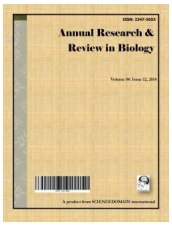

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Bacterial Transformation: What? Why? How? and When?

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Authors' contributions

All the authors contributed equally for the work. Author SC designed the study, prepared the framework and drafted the manuscript. Author MD helped in the preparation of table for comparison, critical analysis. Author HR managed the literature search. All authors read and approved the final manuscript.

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ABSTRACT

Transformation is one of the few options for horizontal gene transfer. Though transformation is a natural process, yet only a handful of the organisms are able to perform it naturally. The process of bacterial transformation is also a step of pivotal importance in the field of genetic engineering. The rDNA which is an exogenous DNA, is required to be inserted and expressed in the suitable host. However, majority of the hosts are unable to take up exogenous DNA. Thus, it requires some artificial methods too. The induction of the ability to take up such DNA is called competence. Several methods are being tried since the inception of its concept, but none of them are found to be universal. Therefore, there is a constant requirement of newer methods having advantage and efficiency over the existing ones. The conventional method involves $CaCl_2$ treatment followed by heat shock for achieving transformation. There is also employment of device oriented high end methods like electroporation or ultrasound mediated transformation etc. The efficiency of such methods varied widely and is often specific to a host. Thus, this review is focused on the necessity of transformation and various options that are available to researchers for performing bacterial transformation. It also attempts to strike a comparative study of the existing techniques.

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Keywords: Bacterial transformation; competence; gene transfer; electroporation; ultrasound; micro-shock wave.

1. INTRODUCTION

Bacterial transformation is an essential and unavoidable step in molecular biology and recombinant DNA technology. Though there are several methods of gene delivery in eukaryotic system, introducing the exogenous DNA in bacteria still remains a challenge. However, there are several limitations of the existing techniques that open up scope for further research. This review attempts to address the basic questions of transformation stating the basics, requirement and the options available. Highlight on the principle of several methods of transformation and a comparative study of the available techniques is also presented. Though the system, host and vector varied largely making it difficult to compare, the attempts in bringing higher efficiency for the process is to be attributed and acknowledged.

2. TRANSFORMATION: AN OVERVIEW

In this first section, we would attempt to understand the basics of transformation along with the system, importance, gap of knowledge and the alternatives available in performing bacterial transformation.

2.1 What is Transformation?

Transformation is a process of transferring exogenous genetic material from one source to a suitable host preferably bacteria. It is one of the methods of horizontal gene transfer apart from conjugation and transduction (Fig. 1). The exogenous DNA is also sometimes referred to as pure DNA in the environment that gets transferred to a bacterial host.

2.2 Why do we Need Transformation?

With the advent of molecular biological studies, there has been tremendous growth of genetic engineering and synthetic biology [1]. From *in vivo* diagnostics [2] to gene therapy [3], there is a constant requirement for the introduction of exogenous genetic material in the host. As a result, the process of transformation holds key step in molecular biology.

2.3 Qualitative versus Quantitative Transformation

Since, the report of Griffith's experiment in 1928, [4] there has been continuous advancement in understanding the phenomenon of transformation; however, his experiments revealed qualitative aspect. Hotchkiss [5] showed the precise quantification for the process in 1957. A published guest commentary by Lacks S.A., 2003 [6] gives a vivid description on the historical aspects, emphasizing breakthroughs and personalities involved. The present era has moved to quantitative transformation aiming to bring about better transformation efficiency than the existing one.

