



## Transformation of Antibiotic Resistant Gene from *Staphylococcus* Species to *E.coli Dh5 Alpha* Sensitive Strain

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### Abstract

Natural genetic transformation is the active uptake of free DNA by bacterial cells and the heritable incorporation of its genetic information. In the current work, Transformational insertion of an Antibiotic resistant gene (Ampicillin) from the native bacteria transformed into the sensitive strain of *E. coli* DH5 alpha. The bacteria were isolated from stagnant water and were tested for their antibiotic resistance. The resistant bacteria which showed a good growth in the antibiotic media is pure cultured and used for further analysis. The resistant bacteria were identified based on Biochemical analysis as per Bergey's manual. The bacterium was identified to be *Staphylococcus aureus*, whose blaR1gene was responsible for antibiotic resistance. The Plasmid DNA was extracted from the resistant strains and was inserted into the previously prepared competent sensitive *E. coli* DH5 alpha strains. The transformation was performed and the cells were further incubated for division and multiplication. The final cells obtained were tested for the transformation efficiency using Blue White Screening on X Gal media. The presence of all white colonies indicated a positive and successful transformation. In order to confirm the transformation efficiency at the molecular level the gene blaR1 was targeted. In case of successful transformation this gene must be available in the transformed culture. Thus the gene sequence was retrieved from the database and the primer was designed. The plasmid DNA from the transformed cells was extracted and was amplified using the above designed primer. Successful amplification and band in the post PCR Gel electrophoresis indicates a successful transformation at the molecular level. The work can be used for the transformation of the genes responsible for some special user interested qualities into the existing strains so as to meet the industrial demands and produce recombinant strains of bacteria.

### Keywords:

Transformation, Competent, Resistance, Plasmid, Genomic DNA

### Introduction:

#### Bacterial transformation

In molecular biology, TRANSFORMATION is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material (exogenous DNA) from its surroundings and taken up through the cell membrane(s). Transformation occurs naturally in some species of bacteria. For transformation to happen, bacteria must be in a state of competence which might occur as a time-limited response to environmental conditions such as starvation and cell density.

#### Plasmid:

Apart from genomic DNA in bacteria, it also contains extra-chromosomal DNA in them. This extra-chromosomal DNA is called as PLASMID. These plasmids are known to have the genes for the many antibiotics. There may be antibiotic susceptible genes and antibiotic resistant genes or even multi-drug resistant genes.

Normally bacteria cannot grow in presence of antibiotics as they are susceptible to them. These are called as antibiotic susceptible bacteria. But there are some bacteria that are capable of growing in presence of some antibiotics. These are called as antibiotic resistant bacteria.

#### *Staphylococcus* species:

*Staphylococcus* species which is resistant to ampicillin is a genus of Gram-positive bacteria. Under the microscope, they appear round (cocci), and form in grape-like clusters. It is a facultative aerobe.

The *Staphylococcus* genus includes at least 40 species. Of these, nine have two subspecies and one has three subspecies. Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms. Found worldwide, they are a small component of soil microbial flora. Ampicillin is an antibiotic that acts as an irreversible inhibitor of the enzyme trans peptidase, which is needed by bacteria to make their cell walls

It inhibits the third and final stage of bacterial cell wall synthesis in binary fission, which ultimately leads to cell lysis; therefore ampicillin is usually bactericidal.

In recent years, antibiotics resistant bacteria have become a great concern to the medical community. There has been a great increase in the number of species that have acquired resistance to antibiotics, as well as an increase in the kind of antibiotics. Antibiotics work in variety of ways, some antimicrobial agents inhibit bacterial cell wall synthesis. These agents include  $\beta$ -lactam compounds such as penicillin (e.g. penicillin G, ampicillin and methicillin), cephalosporins and as well as monolactams and  $\beta$ -lactamase inhibitors. Beta-lactams inhibit the final stage of murein synthesis. This, by some undetermined mechanism, triggers murein hydrolases to lyse the cell. A related group of antibiotics that prevent a different step in cell wall synthesis are the glycopeptides, vancomycin etc. Other agents have an antibacterial effect by inhibiting protein synthesis. Representatives of this group include the aminoglycosides, tetracyclines, macrolides and chloramphenicol which interfere with ribosome function. In addition, there are antibiotics that inhibit DNA synthesis, including quinolones, fluoroquinolones and sulfonamides.

## **Materials and Methods**

### **Sample collection:**

In order to obtain the resistant bacteria from its natural habitat a stagnant water zone has been selected near LB Nagar. 10ml of water have been collected from the stagnant water zone which was later used for the serial dilution and isolation of the microbes.

