboratory conditions at 31ºC. Every 7 days 1 g of soil was taken, DNA purified from it and then that DNA was used for transformation with the *E. coli* DH5α competent cells and the results showed that DNA would persist till 35 days and it had transforming ability.

Key words: Horizontal gene transfer, plasmid pUC18, persistence, abiotic.

INTRODUCTION

Genetically engineered microbes (GEMs) are increasingly being deployed for bioremediation purposes. In recent years there has been introduction of gems to perform better, not only in the term of bioremediation efficiency, but also in an environmentally safe manner. Generally such applications have been considered for the improvement of suitable method, survival of GEMs and better competitive ability of the engineered microbes (Stephenson and Warnes, 1996).

Horizontal gene transfer refers to the transfer of genes of genetic material directly from one individual to another by process similar to infection (Lorenz and wackernagel, 1994) and distinct from the normal process of vertical gene transfer from parent to offspring which occurs during reproduction. Genetic engineering bypasses reproduction altogether by exploiting horizontal gene transfer. So genes can be transferred between distant species that would never interbreed in nature. Completely new, exotic genes can therefore be introduced into crops. Horizontal gene transfer occurs frequently in nature and plays a significant role in bacterial evolution (Mae-wan, 1999).

Transformation, conjugation and transduction are different methods of genetic exchange that introduce sequence of DNA that share very little homology with the remaining DNA of the recipient cell (Davison, 1999). The influence of various a biotic factors on transformation has been evaluated in terms of their influence on transfer frequency, although gene transfer in soil is limited. Optimal transfer frequencies have normally been observed at different temperatures, soil, pH, nutrient concentrations and moisture contents which are required for optimal bacterial growth (Trevors et al., 1986; Cresswell et al., 1992).

Most experiments are done in *E. coli* and in the present study the bacterial strain used was *E. coli* strain DH5α. Recipient DNA used was pUC18 plasmid DNA was purified from *E. coli* strain JM83. Other studies on horizontal gene transfer from genetically modified plants (GMP) to soil associated bacteria (Nielsen et al., 1997), horizontal gene transfer by natural transformation in bacteria (Davison, 1999), binding of DNA from *Bacillus subtilis* on clay mineral montmorillonite, and the ability of

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bound DNA degraded by DNase I have already been reported (Khanna and Stotsky, 1992).

There have been few studies on transformation on the mode of gene transfer in soil. Reanney et al. (1982), Stewart and Sinigalliano (1989), Stotzky (1989) and Stotzky and Babich (1986) have revealed the occurrence of horizontal gene transfer in soil. A perusal of available literature revealed that there are plenty of published data on various aspects of horizontal gene transfer in nature. But data related to the DNA retaining capability of different soils is not available in the literature. Hence, the present study was conducted to analyze the pUC18 plasmid DNA retaining capability of biotic soils under laboratory microcosm.

MATERIALS AND METHODS

Bacterial strain, plasmid and media

The recipient bacterial strain used was DH5α grown in Luria-Berani agar. PUC18 plasmid DNA used in this study was purified from *E. coli* JM83 containing the plasmid DNA.

Experimental design

The plasmid DNA pUC18, purified from *E. coli* mixed with the sterile soils and experimental setup was maintained in the laboratory conditions at 31 ± 2ºC and the moisture content of the soil was less than 20%. Every 7 days, an aliquot of soil was taken and soil extract was prepared with sterile water the DNA was isolated and used for transformation using competent *E. coli* cells and the transformants were selected on the Luria-Bertani agar supplemented with 50 µg ampicillin per ml. The transformants were scored after 17 h of incubation at 37ºC.

Transformation and purification of PUC18 DNA

Competent cell was prepared then PUC18 plasmid DNA was transformed. Plasmid DNA was isolated from the transformants and DNA pellet was gently mixed with equal volume of equilibrated phenol resulting formation of milky complex which was centrifuged at 5000 rpm for 5 min at 4ºC (Sambrook et al., 1989). The upper layer containing DNA was transferred into another eppendorf tube in which equal volume of chloroform : isoamyl alcohol (24:1) was added, mixed well and centrifuged at 5000 rpm for 5 min. Then upper layer was transferred into another tube and DNA was precipitated by adding 0.3 M sodium acetate (pH 4.6) and equal volume of isopropanol. The DNA was collected by centrifugation at 1000 rpm, 5 min at 10ºC. The pellet containing DNA was washed with 70% ethanol and the pellet was air dried and resuspended in Tris EDTA buffer.

The soil microcosm set up

A tray consisting of 2 kg of sterile soil was used as a microcosm set up for each soil. The soil was constantly moisture twice a week by sterile distilled water. The pH of the soil was 6. The black soil was light black in color; whereas the loose sand soil was dark yellowish in color. Both of them have less porosity. Silt soil seems to be like powder where as loose sand soil was mixed with small tiny stones. The red soil was dark red in color but river soil was light biscuit in color with very small tiny stones.

Inoculation of PUC18 plasmid DNA in soil

100 µl (2 µg) of PUC18 plasmid DNA was diluted up to 1 ml with Tris EDTA buffer. This was inoculated in the soil. The inoculated DNA was thoroughly mixed in the soil whose pH was known. The inoculated soil samples were maintained safely for 2 months in the laboratory conditions.

