FEMS Microbiology Letters, 364, 2017, fnx081

doi: 10.1093/femsle/fnx081
Research Letter

RESEARCH LETTER – Biotechnology & Synthetic Biology

Finding a facile way for the bacterial DNA transformation by biosynthesized gold nanoparticles

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One sentence summary: Biogenic gold nanoparticles mediate transformation in both Gram-negative and Gram-positive bacterial cells.

Editor: Andre Klier

ABSTRACT

The major problem encountered during genetic manipulation of bacteria is the inability to get transformed because of their natural non-competency. In this study, to overcome this problem, a cost-effective method was developed by combining the properties of gold nanoparticles (GNPs) and the Yoshida effect. Various parameters, including GNP:plasmid ratio, pH and time, were optimized for stability of the GNP–plasmid conjugate. With non-competent Gram-negative cells, the efficiency ranged between 0.1 and 0.45 $\times$ 10^4 transformants μg^{-1}, while the range was (0.02–0.2) $\times$ 10^4 transformants μg^{-1} with Gram-positive bacteria. GNPs can serve efficiently as a vehicle for better transformation in bacteria.

Keywords: gold nanoparticle; transformation; competent; plasmid; bacteria; optimization

INTRODUCTION

Bacteria have widely been manipulated genetically for several biotechnological applications. With the help of genetic engineering, they can be programmed to synthesize desired protein, enzymes, antibodies and drugs. The most important part of genetic manipulation is insertion of the gene of interest into the bacterial host. Therefore, the emphasis has always been given on discovering efficient means of transformation by which DNA is introduced into bacteria (Mendes *et al.* 2015). Several techniques such as electroporation, chemical transformation, biolistic transformation, electron spray and sonoporation have been widely used for transformation studies (Okubo *et al.* 2008; Yoshida and Sato 2009) but all have their own limitations (Aune and Aachmann 2010). There is a need for chemically competent and electrocompetent cells for chemical transformation and electroporation, respectively. Competency is found naturally in some bacteria, but most species require a tedious procedure to make them competent. While chemo-transformation is limited due to the tediousness and limitation of host range, electroporation is a costly affair (Naqvi *et al.* 2012). Similarly, biolistic transformation and sonoporation cause injury to the tiny cells (Rivera *et al.* 2014). Recently a new phenomenon called the Yoshida effect (Yoshida 2007) has been discovered which is based upon sliding friction force at the interface between hydrogel and an interface-forming material when a colloidal solution and bacterial cell is mixed. A few successful transformations have been reported in *Escherichia coli*, *Pseudomonas* and *Streptococcus* species based upon this phenomenon (Yoshida 2007; Rodríguez-Beltrán *et al.* 2012). The Yoshida effect has been observed in multi-walled carbon nanotubes, maghemite (γ-Fe_2O_3), chrysotile and α-sepiolite, having nanodimensions of 10–50 nm (Yoshida 2007; Tan, Fu and Seno 2010; Rodríguez-Beltrán *et al.* 2012; Elabed *et al.* 2016).

Gold nanoparticles exhibit several unique properties which make them a wonderful candidate for several applications (Mishra *et al.* 2014; Kumari *et al.* 2016). Their facile synthesis, ease of functionalization with several molecules,

Received: 1 February 2017; **Accepted:** 21 June 2017

biocompatibility and inherent non-toxicity (Li *et al.* 2016) provide them with an additional advantage to be used as drug and nucleic acid cargo molecules. Several successful attempts have been made to deliver drugs and nucleic acid using gold nanoparticles as carriers in animals (Fong *et al.* 2016) and have gained popularity as non-viral vectors. The electrospray of gold nanoparticles has also been attempted successfully for transformation of non-competent *E. coli* (Lee *et al.* 2011). The nano-sized particles enter cells through endocytosis and can easily overcome the nuclear pore complex barrier owing to their small size (Naqvi *et al.* 2012). Though there are reports of using gold nanoparticles in animal and plant transformation, their ability for gene delivery in both Gram-positive and Gram-negative bacteria has been little explored.

