



Transformation of Penicillin Resistance Plasmid from Penicillin Resistant soil Bacteria to E.coli DH5 α

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Abstract:

Transformation, an active uptake of free DNA by bacterial cells and the heritable incorporation of its genetic information is one of the most adventurous mechanism that can be manipulated with the technology to produce genetically modified and manipulated organisms that can fulfil the needs of the user.. Only more recently has it been considered that transformation may be a powerful mechanism of horizontal gene transfer in natural bacterial populations. With the advent of the technologies like RFLP, Amplification, Electrophoresis the act of addition and deletion of the genes into the native genome of the organisms has been taking up a major hand. The current work is an attempt to add the important antibiotic resistant R plasmid having penicillin resistance gene from the natural host to the *E. coli* cells which lack the property of Penicillin resistance. Once the transformation is performed the cells are cultured and tested for their antibiotic resistant property. If the cells are successfully transformed they too exhibit the resistant action against the antibiotic penicillin.

Keywords: transformation, plasmid, penicillin gene, vector

Introduction:

"Transformation" is simply the process where bacteria manage to "uptake" or bring in a piece of external DNA (somehow or another)^[1]. Usually, this process is used in the laboratory to introduce a small piece of PLASMID DNA into a bacterial cell .Nearly everything we know about transformation and competence comes from experiments carried out on four species: *S. pneumoniae*, *Bacillus subtilis*, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*. Thus we can view the process of transformation as passing through four stages: (i) Competent cells sporting a limited number of DNA-binding receptors attach duplex DNA to the cell surface. (ii) The DNA is processed by endonucleolytic cleavage and,

usually, by exonucleolytic removal of one of the strands. (iii) The other strand is drawn through a pore into the cytoplasm, possibly in concert with the exonucleolytic degradation^[2]. The entering single strand of DNA undergoes homologous recombination with the chromosome, creating a new recombinant if the recombining partners carry different alleles. A plasmid is a DNA molecule that is separate from, and can replicate independently of, the chromosomal DNA^[3]. They are double-stranded and, in many cases, circular. Plasmids, usually occur naturally in bacteria, but are sometimes found in eukaryotic organisms.

There are two types of plasmid integration into a host bacterium: Non-integrating plasmids replicate as with the top instance, whereas episomes, the lower example, integrate into the host chromosome. Plasmids used in genetic engineering are called vectors^[4]. Plasmids serve as important tools in genetics and biotechnology labs, where they are commonly used to multiply (make many copies of) or express particular genes. Many plasmids are commercially available for such uses. The gene to be replicated is inserted into copies of a plasmid containing genes that make cells resistant to particular antibiotics and a multiple cloning site(MCS, or polylinker), which is a short region containing several commonly used restriction sites allowing the easy insertion of DNA fragments at this location^[5]. The plasmids are later inserted into bacteria by a process called transformation. Then, the bacteria are exposed to the particular antibiotics. Only bacteria that take up copies of the plasmid survive, since the plasmid makes them resistant.

Material and methods:

1. SAMPLE COLLECTION: The soil sample was collected from a hospital, LB Nagar, Hyderabad. One gram of soil was used for the isolation of bacteria.

2. Isolation of bacteria from soil by serial dilution method:

10 fold serial dilution methods has been employed for obtaining isolated colonies on to the nutrient agar plate so as to proceed for the further procedure.

3. Pure Plate Preparation: Based upon the colony morphology and plate characteristics the isolated colonies are selected that are plated onto the fresh media plate by the process of streaking. The media used here is the basic media Nutrient agar with the antibiotic penicillin added. This enables the growth of only the penicillin resistant strains on to the petri plate.

4. Sub Culturing on penicillin agar media:

25ml of nutrient agar media is prepared and autoclaved to which 20 ul penicillin was added. The media is than poured into petri plate and allowed to solidify. Aloop full of the selected bacterial colonies are taken and are inoculated onto the nutrient agar late. It was kept in incubator at 37°C, overnight.

5. Isolation of plasmid DNA:

To perform the transformation of the selected plasmid the plasmid must essentially be extracted out of the cells. This naked plasmid must than be used to transform the target cells. In this step the bacterial cells are collected from the broth and the plasmid DNA is extracted from the cells by routine laboratory protocols. This plasmid DNA which harbours the Penicillin resistant gene is later used for the transformation of the penicillin sensitive *E.coli* cells.

6. Transformation:

10ml of nutrient broth was prepared into which the Penicillin sensitive bacteria *E. coli* is inoculated. Broth was kept in incubator at 37°C, overnight. Once the penicillin sensitive cells are cultured they are later used for the preparation of competent cells before proceeding for the transformation.

Competent cell preparation: 1.5ml of culture was taken, centrifuged at 6000rpm for 10min. the pellet was taken and added 1ml of 80mM MgCl₂ and 20mM CaCl₂. It was kept in ice cold condition for 10min and Centrifuged at 6000rpm for 10min.the pellet containing the cells was taken, added 1ml of 100mM CaCl₂, and kept it in ice cold condition for 10min.Then Centrifuged at 6000rpm for 10min, the pellet was taken for transformation. Now these cells

are said to be competent as they are ready to receive an external naked DNA due to the delicate cell wall.

Transformation:

The pellet containing the competent cells was taken to which 1ml of LB broth was added. To this 5µl of Plasmid DNA was added which was extracted in the previous step from the penicillin resistant bacteria. The micro tube was kept in ice cold condition for 10min. Then it was incubated at 42°C for 90second or 2 min in water bath. It was kept in ice cold for 10min.To the pellet 1ml of LB broth was added and kept at 37°C for 1 hour in incubator. This step causes the entry of the necked DNA of the plasmid containing the penicillin resistant genes into the sensitive cells and the cells are said to be transformed.

Screening for the success of transformation:

To detect weather the transformation protocol was successful, a screening test called blue white screening is performed where there is a reference gene called beta galactosidase gene, which when blocked with the insertion of the desired gene would loss its expression. This loss of expression indicates a successful transformation. The loss of expression is read by the indicator IPTG. If the beta galactosidase gene is expressed than the color would be blue whereas if the transformation was successful and the beta galactosidase gene was disrupted the color of the colonies is white. Based upon the expressivity of the gene and color change the accuracy of the transformation is detected.

Results and Discussion:

Fig 1: Serial dilution for master plate preparation



The fig 1 shows the isolated colonies obtained on the master plate from which the cultures are selected fro the pure culture preparation

Fig 2: Pure culture preparation for the isolation of penicillin resistant bacteria



Extraction of Plasmid DNA from the resistant strains:

The penicillin resistant strains that were cultured on the penicillin media were used for the extraction of the plasmid DNA containing the penicillin resistant gene. The extracted plasmid was later used for the transformation

Transformation of the sensitive cells with the resistant plasmid extracted:

The Penicillin resistance plasmid extracted from the previous step has been used for transforming the penicillin sensitive *E.Coli* cells. This transformation imparts penicillin resistance to the sensitive *E.coli* cells.

Screening for the Accuracy of Transformation:

To detect the rate of success in the transformation protocol Blue white screening has been performed.

Fig 3: The petriplate showing all the cells to be white colonies indicating the success in the transformation protocol.



Conclusion:

The objective of the work is to apply the mechanism of natural gene transfer, transformation to impart the

antibiotic resistant nature to the sensitive cells. This technique being the natural mechanism would enable the sensitive strains to survive and fight against the antibiotic producing fungi. This technique may be employed in producing the resistant strains for these organisms that can act as an important bioreactors or sources for the production of industrially important enzymes or proteins.

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