Recovery of PUC18 DNA from soil

To 1 g of soil, 3 ml of sterile distilled water was added. Then 1.5 ml of 3 M sodium acetate was added to the above extract and incubated at -20ºC for 10 min. The sample was centrifuged at 15000 rpm at 10ºC for 10 min. To the supernatant, isopropanol was added and centrifuged and the DNA pellet was washed with in 70% ethanol, and sample was centrifuged and resuspended and stored in Tris EDTA buffer (Stotsky and Babrich, 1986). The isolated pUC18 plasmid DNA was used for transformation into competent cells of *E. coli* strain DH5α and the number of transformants in each soil types were recorded and analyzed.

RESULTS AND DISCUSSION

When horizontal gene transfer studies were conducted in different soils, plasmid transfer was significantly fluctuating. Number of transformants obtained from 1 g of different soils incubated with pUC18 plasmid DNA was taken as a measure of DNA retaining capability of particular soil. The transformed *E. coli* strain DH5α was able to grow in the ampicillin supplemented Luria-Bertani agar plate since it has the plasmid DNA pUC18. In our experiment after the transformation and incubation for 17 h, transformed colonies were scored. The whole process was continuously done for 8 weeks. The transforming capacity of the pUC18 plasmid DNA incubated in the sterile soil was studied and the results showed a fluctuation of retaining capability (Figure 1). The gene transfer studies conducted on soil microcosm demonstrate that the persistence of pUC18 plasmid DNA was identified using transformation principle via Luria-Berani-agar plating technique. Transformants are counted using colony counter and pUC18 was isolated and identified via agarose gel electrophoresis. The persistency was confirmed by repeated experiments and similar data was obtained.

In our experiment we took different kinds of soil from different areas of Tamilnadu, India and sterilized by autoclaving using autoclave. *E. coli* DH5α strain was used for checking transforming frequency because *E. coli* will have a short generation capacity (Recorbet et al., 1993; Khana and Stotzky, 1992). Many studies have evaluated gene transfer on agar plates. However, data on the transfer and expression of large, catabolic plasmids in soil are limited, particularly in non sterile soils. Studies

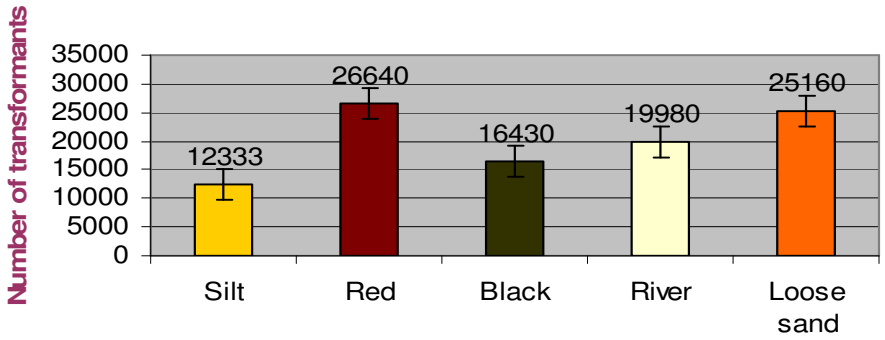

Figure 1. Persistence of plasmid DNA in different soils. Each column represents the mean ± standard error of number of transformants counted by colony counter. Number of the *E. coli* transformants of pUC18 DNA isolated from different soil microcosm on 34th day.

utilizing sterile soils once useful to evaluate the influence of the biotic environmental parameters on gene transfer when compared to non sterile systems. The present study done in pUC18 explains that free plasmid molecules in soil could transform soil bacteria (Lawrence, 2002).

This study was similar to the experiment of Neilson (Neilson et al., 1994), into the way of selecting the transformants of pUC18 plasmid DNA on agar plates. A biotic soil moisture condition was maintained where using of distilled water (Gebhard and Smaller, 1999). This experiment was done in sterile soil condition. In contrast to the observations made with plasmid DNA, the transformation efficiency of chromosomal DNA on sand was as high as in liquid (Lorenz and Wackernagel, 1987; Khanna and Stotsky, 1992). Transformation by DNA desorbing loam sand during 30 min of incubation with a cell-free filtrate of a comparison with the transformation frequency obtained directly in the microcosm, supporting the interpretation of a direct uptake of DNA from mineral grains (Chamier et al., 1993).

Thus the present study reveals the DNA retaining capability of plasmid DNA fluctuated in different soils. The results were encouraging in that extracellular DNA such as pUC18 plasmid could persist in the natural soil for more than a month and it retains the transformable molecular nature. Hence soils having such plasmid DNA are the potential source for the horizontal gene transfer among bacteria. Moreover these data explains that red soil has a good capability to retain the plasmid DNA and persistency DNA in soils as follows: Red > Loose sand > River > Black > Silt. In spite of this persistency of the DNA in these soils, correlations of the capability of persistency of DNA in these soils are still unknown to study the horizontal gene transfer mechanism.

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