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Use of β-galactosidase (*lacZ*) gene α-complementation as a novel approach for assessment of titanium oxide nanoparticles induced mutagenesis

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ABSTRACT

The mutagenic potential of titanium dioxide nanoparticles (TiO_2-NPs) of an average size 30.6 nm was investigated using β-galactosidase (*lacZ*) gene complementation in plasmid pUC19/*lacZ*$^-$ *Escherichia coli* DH5α system. Plasmid pUC19 was treated with varying concentrations of TiO_2-NPs and allowed to transfect the $CaCl_2$-induced competent DH5α cells. The data revealed loss in transformation efficiency of TiO_2-NPs treated plasmids as compared to untreated plasmid DNA in DH5α host cells. Induction of multiple mutations in α-fragment of *lacZ* gene caused synthesis of non-functional β-galactosidase enzyme, which resulted in a significant number of white (mutant) colonies of transformed *E. coli* cells. Screening of mutant transformants based on blue:white colony assay and DNA sequence analysis of *lacZ* gene fragment clearly demonstrated TiO_2-NPs induced mutagenesis. Multiple alignment of selectable marker *lacZ* gene sequences from randomly selected mutants and control cells provided a gene specific map of TiO_2-NPs induced mutations. Mutational analysis suggested that all nucleotide changes were point mutations, predominantly transversions (TVs) and transitions (TSs). A total of 32 TVs and 6 TSs mutations were mapped within 296 nucleotides (nt) long partial sequence of *lacZ* gene. The region between 102 and 147 nt within *lacZ* gene sequence was found to be most susceptible to mutations with nine detectable point mutations (8 TVs and 1 TSs). Guanine base was determined to be more prone to TiO_2-NPs induced mutations. This study suggested the pUC19/*E. coli* DH5α *lacZ* gene α-complementation system, as a novel genetic approach for determining the mutagenic potential, and specificity of manufactured NPs and nanomaterials.

1. Introduction

Extensive applications and an upsurge in synthesis of nanoparticles (NPs) in recent years pose a serious concern due to potential human and environmental risks [1–3]. Rapid commercialization of engineered NPs for use in a variety of industrial, chemical, medical and diagnostic applications presents a challenge to NPs manufacturers and regulators related to the safety of nanomaterial products. Indeed, the biological responses to NPs may exceed those elicited by micron-sized particles [4,5] due to their small size, high number per given mass, large specific surface area, and generation of free radicals [6]. It has been shown that NPs of titanium dioxide (TiO_2) and silica can enter the nucleus [7,8] where they produce intranuclear protein aggregates that can lead to inhibition of replication, transcription, and cell proliferation [9]. The titanium dioxide nanoparticles (TiO_2-NPs) have been reported to induce oxidative stress, and DNA damage in Chinese hamster ovary (CHO) cells, Syrian hamster embryo fibroblasts, human lymphoblastoids and human bronchial epithelial (BEAS-2B), and human amniotic epithelial (WISH) cell lines [10–15]. These NPs have also shown to induce apoptosis in human lymphocytes, U937 human monoblastoid cells, A549 alveolar epithelial cells, NRK-52E, LLC-PK1 renal proximal cells, and A431 human epidermal cells [16–19].

TiO_2-NPs are reported to be toxic to both Gram-negative and Gram-positive bacteria, and the antibacterial properties of these NPs have been exploited in water treatment reactors [20,21]. However, limited studies have been performed on the assessment of the mutagenic potential of TiO_2-NPs [22–24] in bacteria. Warheit et al. [25] have used the Ames bioassay to test the potential mutagenicity of TiO_2 using various bacterial strains. Pan et al. [26] compared the mutagenicity of several metal oxide NPs (TiO_2, Al_2O_3, Co_3O_4, CuO, and ZnO) to define the toxic potential of this group of nanomaterials. Recently, Kumar et al. [27] demonstrated the weak mutagenic potential of ZnO and TiO_2-NPs in *Salmonella typhimurium* strains TA98, TA1537 and *Escherichia coli* (WP2*uvrA*) of Ames test, underscoring the possible carcinogenic potential similar to certain mutagenic chemicals. Many chemical mutagens and carcinogens produce characteristic DNA adducts and base changes, allowing a correlation between the mutational spectrum of the

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mutated genes in tumors and the carcinogenic agents [28]. Therefore, it is important to identify the mutational specificity in DNA, damaged by a vast range of available metal oxide NPs and nanomaterials. In this study, we have chosen nano-sized TiO_2 (30.6 nm), a naturally occurring mineral, extensively being used in domestic and cosmetic products including food colorants, ceramics, antifouling paints, coatings, and additives in pharmaceuticals [29,30] to investigate its genotoxicity and mutagenicity.