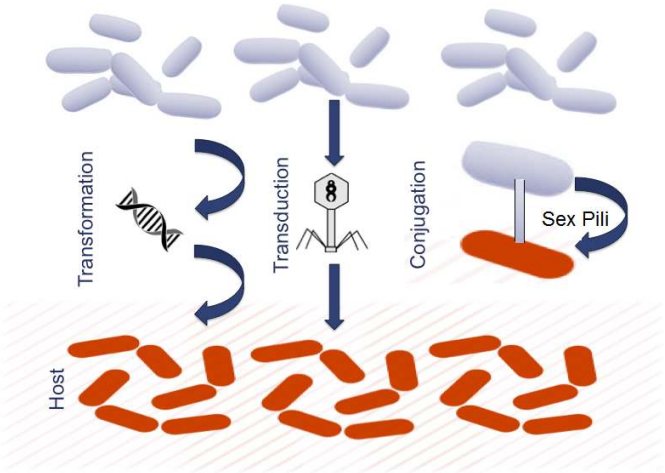


Fig. 1. The methods of horizontal gene transfer in bacteria

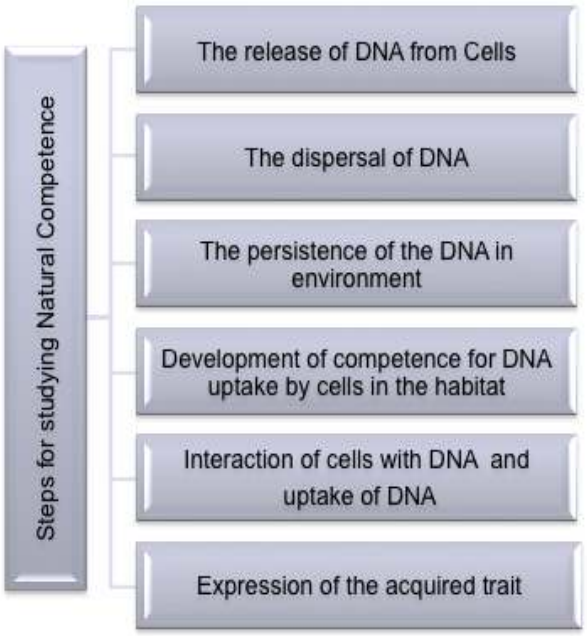


Fig. 2. Scheme of steps involved in studying natural transformation of bacteria

2.4 Natural Transformation

The process by which bacteria are able to take up and integrate exogenous free DNA from the environment is termed as natural transformation in bacteria [7]. The significance of natural transformation [8] lies in the ecological, evolution and adaptation aspects where such horizontal gene transfers by natural means promote genetic variation and even evolution of virulence factors [9]. Natural competence is also exploited as a genetic tool [10].

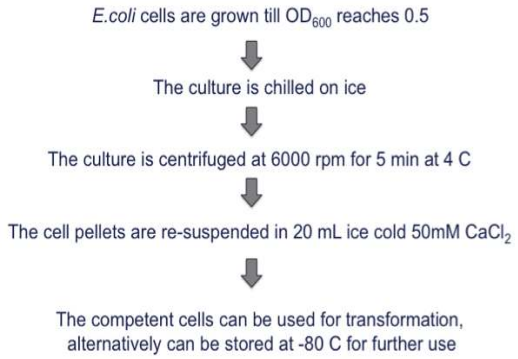
The complex process has been dissected to several events as shown in Fig. 2 for experimental investigation of strategies and techniques in environmental simulation [11]. Lorenz et al. [7] has given a detailed account on the bacterial gene transfer by natural genetic transformation in the environment.

2.5 Lacunae and Challenges

There has been no less effort spend on finding a suitable method for this key process, yet no single technology solves all the problems. Therefore a universal and optimal method still remains as warranted. This problem persists and challenges scientists working in related and allied field.

3. DEVELOPMENT OF COMPETENCE

The fundamental process of transformation depends on host as well as the method used. The competence of cell (either natural or artificially acquired) remains as the key for the process. Most of the techniques of bacterial transformation depend on the following methods for the development of competence in the host in order to facilitate the entry of exogenous DNA; 1) $CaCl_2$ treatment of the host cell and 2) PEG mediated bacterial transformation [12]. The protocol for $CaCl_2$ mediated method of competence development is illustrated in Flowchart 1.



