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Bacterial Gene Transfer in Soil from a Hospital Environment

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Abstract: Of the three proposed mechanisms for the transfer of genetic material in Prokaryotes (transduction, transformation and conjugation) via Horizontal Gene Transfer (HGT), transformation has been considered as a less important event since it has low frequency and also the possibility of enzymatic degradation of naked DNA. Later it was revealed that binding of naked DNA to mineral surfaces not only stabilizes the DNA, it also protects the DNA from enzymatic degradation. Natural transformation has been reported as a means of HGT in different environments such as river water, spring water and some soil microcosm. On this line, the present study was aimed at analyzing the possibility of natural transformation as a means of HGT in a hospital environment. Our study reveals the presences of transformable cell free DNA in these environments and it also confirms the possibility of gene transfer via HGT among microorganisms in hospital environment.

Key words: DNA · soil · horizontal gene transfer · hospital environment

INTRODUCTION

Prokaryotes reproduction (binary fission), limits the genetic diversity, as the daughter cells receives identical copies of genomic DNA. In contrast the sexual reproduction in eukaryotes allows more diversity in genetic makeup possibly by random assortment of alleles and homologous recombination [1]. But it was estimated that up to 25% of typical bacterial genome is obtained from other cells [2]. This acquisition of foreign DNA may occur by any one of the three following mechanisms: transduction by viruses, natural DNA transmission by transformation and conjugation. Transformation is a different process than the other methods as it involves in the transmission of cell free DNA, which is available in all environments that may be excreted by living bacteria or liberated during autolysis [3].

Information on transformation has been obtained primarily during studies performed in the laboratory conditions that optimize the process [4-5]. Natural transformation has been neglected in the case of soil and natural habitats. It has viewed as an unimportant mechanism for the transfer of genetic material because of the fact that the naked DNA in the soil and natural environment were susceptible to microbial degradation. Apart from this, some other factors which influence the successful expression of foreign DNA include the sequence homology between the incoming DNA and the recipient cell, the frequency of transformation and importantly their physiochemical conditions that are needed for transformation process. Adsorption and binding of DNA to clay materials and other particulate materials may protect DNA against the degradation and enhances its persistence in the adverse condition [6]. The DNA becomes 100-1000 times more resistant to the enzyme DNase [7-8]. These persisted DNA becomes undetected i.e. cryptic in the absence of a host susceptible to transformation by the DNA. However in the presence of a susceptible host, the bound DNA could be taken up and expressed. The long term persistence of cryptic genes in the soil environment as well as the subsequent reappearing of these genes in the native and added population of the environment should be studied not only for assessing the risks associated with this but also for the study of role of transformation in the evolution of prokaryotes. Hence an attempt was made in this experiment to study the bacterial gene transfer in soil from a hospital environment.

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MATERIALS AND METHODS

Bacterial strain: Bacterial strain, *E. coli* DH5α, was obtained from School of Biotechnology, Madurai Kamaraj University, Madurai, India.

Media and antibiotics: Bacterial strain was grown in Luria Bertani medium. The bacterial strain was selected by supplementing antibiotics viz, ampicillin, chloramphenicol, ciprofloxacin, streptomycin, gentamycin and tetracycline. The broth cultures were grown at 37°C in a rotary shaker platform at the aeration speed of 150-200 rpm. Agar cultures were grown by incubating the plates at 37°C for 16 h.

Experimental design: A waste dumping ground of Government Rajaji hospital, Madurai, India, was selected for the study of natural horizontal gene transfer. Soil sample was collected aseptically from the site and 1g of soil is well mixed with sterile distilled water. Soil extract was serially diluted and spread on to Nutrient Agar (NA) plates containing different antibiotics such as ampicillin, chloramphenicol, ciprofloxacin, streptomycin, gentamycin and tetracycline. The resistant colonies were selected from these plates and tested once again on the respective antibiotic supplemented plates. DNA from these isolated resistant strains was extracted [17] and transformation in soil microcosm was done according to Nielson *et al.* [9]. Transformants were scored on the next day. Naked DNA extraction from the soil sample was done [18] and transformation of naked DNA was performed [17].