In this study, using characteristics of the Yoshida effect and properties of gold nanoparticles, successful attempts were made to transform both competent and non-competent cells.

MATERIALS AND METHODS

Strains, plasmids and nanoparticles

Plasmid pLITMUS 38i and pCAMBIA 1304 were used in this study (Mishra *et al.* 2012). Competent *E. coli* DH5α was used because of its ampicillin and kanamycin sensitivity.

The gold nanoparticles (GNPs, spherical, 7–24 nm) used were biosynthesized by *Trichoderma viride* as in earlier provided conditions (Kumari *et al.* 2016). The stock solution of GNP (10 μg mL^{-1}) was prepared and diluted further for experiments.

Non-competent Gram-negative strains *Pseudomonas putida* (NBRI RA, NBRI C19), *Ochromobactrum* sp. and Gram-positive strains *Paenibacillus lentimorbus* (NBRI CHM12), *Rhodococcus baikonurensis* (NBRI D9) and *Bacillus macerans* (NBRI SN13) used in this study were procured from the lab repository of the Division of Plant Microbe Interactions, CSIR-NBRI. Strains were checked for their sensitivity with kanamycin and ampicillin. Toxicity of GNP was checked by colony-forming units (CFU) studies (Mishra *et al.* 2014).

Parameter optimization for GNP–plasmid conjugation

GNP volumes (1, 2, 10, 25, 50 and 100 μL) were taken and mixed with 1 mL of plasmid (10 ng μL^{-1}). Different plasmid concentrations (1, 10, 25, 50, 75, 100, 250 ng) were conjugated with 25 μL GNPs. Effects of conjugation of GNP–plasmid and their subsequent release at different time intervals (0, 1 and 4 h) and at different pH (5, 7 and 9) were also studied and checked on 0.8% agarose gel (Naqvi *et al.* 2012).

Transformation of competent cells

For transformation of competent DH5α, GNP–plasmid (pLITMUS 38i) conjugates were prepared. In one set, plasmid (100 ng μL^{-1}) was mixed with 1, 2, 10, 25, 50 and 100 μL GNPs. In another set, 1, 10, 50, 100, 250, 500 and 1000 ng plasmid were mixed with GNPs (25 μL).

Further, prepared GNP–plasmid conjugates were mixed directly with 100 μL of DH5α (absorbance 0.4) to which 400 μL Luria Bertani Broth (LB) was added. The mixture was vortexed vigorously for 5 min and kept for 1 and 16 h (37°C, 180 rpm). One hundred microliter volumes of incubated cultures were spread on Luria Bertani Agar (LA) + ampicillin plates and observed for growth. Strains transformed with the heat shock method served as positive control while mixed with plasmid without GNP served as negative control.

Transformation of non-competent cells

Plasmids, pLITMUS 38i and pCAMBIA 1304 (100 ng μL^{-1}) were conjugated separately with GNP (25 μL). Each strain (50 mL) was grown in a 500 mL flask until the absorbance reached 0.4. Further, cells were centrifuged at 5000 rpm for 5 min and the pellet was suspended in 5 mL Nutrient Broth (NB). Suspended culture (100 μL) was mixed with GNP–plasmid conjugate. Heat shock (50°C) was given to the prepared mixture for 2 min and vortexed vigorously for 5 min. To this, 400 μL LB was added and further kept for 1 and 16 h (28°C at 180 rpm) and spread on culture plates as earlier. Blue white screening and kanamycin resistance were used as selectable markers. Strains transformed with conventional heat shock method served as negative control.

To confirm the transformation of pCAMBIA 1304, *gfp* gene amplification through semi-quantitative PCR (TC 3000, Genei, Bengaluru, IN) was done.