Flowchart 1. The method of competent cell development by $CaCl_2$ treatment

4. OPTIONS AVAILABLE

The following section illustrates various options available in the transfer of exogenous DNA into a suitable host. The basic scheme of achieving competence in bacteria (Fig. 3) and common options available for transformation (Fig. 4) are illustrated.

4.1 Ultrasound Treatment

The ultrasound mediated method for gene transfer has recently been utilized in eukaryotic system [13,14]. The ultrasound mediated DNA delivery (popularly known as UDD) is a non-invasive technique without requiring any direct physical contact [15]. The principle is based on the cavitational effect, generating reversible porosity in the cell membrane [16]. The efficiency of the method was found to be even superior to conjugation and even electroporation, while delivering plasmid pBBR1MCS2 in *Pseudomonas putida* UWC1 [15] using a 40k Hz ultrasound apparatus. Further to this, low frequency ultrasound process was found to be more effective than the hi-frequencies. Temperature, plasmid and cell concentrations also affect the efficiency of the UDD process.

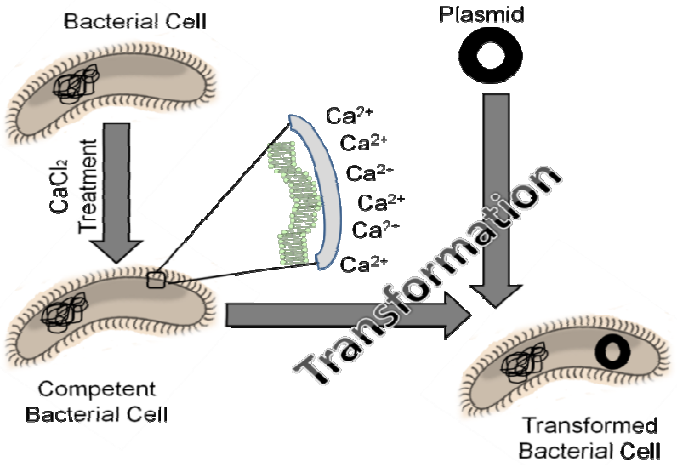


Fig. 3. The method of competence development by $CaCl_2$ treatment

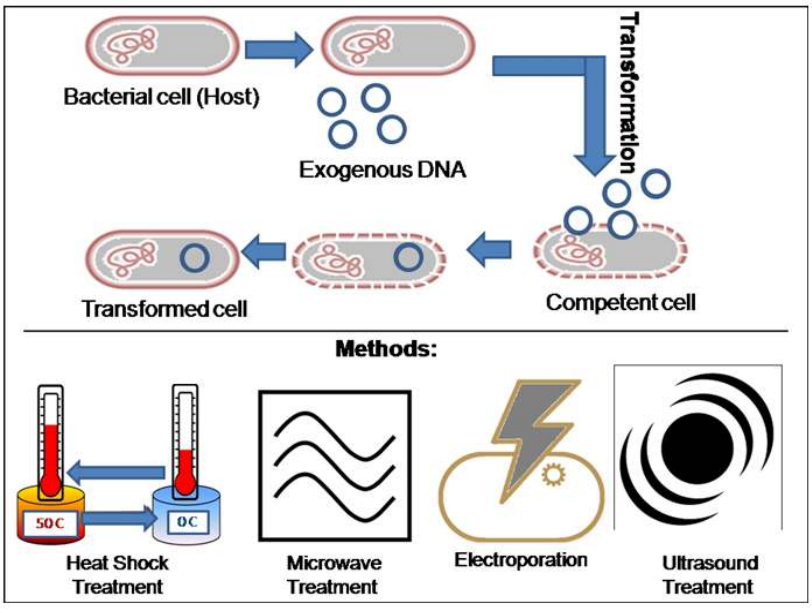


Fig. 4. Various methods available for bacterial transformation

4.2 Electroporation

The non competence of most of the bacterial strains led to alternative method of bacterial transformation employing electric current. The principle lies in the distortion of the membrane allowing uptake of the exogenous DNA. The formation of small localized holes due to the electric current aids the process of transformation. Though, initially it was employed for eukaryotes, it has long been targeted for the process of bacterial transformation [17]. This method is therefore independent of the host, both prokaryotes or eukaryotes [18] and posses high efficiency [19] and commercially viable [20]. The process of electroporation is apparatus driven employing a capacitor discharge device producing exponentially declining pulses in field strength between 125 to 6250 V/cm. Conductivity of the buffer and the choice of capacitor determines the duration of the pulse while the voltage is set gradually between 50-2500 V. Lakshmi Prasanna et al. [21] presents a detailed account on the principle and application of electroporation along with practical considerations.

4.3 Micro-shock Wave