To the best of our understanding, no significant information on mutational analysis encompassing the extent and nature of TiO_2-NPs induced mutations is available in literature. Further, the Ames tester strains designed for assessing the chemical mutagenesis have some limitations with NPs. Particularly the strains do not account for the physical interactions of the particles with DNA that could lead to mutation, which is contrary to the mechanism of chemical induced mutations, and are also known to generate false positive results [31,32]. This has prompted us to utilize the pUC19/*lacZ*$^-$ *E. coli* DH5α complementation as a simple and effective assay system for assessment of NPs induced mutagenesis. The mutational analysis was performed within the partial sequence of a 296 nt long α-fragment of *lacZ* gene insert in a commercially available 2.686 kb vector plasmid pUC19. The TiO_2-NPs induced DNA lesions in plasmid were processed (repaired and replicated) with in the *E. coli* cells, and validated the functionality of plasmid pUC19/*E. coli* DH5α complementation assay, as a useful tool for studying the NPs induced mutagenesis.

2. Materials and methods

2.1. Materials

Powdered polyhedral rutile TiO_2-NPs, average size 30.6 nm, was a kind gift from the Centre of Excellence in Nanomaterials, Department of Applied Physics, AMU, Aligarh, India. TiO_2-NPs in Milli-Q water were sonicated using Pro Scientific Inc., USA for 15 min at 40 W to obtain a homogeneous suspension before the treatments. Plasmid pUC19 was prepared using QIAGEN plasmid Mini Kit, QIAGEN, USA. Ampicillin, $MgCl_2$, $CaCl_2$, 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) and isopropyl β-D-1-thiogalactopyranoside (IPTG) were purchased from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals were of analytical grade.

2.2. Effect of TiO_2-NPs on viability of E. coli DH5α

Freshly grown cells (2×10^8 CFU/ml) of *E. coli* DH5α (F$^-$, *endA1*, *glnV44*, *thi-1*, *recA1*, *relA1*, *gyrA96*, *deoR*, *nupG*, Φ80d*lacZ*ΔM15, Δ(*lacZYA-argF*), U169, *hsdR17*(rK$^-$ mK$^+$), λ$^-$) stock were treated with increasing concentrations of 5, 10, 25, and 50 μg/ml of TiO_2-NPs in a total volume of 1 ml for 6 h at 37 °C. The aliquots of 0.1 ml of the treated and untreated control cells were serially diluted and plated on LB agar plates. The plates were then incubated at 37 °C for overnight. The colonies appeared on the plates were recorded as colony forming units (CFU) per ml. The percent survival was plotted as a function of increasing concentrations of TiO_2-NPs.

2.3. Preparation of competent E. coli cells

Cells from the freshly grown 100 ml culture (OD_{600} ~0.5) of *E. coli* DH5α were harvested by centrifugation at 2000 rpm for 8 min at 4 °C. The cells were gently suspended (1/10 volume) in 10 ml of ice-cold 10 mM $CaCl_2$ and subjected to centrifugation at 2000 rpm at 4 °C for 8 min. The cells were resuspended in 10 ml of cold 10 mM $CaCl_2$ and then kept on ice for 30 min. Cold suspension was again centrifuged at 2000 rpm for 8 min at 4 °C, and the cells were resuspended in 1 ml of cold 10 mM $CaCl_2$. The competent cells were used immediately for transformation.