Mechanism of transformation

To get an insight into the mechanism of bacterial transformation, transmission electron microscopy was carried out following the protocol of Kumari *et al.* (2017). Briefly, GNP-mediated transformed *E. coli* DH5α were picked from the plate, washed with 0.1 M sodium cacodylate buffer and fixed with 2% paraformaldehyde and 2.5% glutaraldehyde mixture for 2 h at 4°C. Sample blocks were cut by an ultramicrotome (Leica EM UC7, Vienna, Austria), mounted and stained with uranyl acetate and lead citrate. Prepared samples were examined by a transmission electron microscope (Tecnai G2 Spirit, FEI, Hillsboro, OR, USA) equipped with a GatanOrius camera. Non-transformed *E. coli* DH5α colonies growing on Luria Bertani agar plate served as control.

RESULTS AND DISCUSSION

Strains and antibiotics sensitivity

All strains tested were kanamycin sensitive except *P. lentimorbus* (Table 1), so for transformation with pCAMBIA 1304, *gfp* gene amplification and kanamycin resistance were used as selectable markers. For transformation of *P. lentimorbus* with pLITMUS 38i, blue white colony screening was set as selection marker.

Cell toxicity assay

GNPs were not toxic to any strains at 1 μg mL^{-1}, the highest concentration used (Fig. S1 in the online supplementary material). GNPs are widely used in drug delivery and therapeutics because of their low toxicity (Arvizo, Bhattacharya and Mukherjee 2010) as compared to other metal nanoparticles.

Parameter optimization for GNP–plasmid conjugates

Maximum fluorescence of GNP–plasmid conjugates were observed with 10, 25 and 50 μL GNP (Fig. 1a) and 100 and 250 ng plasmid (Fig. 1b). The conjugates were clearly visible on agarose gel at different pH and time intervals (Fig. 1c).

The strong negative charge of nucleic acids makes cationic GNP a perfect partner for self conjugation (Ding *et al.* 2014).

Table 1. Antibiotics sensitivity and selection marker.

Strain	Ampicillin	Kanamycin	Selection marker
Pseudomonas putida (RA)	+	–	Kan/GFP gene amplification
Pseudomonas putida (C19)	+	–	Kan/GFP gene amplification
Ochromobactrum sp.	+	–	Kan/GFP gene amplification
E. coli DH5α	–	–	Am/Kan/GFP gene amplification
Bacillus macerans (SN13)	–	–	Kan/GFP gene amplification
Paenibacillus lentimorbus (CHM12)	+	+	Blue white selection/GFP gene amplification
Rhodococcus baikonurensis (D9)	+	–	Kan/GFP gene amplification