The limitations of electroporation in terms of expenses and other technical feasibility have accelerated the development of newer technique giving rise to micro-shock wave based method for bacterial transformation. However, the method is device oriented and several prototypes have been designed so far. Micro-shock waves can be produced in a laboratory by pulsed laser beam focusing, electrohydraulic method, piezoceramic method, and controlled explosions. Application of underwater shock wave generator is also employed for bacterial transformation [22]. The transfer of micro-shock wave to the transformation vessel could be achieved via metal foil, making it an essential factor. However this method did not rule out the requirement of $CaCl_2$ treatment. An optimal plasmid and cell concentration also contributes significantly in obtaining higher transformation efficiency. In an attempt to utilize micro shock wave for bacterial transformation $p^{FPV-mCherry}$ expression vector of 5.37 kb encoding mCherry protein was transformed in *E. coli* DH5a, *S. typhimurium*, and *P. aeruginosa* [23].

4.4 Microfluidic System Based Electro-Transformation

Electroporation is a common gene delivery tool for bacterial transformation. In spite of, its broad range specificity, it is lagging behind with successful outcome of transformed cells. This is due to insufficient knowledge of an optimized protocol based improved designed tool to mitigate Joule heating and associated cell death. Microfluidic electroporation [24] is an ideal miniature form of DNA assembly (genetic transformation of microbes) that has come out as beginners choice. This method have many other advantages like single-cell manipulation and analysis, usage of nano or picoliters reaction volumes, thereby reducing costs, high-throughput execution of parallel experiments, automated routine liquid handling, integration of multiple biological processes in a single system, and programmability for complex protocols. This device mainly works with fluid channels based geometrically defined models with single cell or multi cell systems and applied largely recent days in characterization studies of bacteria and recombinant expression systems [25].

4.5 Electrospray Technique

This process was previously known as electro hydrodynamic atomization. Electrospray employs an electric field to disperse and accelerate liquid droplets or fine particles. An advantage of this method is that, it does not require prior treatment for competent cells. The principle employs gold nanoparticles to be conjugated with DNA that can increase the momentum of the plasmid during electrospray, resulting in higher amount of penetration through cell wall. The permeability of cellular membrane can be increased without disruptive damage, that serves the purpose. The gene encoding green fluorescence protein (GFP) containing expression vector pET30a- GFP were transformed in *E. coli* BL 21 (DE3) strain employing this method [26]. The cell growth stage, size and amount of nanoparticles sprayed emerged as important factors determining transformation efficiency.

4.5.1 Modifications over existing methods

Several techniques along with their modifications and optimizations are available, yet the search for method for transforming ‘difficult to transform bacteria’ is persistent.