2.4. Treatment of plasmid DNA with TiO_2-NPs and transformation in competent DH5α cells

Plasmid pUC19 DNA (~100 ng) in Tris–HCl buffer (0.1 M, pH 7.5) was treated with increasing concentrations of 5, 10, 25, and 50 μg/ml of TiO_2-NPs, in a final volume of 50 μl at 37 °C for 2 h. The untreated and treated plasmid DNA were then incubated separately with 100 μl of competent (*lacZ*$^-$) *E. coli* DH5α host cells each at 4 °C for 30 min, followed by 90 s heat shock at 42 °C. To this nutrient broth (500 μl) was added and the cells were further incubated at 37 °C for 1 h for expression of antibiotic resistance. Cells were then plated on Luria broth (LB) agar plates containing ampicillin (60 μg/ml), X-gal (40 μg/ml) and IPTG (0.1 mM). The plates were incubated overnight at 37 °C. After incubation, the plates were transferred to 4 °C to allow the blue collared colonies to develop. Colonies were then counted to score the number of mutants (white colonies) versus the number of normal (blue) colonies of Lac$^+$ bacteria. This genetic event, in which the α fragment of *lacZ* gene derived from plasmid and ω fragment derived from the host cells constructs a functional gene producing β-galactosidase, is called α-complementation [33]. The *E. coli* DH5α host cells, which undergo α-complementation, are easily recognized due to formation of blue colonies in the presence of a chromogenic substrate, X-gal. However, any mutation in the *lacZ* gene sequence of the plasmid, invariably produces an amino terminal fragment incapable of α-complementation, and therefore, results in formation of white (mutant) colonies.

2.5. Isolation of plasmid DNA from the mutants and its sequencing

Plasmid pUC19 DNA was isolated using QIAGEN plasmid Mini Kit, QIAGEN, USA, accordingly as suggested in the manufacturer's protocol. The purity and amount of DNA was determined by measuring the absorbance at 260 nm and ratio at 260/280 nm using Nanodrop 800 spectrophotometer (Thermo Scientific, USA). The quality of the isolated DNA was further validated on 1% agarose gel. The plasmid DNA was then subjected to sequencing of a fragment of the *lacZ* gene insert in vector plasmid using M13 reverse primer (5′CAGGAAACAGCTATGAC3′) on an automated ABI Prism®377 DNA sequencer (Applied Biosystems Inc., CA, USA) at DNA sequencing facility of the King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

2.6. Multiple sequence alignments and mutation analysis

Partial sequences of a 296 nt *lacZ* gene in mutated plasmids isolated from ten randomly selected bacterial mutants (white colonies) were compared with the sequence of the untreated control. Multiple sequence analysis was performed by using CLUSTALW with default parameters [34]. The point mutations and their distribution across the specific region of *lacZ* sequence were manually identified.

3. Results

3.1. E. coli (DH5α) host cell viability and transformation with TiO_2-NPs treated pUC19 plasmid

E. coli DH5α cells treated with TiO_2-NPs at concentrations of 5, 10, 25, and 50 μg/ml for 6 h exhibited a decrease in the colony forming ability. The percent survival of TiO_2-NPs treated cells declined in a concentration dependent manner from 83.1 ± 3.9 to 38.1 ± 3.1 at 5 and 50 μg/ml, respectively (Fig. 1). Whereas, the pUC19 plasmid DNA treated *in vitro* with identical concentrations of TiO_2-NPs have not exhibited any alterations in its molecular size. Virtually no excision of DNA leading to size reduction or speciation was detected on an agarose gel, as compared with the untreated control (results not shown). The effect of TiO_2-NPs treated plasmid pUC19 DNA on transformation efficiency, using $CaCl_2$ induced competent *E. coli* cells has also been determined. The pUC19 DNA after treatment with TiO_2-NPs at a similar concentration range of 5, 10, 25, and 50 μg/ml were transfected into competent (*lacZ*$^-$)