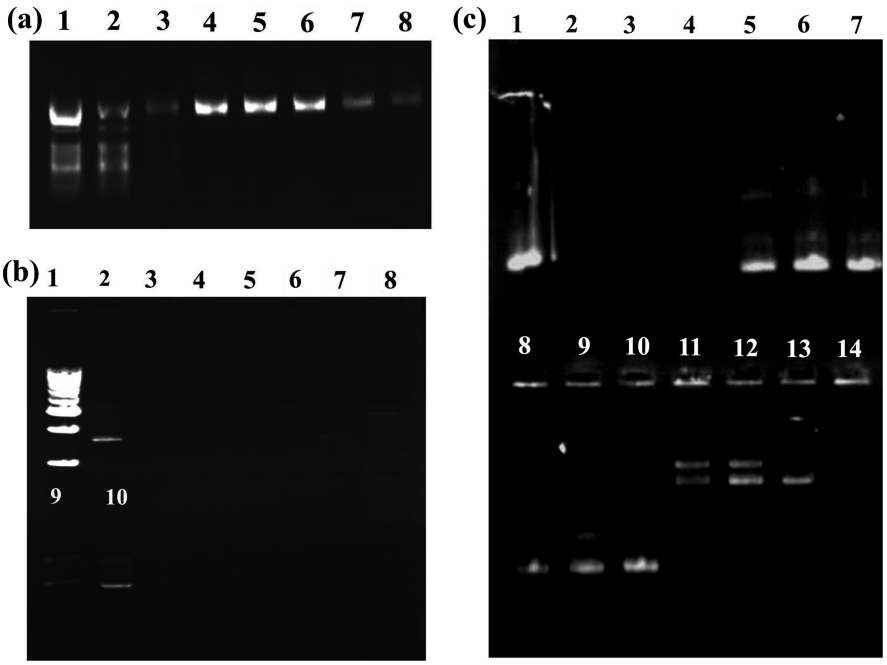

Figure 1. (**a**) Plasmid (100 ng μL^{-1}) with different GNP concentrations. Lane 1, plasmid alone. Lanes 2–8, plasmid:GNP at 1:1, 1:2, 1:10, 1:25, 1:50 and 1:100, respectively. (**b**) GNP (25 μL) with different concentrations of plasmid: Lane 1, 1 kb ladder. Lane 2, plasmid alone. Lane 3, GNP alone. Lanes 4–10: 1, 10, 25, 50, 75, 100, 250 ng plasmid, respectively. (**c**) Plasmid (100 ng μL^{-1}) with GNP (25 μL) at different pH. Lane 1, plasmid alone. Lanes 2–4, GNP alone at pH 5, 7 and 9. Lanes 5–14, plasmid–GNP conjugate at pH 5, 7 and 9 at time intervals of 0, 1 and 4 h.

A number of factors, including surface charge, DNA nanoparticles ratio, pH and incubation time, can affect the self assembly of nanoparticles–DNA conjugate (Rana *et al.* 2012; Lu *et al.* 2015).

Competent bacterial transformation

Plasmid conjugated with 25 and 50 μL GNP showed maximum transformation efficiency (Fig. 2a) with DH5α, as earlier results obtained during parameter optimization.

Maximum transformants were obtained with plasmid (10 ng) and GNP (25 μL), which were 10-fold higher than obtained by heat shock method (Fig. 2b). Due to the small size of GNPs (Kumari *et al.* 2016) and their cationic nature (Muschiol *et al.* 2015), they readily crossed the cell membrane barrier and pulled the conjugated DNA also inside the cell without the need of heat shock.

Non-competent bacterial transformation

Plasmids of two sizes, pLITMUS 38i (2.8 kb) and pCAMBIA 1304 (12.4 kb) were used during transformation studies at two time intervals (1 h, 16 h).

With pLITMUS 38i, all tested bacteria showed transformation efficiency of (0.1–0.45) $\times$ 10^4 transformants (μg plasmid)$^{-1}$. Maximum efficiency was obtained with *E. coli* (0.45 $\times$ 10^4 transformants μg^{-1}) (Fig. 2c).

With pCAMBIA 1304, transformed bacteria were obtained after 16 h only ((0.2–1.7) $\times$ 10^4 transformants (μg plasmid)$^{-1}$) (Fig. 3a). Incubation time and size of plasmids played a crucial role in transformation of bacterial cells. pLITMUS 38i, having smaller size, transformed after 1 h of incubation with two bacterial strains with higher efficiency while larger sized plasmid pCAMBIA 38i transformed only after 16 h of incubation.

The maximum transformation efficiency was obtained with *E. coli* (0.17 $\times$ 10^4 transformants μg^{-1}). No transformed colonies were obtained by heat shock method in any tested conditions. Further, to confirm the transformation, colony PCR was carried out for *gfp* gene amplification. Bands at 700 bp were obtained with all tested (Fig. 3b) bacteria confirming the transformation of bacteria with pCAMBIA 1304 carrying the *gfp* gene.