Table 1. Comparative study of existing methods of bacterial transformation

Sl no	Method	Transformant /Host	Parameters	Remarks	Ref
1	Electroporation (F Factor based)	*pBAC108L/E. coli* DH1OB	Efficiency	10^6 transformants per µg of DNA	[30]
			Pre Bench Work/ Instrumentation required	PFGE; high mol.wt. DNA prepared	
			Special remark	>300 Kbp of human DNA cloning and stable maintenance even after 100 generations	
2	Electrotransformation - microfluidic system	Ampicillin resistance and Green Fluorescent Protein (GFP) encoding DNA plasmids (Parts Registry K176011)/ *E. coli* DH10β and *E. coli* K12 wild	Efficiency	Approx. four times greater / 1.19×10^7 CFU / µg of DNA	[1]
			Pre Bench Work/ Instrumentation required	Microfluidic system / computational models coupled electric, hydrodynamic, and thermal responses in COMSOL Multiphysics v5.1/ MicroPulser™	
			Special remark	Small sample volume needed / flexible transformation platform for both prokaryotes and eukaryotes.	
3	Electroporation	pBR322, pKT230 and pAM401/ *E.coli* HB101, *E. faecalis* OG1X, *P. putida* KT2440	Efficiency	10^4 and 10^5 per µg DNA transferred	[17]
			Any prior workbench/ /Instrumentation required	Growth of cells in glycine-containing medium to early log phase and washing and suspending the cells in osmotic stabilizing buffer	
			Special remark	Transformation system chosen in already established systems	
4	Carbon nanotubes mediated transformtaion – Yoshida effect	pET15b/ *Escherichia coli* DH5α,	Efficiency	15,000 transformants / 100 fold total increase through CNT based	[31]
			Pre Bench Work / Instrumentation required	Vortex mixing	
			Special remark	Enhancement of 10 fold through vortex mixing than sepiolite based CNT nanoparticles mediated transformation	
5	Electrospray of gold nanoparticles	pET30a-GFP and pET30a-Cherry) /*E.coli*	Efficiency	5-7 fold ; 2×10^6 cfu per µg plasmid DNA	
			Pre Bench Work /	Membrane integrity determination using BacLight	[26]

Sl no	Method	Transformant /Host	Parameters	Remarks	Ref
			Instrumentation required	Live/Dead Kit	
			Special remark	Do not loss activity of the biomaterials / noncompetent host cell is sufficient/ role of elctrospray buffer crucial	
6	Ultrasound mediated DNA transfer – cavitation leaded to	pBBR1MCS2/ *Pseudomonas putida* UWC1; *Escherichia coli* DH5α and *Pseudomonas fluorescens* SBW25	Efficiency	$9.8 \pm 2.3X10^6$ transformants per cell; (nine time more efficient than conjugation & four times greater than electroporation) $1.16\pm0.13X10^6$ and $4.33\pm0.78X10^6$ transformants per cell	[15]
			Pre Bench Work/instrumentation required	40 kHz ultrasound apparatus was a standard ultrasonic cleaning bath 375H (Langford Electronics Ltd., Coventry, UK) and 850 kHz ultrasonic bath (Meinhart Ultraschalltechnik, Germany, K80-5); Digital Test Thermometer(Brannan Thermometers, UK).	
				Optimal conditions: ultrasound exposure time of 10 s, 50mM $CaCl_2$, temperature of 22^0C, plasmid concentration of 0.8 ng/ml, *P. putida* UWC1 cell concentration of $2.5X10^9$CFU (colony forming unit)/ml and reaction volume of 500 ml.	
				Addition of calcium chloride to enhance plasmid transfer	
7	Osmolarity Electroporation	pUBxynA / *B. subtilis* IH6140, *B. licheniformis* S89	Efficiency	5000-fold/ $1.4X\ 10^6$ transformants per µg of DNA/ 400 fold with $1.8X\ 10^4$ transformants per µg of DNA	[32]
			Pre Bench Work/instrumentation required	Preparation of electro-competent cells in presence of high concentrations of the osmoticums, sorbitol and mannitol / electric field strength in the range of 12–23 kV/cm	
			Special remark	High osmolarity and high field strength may be a useful approach to improving the transformation efficiency of some gram-positive bacterial species which are able to grow in a high osmolarity medium.	
8	PEG	pBR322; YEp13 / Bacteria (*E.coli* -RR1; HB101; M94;	Efficiency	10^6--10^7 transformants/µg DNA in late log phase; 100%= 1.34×10^6 transformants/	