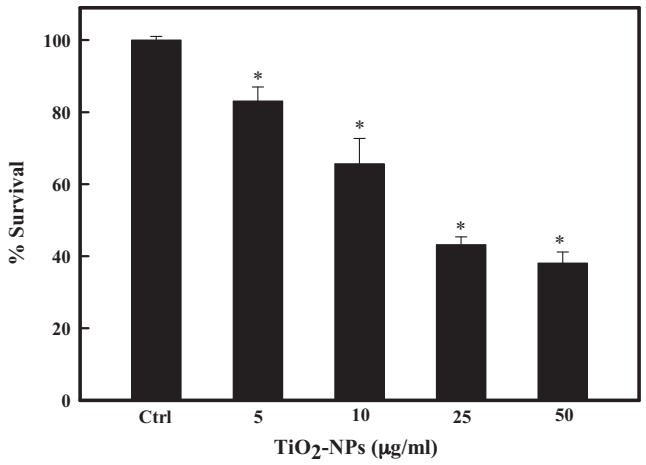

Fig. 1. Effect of TiO_2-NPs on viability of *E. coli* DH5α cells. Values are expressed as mean ± S.D. of three independent experiments done in duplicate. (*) Significant difference from control ($p < 0.01$).

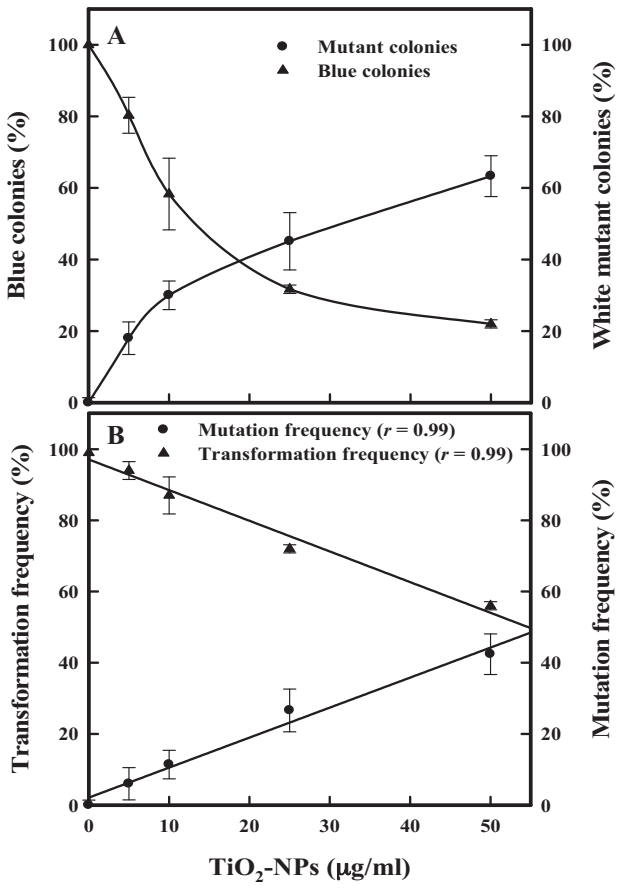

Fig. 2. Transformation capacity of TiO_2-NPs treated plasmid and induced mutagenesis. Panel (A) shows the TiO_2-NPs concentration dependent reduction in number of normal (blue) colonies with concomitant increase in the number of white (mutant) colonies on X-gal/IPTG medium. Panel (B) shows a linear decline in transformation frequency and an increase in mutation frequency as a function of TiO_2-NPs concentration.

E. coli DH5α host cells. The results show a concentration dependent reduction in transformation frequency. The data obtained through blue:white colony assay, relies on the compromised functional activity of β-galactosidase enzyme due to unrepaired or misrepaired base alterations (point mutations) in a partial segment of *lacZ* gene sequence, and thus help demonstrate the TiO_2-NPs induced mutagenesis. A greater number of white (mutant) colonies of transformants were observed with significantly lesser transformation frequency at increasing concentrations of TiO_2-NPs (Fig. 2A and B). The loss of transformation capacity was found to be linearly correlated ($r = 0.99$) with TiO_2-NPs concentration. The extent of reduction in bacterial transformation was recorded as 3.6 ± 2.5, 11.4 ± 5.2, 26.6 ± 1.15 and 42.4 ± 1.2% after 2 h treatment with 5, 10, 25 and 50 μg/ml of TiO_2-NPs, respectively.