Transformation of Gram-positive bacteria is a hurdle because of the inability of DNA to cross the thick cell wall and lack of type IV pili (Yoshida and Sato 2009; Muschiol *et al.* 2015). Though transformation of Gram-negative bacteria was rapid and more efficient as compared to their Gram-positive counterparts with

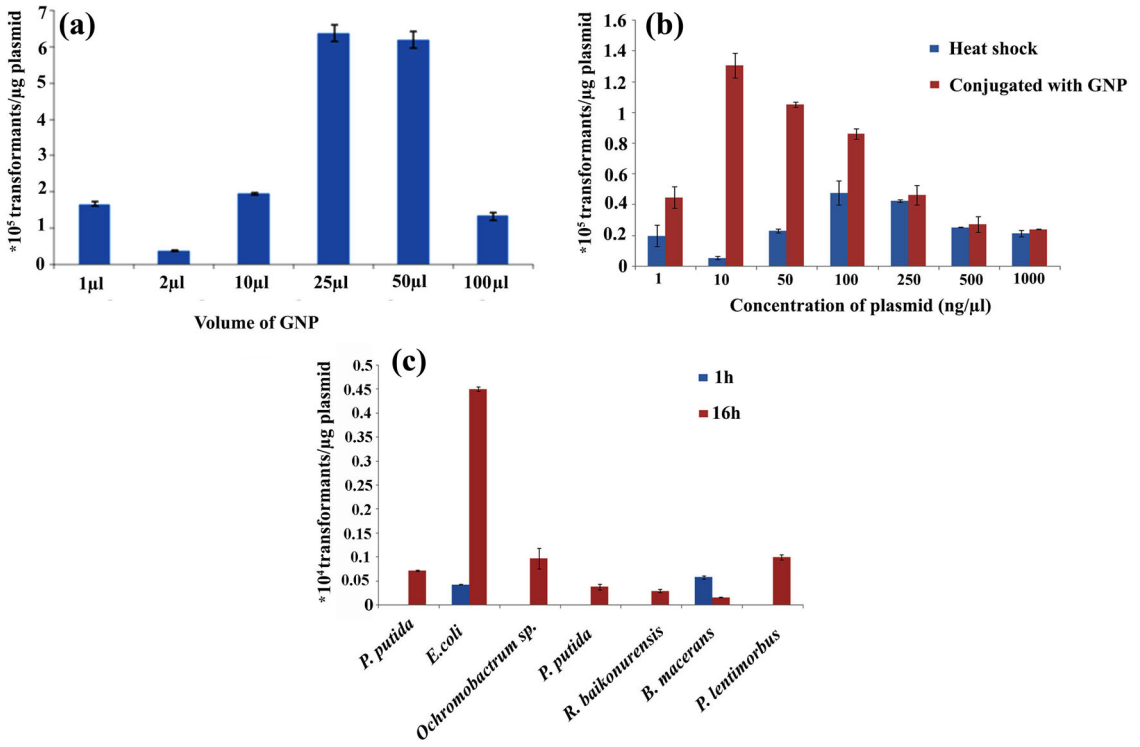

Figure 2. Transformation efficiency with pLITMUS 38i of DH5α with different amounts of GNP (**a**), of DH5α with different concentrations of plasmids (**b**), and with different non-competent strains at different time intervals (**c**).