### **Screening Test for the Ampicillin resistant Starins**

The sample was subjected for serial dilution so as to obtain the isolated colonies on the master plate. However in order to obtain selectively the ampicillin resistant cultures the media used here was Nutrient agar with 0.5% Ampicillin. The plates were prepared and the sample above was spreaded onto the plates. The plates were incubated overnight for the growth of the colonies.

### **Preparation of liquid broth for the resistant colonies**

In order to obtain a uniform suspension of the antibiotic resistant cells the sub culturing was performed in liquid nutrient broth. The positive cultures were included in the broth and incubated overnight.

### **Isolation of Plasmid DNA from the resistant strains**

In order to obtain the resistant gene of the bacteria the complete plasmid has to be extracted. The extracted plasmid DNA can further be used for transformation. The plasmid containing bacterial cells are treated with a solution containing Tris HCl, EDTA and glucose to provide density to the cells and protect the DNA from pH and enzymatic changes. The solution is vortexed and the supernatant discarded. To the pellet SDS and NaOH were added that would enable the lysis of the cell wall and the maintenance of the alkaline conditions respectively. To the above solution 3M Sodium acetate was added and then centrifuged. The clear supernatant containing the DNA is separated and precipitated with isopropanol, the purification steps were performed later.

### **Preparation of Competent Cells:**

In order to perform successful transformation the host cell must be made competent by chemical and mechanical treatments. The alternative heat and cold shock treatment, along with the treatment with chemicals makes the cell weaker and competent to accept the external DNA. The Antibiotic sensitive Ecoli cells were subjected for this treatment.

### **Transformation Process:**

The pellet obtained in the competent cell preparation was taken and 2 $\mu$ l of plasmid was added which was previously extracted from the antibiotic resistant strain. Alternative heat and cold shock treatments are provided so as to pump the external plasmid DNA into the bacterial cells. Once the procedure is complete the liquid is added into a nutrient agar broth so as to allow for multiplication. The transformed cells will be generated after 24hrs of incubation.

### **Screening For transformation:**

After transformation, the efficiency of transformation was tested by performing screening tests. The screening tests can be performed by the following approach:

- Blue-white screening on X-gal media.
- Plasmid extraction from the transformed cells.
- Molecular level screening by sequencing the resistant gene using specific primers.

### **Blue White Screening on XGAL Media:**

Here a special technique based on color development was utilised to check for the transformation efficacy. The plasmid DNA contains X galactosidase gene whose product would digest the galactose enzyme present in the X Gal media. The IPTG added to the media would develop blue

colour in the presence of galactose whereas if the bacteria has produced the galactosidase enzyme which would digest the substrate from the media the colour of the cells would be blue due to IPTG.

#### **Plasmid DNA Extraction:**

This step would check for the presence of plasmid DNA among the cells that were initially non plasmid cells. The regular plasmid DNA extraction was performed for the culture to be tested. The final product obtained was purified and run on Agarose gel. The presence of band conforms that the bacterial cells were transformed and the Plasmid was present in the cells.

#### **Molecular Screening based on Sequencing:**

In order to confirm the efficiency of transformation at the molecular level, the gene sequence responsible for the resistance can be identified and primers would be designed. These are further used for the amplification. The amplification of the producer indicates a successful transformation whereas the contrast would represent no transformation.

#### **Results and Discussion:**

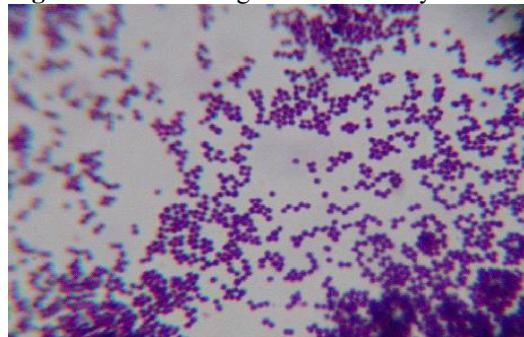
##### **Isolation of Antibiotic Resistant Bacterial Cells:**

The source sample was inoculated onto the antibiotic medium. The colonies produced were all resistant to the antibiotic used. One of the colony was selected and used for pure culture preparation. The Fig 1 shows the pure culture which is antibiotic resistant.

**Fig 1:** Pure culture of Antibiotic resistant bacterial sample

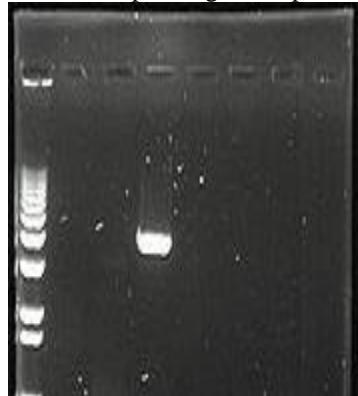


**Fig 2:** Gram's staining result to identify the culture



As per the above microscopic view after gram staining the bacteria has been found to be Gram Positive Cocci in groups. This culture can be processed further for the biochemical tests to identify the species.