3.2. Assessment of mutation profile of TiO_2-NPs in damaged pUC19 plasmid DNA

For determination of the types of mutations and their distribution upon processing of TiO_2-NPs damaged plasmid DNA in *E. coli* cells, the mutant (white) and control (blue) colonies of Lac^+ bacteria were randomly picked and the plasmid DNA isolated. Sequences of the partial α segment of the *lacZ* gene insert in plasmid were obtained using M13 reverse primer (5′CAGGAAACAGCTATGAC3′) from both the blue and white colonies. Sequences of 296 nt long α fragment of a *lacZ* gene consisting of 46 base pairs multiple cloning sites (MCS) derived from plasmids were tagged as TiO_2-W1 to TiO_2-W10 and compared with that of untreated control. The results show that all the DNA sequences from randomly picked mutant colonies exhibited multiple point mutations ranging from 2 to 5, except the colony (TiO_2-W3), which showed only 1 transition mutation at position 150 nt on treated plasmid. The randomly picked blue (control) colonies did not show any mutations.

Table 1
Profile of TiO_2-NPs induced mutations within *lacZ* gene in plasmid pUC19 DNA upon α-complementation in *E. coli* DH5α cells.

E. coli DH5 α mutants	Number of point mutations	Nucleotide changes	Mutation map (nucleotides position on *lacZ* gene)
TiO_2-W1	3	C—G	33
		T—A	135
		T—A	218
TiO_2-W2	3	T—A	23
		*T—C	127
		C—A	263
TiO_2-W3	1	G—T	150
TiO_2-W4	3	T—A	127
		*A—G	176
		*G—A	215
TiO_2-W5	4	A—T	19
		G—C	147
		T—A	180
		G—C	271
TiO_2-W6	2	C—G	107
		T—A	227
TiO_2-W7	3	A—T	102
		G—C	139
		*C—T	267
TiO_2-W8	5	A—T	06
		G—T	18
		A—T	64
		*C—T	66
		G—T	214
TiO_2-W9	4	G—C	106
		A—T	163
		G—T	198
		*A—G	232
TiO_2-W10	4	G—C	73
		C—G	99
		T—A	223
		A—T	256

* Indicates transition mutations.

The *lacZ* DNA sequences from mutants and control plasmids were aligned together and a consolidated map of TiO_2-NPs induced mutations was obtained through multiple sequence alignment analysis, as shown in Fig. 3. The mutational analysis revealed that all nucleotide changes were point mutations, mainly transversions (TVs) and transitions (TSs). Comparative nucleotide sequence analysis revealed a total of 32 transversions and 6 transitions mutations mapped along 296 nucleotides (nt) *lacZ* DNA sequence of the TiO_2-NPs treated pUC19 plasmids (Table 1). The region between 102 and 147 nt within *lacZ* DNA sequence was found to be most susceptible to mutations with nine detectable point mutations (8 TVs and 1 TSs) followed by the sequence regions between 214 and 250 nt and 6–33 nt with 6 point mutations (5 TVs and 1 TSs) and 5 TVs mutations, respectively. Amongst all the four nitrogenous bases, the guanine base was found to be the most susceptible to mutations (31% of detectable mutations) with 9 TVs and 1 TSs, followed by thymine (25% of detectable mutation) with 7 TVs and 1 TSs, adenine (25% of detectable mutation) with 6 TVs and 2 TSs, and cytosine (21% of detectable mutation) with 4 TVs and 2 TSs. Overall, the mutation spectra represented G:C – C:G transversions (12.5%), G:C – T:A transversions (12.5%), G:C – C:A transversions (3.1%) and G:C – A:T transition (3.1%).