RESULTS AND DISCUSSIONS

Different antibiotic resistant strains were isolated from the soil of the experimental site and they were enumerated and listed (Table 1). No resistant strains were isolated against Gentamycin Tetracycline genes in them. The emergence of antibiotics resistant strains confirms the existence of antibiotic resistant genes in them. Transformants obtained in soil microcosm against different antibiotics were enumerated and listed (Table 2). Transformants obtained from the naked DNA transformed with competent cell suspension of laboratory strains of DH 5α were shown (Fig. 1).

Present study demonstrates that transformants could occur in natural environments. Transformation in a variety of aquatic environment and soil microcosm has been reported already [19]. Soil microcosm studies have been performed by simulating the natural environment by adding *E. coli* cells and DNA extracted from natural antibiotic resistant strains. Natural soils are generally poor in nutrients which severely limit the possibilities for bacterial growth and soil sterilization is known to release the nutrients which are presumably differs in quality and quantity between soils [15].This microcosm study also reveals that *E. coli* can develop natural competence. This is in accordance with Baur *et al.*, [14] who reports that natural competence is possible in river water. More number of transformants is obtained for streptomycin which showed the occurrence of higher flow of streptomycin resistant strains. Less number of transformants is obtained for chloramphenicol and ciprofloxacin and indicates the less frequency of gene transfer through HGT in natural environment. Present study also agrees with Orus and Vinas [10] in the fact that the horizontal gene transfer helps the pathogenic strains

Table 1: Number of bacterial strains isolated against different antibiotics

Antibiotics used	Number of bacterial strains isolated (n=3)
Ampicillin	3
Ciprofloxacin	4
Chloramphenicol	5
Streptomycin	6
Gentamycin	Nil
Tetracycline	Nil

Table 2: Number of transformants obtained for different antibiotics in soil microcosm

Source of DNA	Antibiotics used	Number of transformants (n = 3)
DNA isolated from	Ampicillin	824
Antibiotic resistant bacterial	Ciprofloxacin	1160
Strains	Chloramphenicol	2520
	Streptomycin	4000

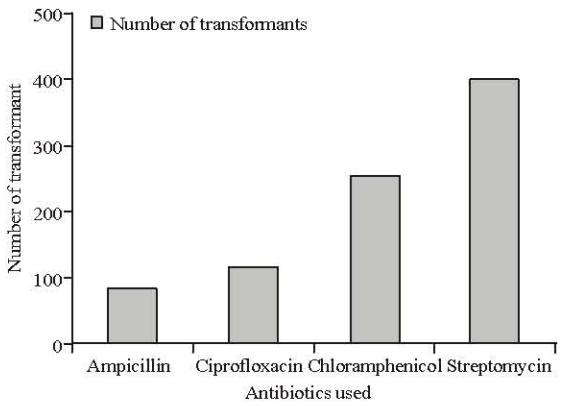

Fig. 1: Number of transformants obtained from cell free DNA against different antibiotics

of *Neisseria* which acquires the altered portions of *pen* A gene-which encodes Penicillin binding proteins from the commensals.This study also explains that the deliberate release of wastes from hospitals along with infected microbes without proper sterilization is leading to the emergence of new drug resistant strains and also the acquisition of new genes which results in more complexity of genomes. This ultimately leads to the microbes being resistant to drugs and create medical problems.

Kroll *et al*., [12] reported the natural genetic exchange between *Haemophilus* and *Neisseria*, important human pathogens that commonly colonize the nasopharynx. There is an intergeneric transfer of chromosomal genes such as two *Haemophilus* core uptake sequences were unexpectedly found forming the terminator of *sod C* in *Neisseria meningitides* and sequence analysis reflects that this virulence gene located next to IS 1106 arise from the horizontal gene transfer from *Haemophilus*. Similarly Nikolich *et al*, [13] demonstrated that natural transfer of a tetracycline resistant gene *tet Q*, has occurred between bacterial genera that normally colonize different hosts. They explained this using Bacteroides, the predominant genus of the human colonic microflora and a distantly related genus *Prevotella* another predominant microflora of rumens and intestinal tract of farm animals. They also found that identical *tet Q* sequences were found in a number of isolates differencing in taxonomy and geographic origin which indicates that the extensive natural gene transmission has occurred.

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