**Fig 3:** The Gel electrophoresis picture showing the band corresponding to the plasmid.



##### **Transformation Efficacy Testing based on Blue White Screening:**

After performing the traditional transformation after the preparation of Compatible cells and plasmid extraction, the cells were incubated for complete transformation. The results of transformation are tested using Blue White screening based on XGal and IPTG Technique. The resulting all white colonies indicated a successful transformation in contrast to the blue colonies indicating an untransformed group.

**Fig 4:** The transformed cells on the X Gal Media



The above figure shows the screening of Blue white colonies on XGAL Media. The colonies all are white colour which indicates a successful transformation.

##### **Molecular level screening based on PCR Amplification:**

The plasmid genes were retrieved from NCBI database against which the primers are designed using Primer 3. These specific primers are used for the amplification of the transformed genome. The

amplified product is rechecked on the gel and the transformation is confirmed.

#### Gene Sequence retrieved form NCBI:

>gi|90969148:8668-10425 Staphylococcus aureus plasmid pSA1379, complete sequence  
ATGGCTAAATTACTAATAATGAGCGTAGTAAGCTTTG TTTATATTCATTGTTAGTATTTTTAGGTACATATT AAAACGCTATTTAATTATAGTTAAATTATAAAGTT GGTATCTAATCTGCTGGATAATCCCTTATCCC AATTAATTCCTTTATAAAATTATAATGTGAATA ATCAAGGCCAACAGTTGAAAGTAACAGATT GAACCATAACATAATACCACCAAAACCTTCAAGAG TTCACAACAGATATCCATAAGTTAATTGGGATTCAAT TGATAATATCTGCACAGTTATTGGATAGTTAGTTA TTATTTAAGTTAAATTGTTGAAAGCCTTATTATATC TAAATATTAAGAACAGTCACTTATCTAACGAA ATGAAAAAAATAAAATAGATACGATACTTTCAACC ATCAATATAAAAATATTGTGATCGAAAGCAGA GGCATTCACTCCAATAACTTTGGTATGGGAAAT ATATTATTGATTCTCATATTAAAAGTGTAA TTGATAAAAGACTAAAATATAATACTGCATGAATAT GCTCATGCTAAAATAGAGATACTTACATTAAATTAT CTTAATATCTCTTATTATTGAGTTACAATCCATT AGACATATTGTAAGAACAGATAATTGATGACAAT GAAGTAGAAGCCGATAGTTGCTCTAATAATATTAA CAAAATGAATTTAAACCTTATGCGGAATCTTATGG ACTCCGTTAAAACCCATTTCACACAAATAATAA TTAAGCCATTCTTAATGGTAAAAAATCATTACTCAA AAGTAGATTAATTAATATAAAGAACCGATCTGAAA AAGCAGTCAAAATTAATTCTAATTGTTATTGTATTGTT ACTTTTTTATCATGATAATTCAAAGCCAGTTTAATG GGGCAATCCTTAACTGATTATAATTAAAAAGCCCT ACAAGCGATTACCAATTAGTGAAGTAAAAT TTTGGTCAATAGTGGATCTTCGTTATGTACAGCAT GAAGAACAGACAATACTATTTATAATGAAAAGAA ATAGAAAAAGATATTCTCCTGATTCACATTACAT TTATTAGCACTGTTGGACTTGACCGTCATATTATAA GTGATAAAAATTACGTATGTCATGGAATCATAACAT TATCTTTGAGTCTTGGATAAGGAAACAAGATTAAA TACAGCAATGCAAAATTCACTGTTAATTGGTACTTCGAAC GTATTAGTAATCAAATACCAAGAAACTATCTCGGCT CAACTCAAGCAATTAAATTATGGTAATGAAAATTGG GAAGTTAAAAGCTATTGGATGGAAGTAGTTGAA AATATCTAATCTGAAACAAGTAATAGTTTAAAATA TGATGGAACAAAATAACCATTAGTAAAAAGCAAA GAATCAATTATCTCTTATTGATTAAGAAAAATGA AAAGTATGAACTGTATGGAAAACAGGTACAGGTATA GTAAACGGGAAGTATAATAATGGTGTGTTGTTAGGTT ACFTAATTACAAATCATGATAAGTATTATTGCTACA CATTATCAGATGAAACCCGTCGGAAAATGCTG AATTAATAAGTGAAGGAAATTAAAAGGAATGGGTGT TTTAAATGACCAATAA

#### Primer designed for the above sequence:

Left Primer: ACCACCAAAACCTATTCAAGAGT

Right Primer: CCTCTGCTTTCGAATCACAAT  
Amplification using PCR was performed to confirm the transformation.