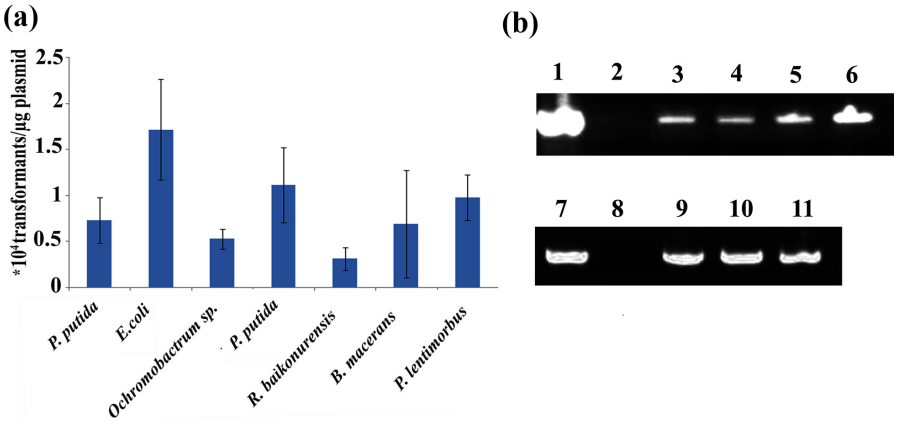

Figure 3. (**a**) Transformation efficiency of pCAMBIA 1304 after 16 h. (**b**) PCR amplification of *gfp* gene. Lanes 1 and 7, positive control; 2 and 8, negative control. Lanes 3–6 and 9–11, PCR of bacteria strains RA, C19, *Ochromobactrum* sp., DH5α, SN13, CHM12 and D9, respectively.

GNP conjugate, all Gram-positive strains showed uptake of DNA as indicated by positive colonies.

In this method, the properties of small GNPs and frictional force of the Yoshida effect were provided by preparing GNP–plasmid conjugates and vortexing them at 50°C for 2 min. This resulted in the creation of pores in the plasma membrane because of the sudden rise in temperature and minimizing the repulsion of plasmid and bacterial membrane, similar to the heat shock method (Panja *et al.* 2008).

Mechanism of GNP-mediated transformation

Transmission electron microscopy was carried out to decipher the mechanism of GNP-mediated bacterial transformation. Granules in the range of 10–20 nm were observed in the GNP-mediated transformed cell (Fig. 4b) that were absent in the control cell (Fig. 4a). When the transverse sections of treated transformed cell were cut, particles accumulated inside the bacterial cytoplasm were clearly visible (Fig. 4c) indicating the formation of nanopores because of the smaller size of GNPs and the Yoshida effect at a sudden increase in temperature. Similar results were obtained by Rojas-Chapana *et al.* (2005) for plasmid delivery in *E. coli* by multi-walled carbon nanotubes.

CONCLUSION

This study demonstrated a simple and reliable method for DNA transformation overcoming the limitations of conventional methods. Using GNPs and the Yoshida effect, and by modulating parameters, transformations in Gram-positive and

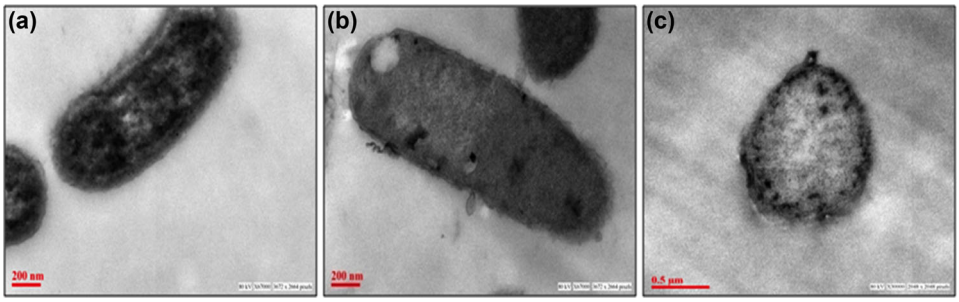

Figure 4. Transmission electron microscopy images of *E. coli*: (**a**) non-transformed control cell, (**b**) GNP-mediated transformed cell, (**c**) transverse section of GNP-mediated transformed cell.

Gram-negative non-competent cells were achieved. Though the transformation efficiency obtained with non-competent cells was lower than the conventional method used with competent cells, this resulted in the transformation of both Gram-positive and Gram-negative cells.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

ACKNOWLEDGEMENTS

MK thanks CSIR for awarding her a Senior Research Fellowship. We thank Ms. Nidhi Arjaria, CSIR-IITR, Lucknow for helping us in TEM analysis.

FUNDING

This study was partially supported by a network project of CSIR 'Root SF BSC0204'.

Conflict of interest. None declared.

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