Sl no	Method	Transformant /Host	Parameters	Remarks	Ref
		ED8767 and yeast –*S. cerevisae* RC-5)		µg pBR322 plasmid DNA. 200-1000 transformants were recovered per µg YEp13 plasmid DNA	[12]
			Any prior workbench/ instrumentation	10 min treatment with DNA for *E. coil* and 1h for yeast in hypertonic medium	
			Special remark	Without cell removal/ functions optimally at 22^0C and is markedly inhibited at 6℃. For yeast the method of choice w hen extremely large numbers of transformants are not required.	
9	Chitin Nanowhiskers	*E.coli*/ pUC18 and *E.coli*/ pUC19	Efficiency	2.1 x 10^6 cfu/ µg of DNA	[33]
			Any prior workbench / instrumentation	Plasmid DNA adsorbtion onto the chitin nanowhiskers/ use of agar gel and polystyrene stick.	
10	Microshock waves	*Addgene plasmid 20956/ E. coli* DH5α, *S. typhimurium*, and *P. aeruginosa*	Efficiency	1 X10^{-5} transformants/cell	[23]
			Any prior workbench/ instrumentation	Polymer tubes of 30 cm/ Invitrogen PureLink HiPure Plasmid Midiprep Kit., NanoDrop ND-1000 Spectrophotometer and agarose gel electrophoresis. (PVDF)-coated needle hydrophone, oscilloscope	
				$CaCl_2$ treatment	
11	Local Heat shock	Ampicillin resistance and a Fluorescent Protein encoding Plasmid DNA / *E.coli* DHα	Efficiency	One-thousandth of volume is required to obtain transformation efficiencies as good as or better than conventional practices	[34]
			Any prior workbench/ Instrumentation	chemically competence of bacterial cells, with $CaCl_2$	
				on-chip local heat shock device with fabrication of micromachining process- MEMS heat shock device, a fluidic cap using molded polydimethylsiloxane (PDMS)	
			Special remark	small volume of sample usage / lab on a chip technology	

1. Role of spontaneous current oscillations during electro spray: In this method, exogenous DNA was transformed into thermophilic anaerobes with the help of spontaneous current oscillations during high efficiency electrotransformation, achieving higher efficiency [27].
2. The role of ethanol during $CaCl_2$ mediated bacterial transformation was elucidated which showed the reduction of transformation efficiency with increase in ethanol concentration. The mechanism showed the ethanol mediated leaching of lipopolysaccharide resulted in the hindrance in the uptake of exogenous DNA [28].

For a more detailed account on comparison, optimization of various methods and factors affecting the transformation of *E. coli* a paper by Chan et al. [29] can be referred. The methods of competent cell generation by $CaCl_2$ based method $MgCl_2$-$CaCl_2$ based method, comparison of heat shock incubation times, effect of various culture media and efficiency of various strains has been experimentally compared in terms of efficiency.

5. COMPARATIVE STUDY

A comparative study between various methods of bacterial transformation is always warranted that might significantly help in the selection of the correct method for intended study. The variations of system, insert, host etc do not allow uniformity in the comparison, since multiple parameters are to be considered. Despite of such, the results obtained by various research groups can throw light on the effectiveness of the process and its efficiency.

Table 1 enlists various methods of bacterial transformation from published literature and attempts to analyze them based on common parameters.

6. CONCLUSION

Bacterial transformation is an unavoidable step in molecular biology as well as genetic engineering. With the advancements of the gene manipulation technology, there is a wide variety and size range of recombinant DNA finding a way to be inserted in a suitable bacterial host. The non competence of most of the bacterial strains raises a challenge to overcome the obstacle. Concepts and technologies across domains have significantly contributed in the search of a solution. Most of the techniques are initially dependent of the generation of competence and later uses various technologies to increase the transformation efficiency. Electric impulse, ultrasound, micro-shock wave, electrospray etc aided the process of bacterial transformation bringing in higher transformation efficiency. However, there are several limitations to the existing techniques generating scope for further research in this topic.

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COMPETING INTERESTS

The authors declare that they do not have any competing interests.

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