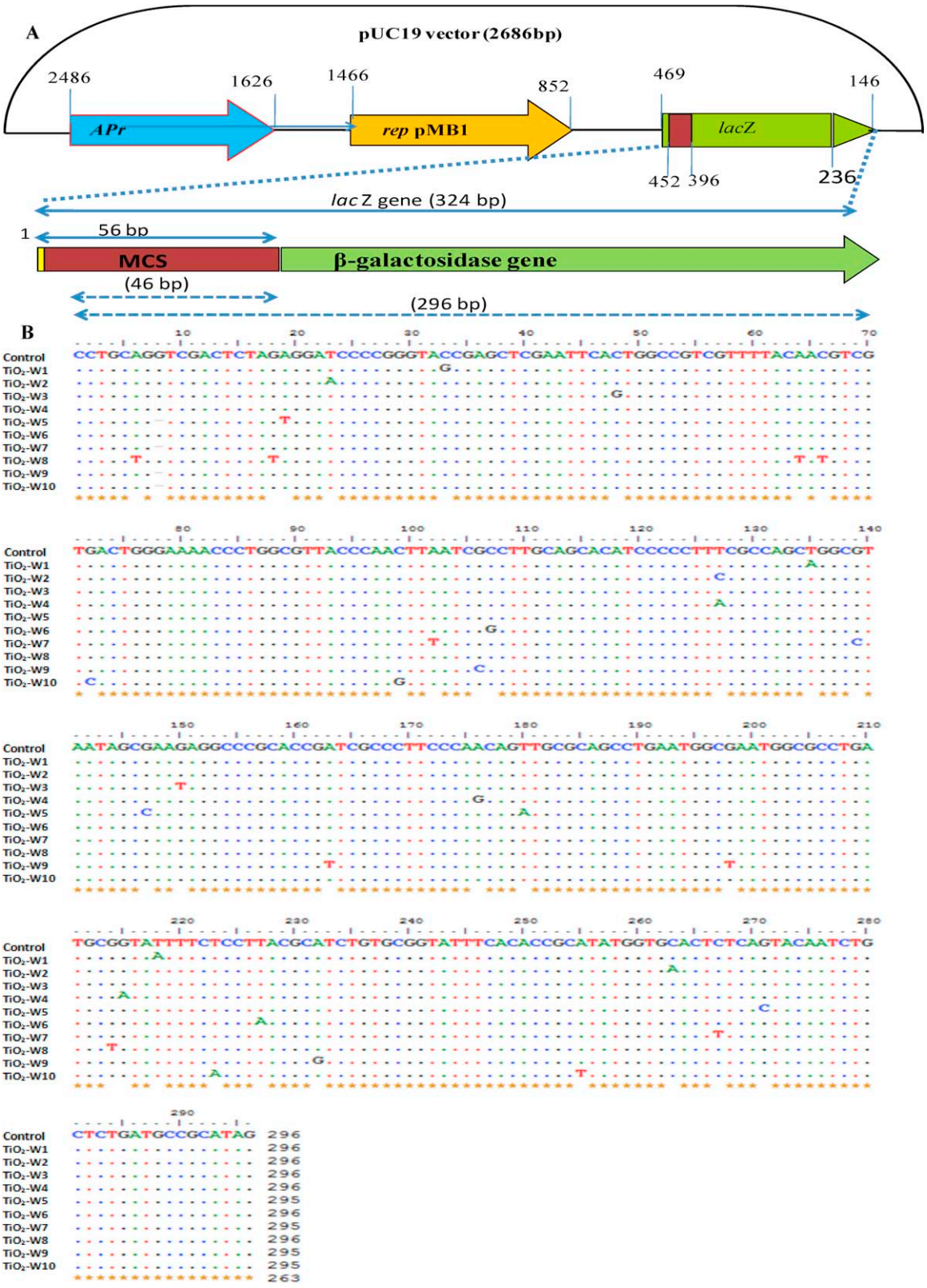

Fig. 3. Multiple sequence analysis of β-galactosidase (*lacZ*) gene indicating the map of TiO_2-NPs induced mutations. Panel A shows the genetic organization of *lacZ* gene in a segment of pUC19 plasmid DNA. The dotted lines represent the *lacZ* gene amplicons of 296 nt including a 46 nt fragment of multiple cloning site (MCS) sequence. The gene sequences from untreated control and ten randomly selected mutant colonies, designated as TiO_2-W1 to W10, were aligned using CLUSTALW with default parameters for detecting the point mutations.