PCR Product was sequenced using the same primers and the sequence obtained was as follows:

#### Sequence of amplicons obtained:

>Amplicons sequence

ATAAATACCAACCAACCTATTCAAGAGTTACAAACAG ATATCCATAAGTTAATTGGGATTCAATTGATAATATC TGCACAGTTATTGGATAGTTAGTTATTATTTAAG

GTTTAAATTTGAAAGCCTATTATCTAAATATT AAAGAAACAGTCACTTATCTAAACGAAATGAAAAAA AATAAAATAGATACGATACTTTCAACCATCAATATA AAAAAAAATTGTGATTGAAAG

#### Comparison of amplicon sequence with the database sequence:

data	1	2	3	4	5	6	7	8	9	10
Amplicons	1 -----									0
data	151 ATCCAAATTCTCTTTATAAAATTAAATGTAATCAAGC									200
Amplicons	1 -----									0
data	201 GCCCACAGTTGAAAGTAACGATTCGAAACATACATAATACCA									250
Amplicons	1 -----									10
data	251 CAAACCTTCAAGGTTGACACAGATTCGATAGTTAAATTGGGAT									300
Amplicons	11 CAAACCTTCAAGGTTGACACAGATTCGATAGTTAAATTGGGAT									60
data	301 TCAATTGATAATCTGCACAGTTATTGGATAAGTTAGTTATTATTT									350
Amplicons	61 TCAATTGATAATCTGCACAGTTATTGGATAAGTTAGTTATTATTT									110
data	351 AAGGTTAAATTGAAAGCCTTATTATCTTAAATTTAAAGAAC									400
Amplicons	111 AAGGTTAAATTGAAAGCCTTATTATCTTAAATTTAAAGAAC									160
data	401 AGTACCTTATCAACGAAAATGAAAAATAAAATGATCGATACCT									450
Amplicons	161 AGTACCTTATCAACGAAAATGAAAAATAAAATGATCGATACCT									210
data	451 TTCAACCCATATAAAATAATTGTTGAAAGCAGAGGCTAT									500
Amplicons	211 TTCAACCCATATAAAATAATTGTTGAAAGCAGAGGCTAT									250
data	501 TCAATCTCCAATAACTTTGGATGGAAATATTCTTGTGATTCTA									550
Amplicons	251 -----									250
data	551 GTTCAT 556									
Amplicons	251 -----									250

Emboss tool shows that a part of the data base sequence was completely present in the amplified product. This indicates that the transformation was successful.

#### Conclusion:

The current work aimed to study the role of genetic transformation in imparting a user desired quality into the known microbe for its use at the commercial scale. Here the sample collected was stagnant water from a nearby area at LB Nagar. The sample was used for the isolation of the organisms from the source. The organisms thus isolated were screened for the antibiotic resistance by culturing on a specialized media with antibiotic under study. The bacteria that were resistant are used for further study by replicating into a pure culture. The bacteria were used for the extraction of Plasmid as the antibiotic resistant gene would be present in the extra chromosomal material only. The extracted plasmid was used to transform the E coli DH5 alpha strain which were made competent in the earlier step. The transformed cells were then checked for the success in the technique. Several molecular techniques including PCR was used for the confirmation of transformation. The study also focused on the use of various molecular biology techniques in the identification of chirality in the DNA samples.

#### References:

- B H Olson, T Barkay and R R Colwell, "Role of plasmids in mercury transformation by bacteria isolated from the aquatic environment", Applied and Environmental Microbiology

2. Stanley N. Cohen, Annie C. Y. Chang, and Leslie Hsu, “**Nonchromosomal Antibiotic Resistance in Bacteria: Genetic Transformation of Escherichia coli by R-Factor DNA**”, PNAS- Vol. 69 no. 8 Stanley N. Cohen, 2110–2114
3. J. Maynard Smith, “**Estimating the minimum rate of genetic transformation in bacteria**”, Journal of Evolutionary Biology, Version of Record online: 11 DEC 2002
4. Nina Irwin and Kaaren A. Janssen, “**Molecular Cloning**”, Volume 1
5. The NCBI handbook [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2002 Oct. Chapter 18, The Reference Sequence (RefSeq) Project. Available from <http://www.ncbi.nlm.nih.gov/books/NBK21091/>
6. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG (2012) Primer3 - new capabilities and interfaces. Nucleic Acids Research 40(15):e115, Koressaar T, Remm M (2007) **Enhancements and modifications of primer design programPrimer3** Bioinformatics 23(10 ):1289-91