4. Discussion

The mutagenic potential of TiO_2-NPs has been assessed through blue:white colony screening, and mutation mapping in a specific fragment of *lacZ* gene insert in pUC19 DNA processed in transformed *E. coli* DH5α host cells. The data revealed that the *in vitro* TiO_2-NPs treatment to plasmid pUC19 DNA resulted in a significant loss in transformation efficiency and increased mutagenesis. The survival data also explicitly suggested the toxic effects of TiO_2-NPs on *E. coli* DH5α cells, which supports the earlier toxicity studies on the Gram-negative and Gram-positive bacteria [35]. Indeed, the nano-sized TiO_2 at a higher concentration range of 100–1000 μg/ml has been reported as detrimental, and can disinfect the water containing 10^5–10^6 *E. coli* cells per ml within 30 min under illuminated conditions [20,21]. In our studies, the reduction in colony forming ability of DH5α at a selected dose range of 5–50 μg/ml TiO_2-NPs is attributed to decrease in viability due to cellular and genetic damage. Therefore, a similar dose range of TiO_2-NPs was chosen for treatment of plasmid pUC19, to determine the nature and extent of induced mutations. The results of transformation studies corroborates well with our earlier studies on reduction in transformation frequency of pBR322 DNA in *E. coli* AB1157 cells at pH 10.0, due to structural distortions and development of strand breaks (S1 nuclease sensitive sites) in super-coiled closed circular DNA [36]. The results also support the studies of Chakrabarty et al. [37] and Ahmad et al. [38], who have demonstrated significant reduction in transformation frequency of RP 1 DNA treated with DNase or by denaturation and sonication, and in plasmid Bluescript SK(+) treated with 200 μM resveratrol, respectively. Thus, the reduction in transformation of *E. coli* DH5α host cells with the treated plasmid DNA can be considered as an end-point for determining NPs toxicity, possibly due to induced DNA damage/structural alterations in plasmid DNA.

To elucidate the extent and nature of TiO_2-NPs induced DNA damage and/or mutagenesis, the molecular size of the untreated and TiO_2-NPs treated plasmids, isolated from control and mutant *E. coli* cells was analyzed on an agarose gel. Comparative analysis revealed no excision of DNA, which suggests that toxic effects of TiO_2-NPs on plasmid DNA *per se* encompass the structural alterations and/or point mutations. Wang et al. [22] through the hprt forward mutation assay and comet assay have demonstrated that the TiO_2-NPs are capable of inducing point mutations and DNA strand breakages. TiO_2-NPs are also known to induce oxidative damage, micronuclei formation, and increased hydrogen peroxide and nitric oxide production in human bronchial epithelial cells [14]. This may lead to mutagenesis, if the lesions in DNA due to chemicals or oxidative insults are not repaired or replicated with sufficiently high fidelity. In order to understand the TiO_2-NPs induced mutagenesis, we have employed plasmid pUC19-*E. coli lacZ* gene complementation system, as an effective one-step approach for delineating the mutational specificity of NPs. Similar approach using a pSP189 shuttle vector plasmid has been extensively used for determining the mutation profiles of a variety of chemicals such as nitric oxides, peroxinitrites, and sodium nitrite [39–41]. The procedure referred as *supF* assay generally involves two cycles of transformations involving the human cell line Ad293 and indicator bacterial strains *E. coli* MBM7070 or MBL 50 [41]. Many studies have employed this assay system for the advantage of picking up the mutations in *supF* gene by initially processing the damaged plasmid through a replication and repair machinery in cultured human cells followed by processing and screening in bacterial cells based on blue:white colony assay.

In this study, we have exploited a simple, one-step process for assessment of NPs-induced mutagenesis, where the TiO_2-NP damaged plasmid pUC19 DNA carrying an α-fragment of *lacZ* gene with the regulatory sequences and coding information for the first 146 amino acids of β-galactosidase, was allowed to be processed in *E. coli* cells. The carboxy terminal portion of the β-galactosidase is encoded by the bacterium *E. coli* DH5α, which is used as a host for such vectors. In isolation, neither the plasmid-coded nor the host-coded fragments are themselves active, but in combination they can associate to form an enzymatically active protein. It is feasible to replicate the damaged plasmid DNA directly in *E. coli*, which results in mutations being generated during DNA lesions processing (repair and replication) in bacterial cells [41]. Ahmad et al. [38] have demonstrated the development of point mutations (deletions/substitutions) in resveratrol treated Bluescript SK(+) plasmid, consequent upon the damage processing in the DH5α cells.

The types and distribution of TiO_2-NPs induced mutations in a partial (296 nt) fragment of *lacZ* gene insert were analyzed through sequencing and multiple sequence alignment analysis. The data revealed multiple point mutations, predominantly TVs and TSs in plasmid *lacZ* gene sequences. It is well known that point mutations and deletions are the possible factors for inactivation of tumor suppressor genes and activation of oncogenes [42]. The potential of TiO_2-NPs to elicit mutagenicity in metabolically more competent eukaryotes, as well as to induce frame shift mutations in TA98, TA1537 and oxidative mutagenic response in *E. coli* (WP2 uvrA) strains, both in presence and absence of S9 fraction, have been suggested earlier [25]. Pan et al. [26] have also reported an increase in mutagenic potential of TiO_2, when the particle size is reduced to lower than 100 nm. This corresponds well with our results, where TiO_2-NPs of primary particle size 30.6 nm exhibited significant mutagenesis.

Mutagenicity of TiO_2-NPs is a matter of health concern particularly for the reason that these NPs readily cross the cellular barrier and distribute in body organs [22,25]. Our recent study explicitly demonstrated the TiO_2-NPs induced changes in the levels of oxidative marker enzymes (GSH and catalase), intracellular ROS generation and consequent DNA damage in human amniotic epithelial (WISH) cells [15]. Earlier, Xu et al. [43] have suggested that TiO_2-NPs uptake by cells can induce kilo-base pair deletion mutations in a transgenic mouse mutation system. Peroxynitrite anions (ONOO—) have been identified as an essential mediator involved in mutations induced by TiO_2-NPs in gpt delta transgenic mouse primary embryo fibroblasts [44]. The nano-sized TiO_2 exposure has also been reported to increase the production of nitric oxide (NO) and oxidative DNA damage in human bronchial epithelial cells [14]. Most likely, NO reacts with superoxide anions ($O_2^{\bullet -}$) and can be rapidly converted into more reactive nitrogen compounds such as peroxynitrite (ONOO—) that can cause nitration of proteins, hydroxylation or nitration of DNA, and mutations [40]. The mutation spectra induced by ONOO— suggested the clustering of mutations within 5′region of the *supF* gene with 21-fold increased mutation frequency in treated pSP189 plasmid replicated in bacteria [40]. The pattern of mutations reported in *supF* gene in ONOO— treated plasmid pSP189, predominantly involves G:C – T:A transversions (65%), G:C – C:G transversions (28%) and G:C – A:T transitions (11%) in *E. coli* MBL 50 cells. Whereas, the mutation spectra induced by TiO_2-NPs revealed G:C – T:A and G:C – C:G transversions (12.5% each) in TiO_2-NPs treated pUC19 plasmid replicated in *E. coli* DH5α. Additionally, the NPs treatment induces T:A – A:T (21.8%) and A:T – T:A (18.7%) transversions, which were not recorded with ONOO— treatment [40] but were induced to the extent of 10% of total mutations with 1 M nitrite, pH 6.4 [39]. Since, guanine base in genomic DNA is highly susceptible to oxidative stress due to its lowest oxidation potential, therefore, G:C – T:A and G:C – C:G transversion mutations might occur more frequently under oxidative conditions. Also, the mutagenic lesion 8-oxo-7,8-dihydro-guanine (8-oxoG), which can pair with adenine (A) may cause G:C – T:A transversion mutations [44]. However, the molecular basis of G:C – C:G transversions is not clear. Furthermore, the

plausible reason for T:A – A:T and A:T – T:A transversions could be the SOS dependent mutator activity of recA⁻ *E. coli* DH5α. The increased SOS dependent mutator activity due to defect in the ∈ (3′ → 5′ proofreading) subunit of DNA polymerase III, has been attributed to a specific increase in A:T – T:A, A:T – C:G and G:C – T:A transversions [45]. The differences in mutation spectra induced with the oxidants like ONOO⁻, nitric oxide, sodium nitrite following replication of a damaged shuttle vector in dual hosts system (human Ad293 cells and *E. coli* MBL 50/MBM7070) *vis-a-vis* TiO_2-NPs damaged pUC19 vector, processed in *E. coli* DH5α alone, could be due to differences in the nature and extent of induced DNA damage, as well as the fidelity of DNA repair and replication machinery of the host cells.

5. Conclusions

The study provided a direct evidence of the TiO_2-NPs induced mutagenicity through DNA sequence analysis, and suggested that the metal oxide NPs induced mutations can be easily detected through the normal processing of DNA lesions by the repair and replication machinery of indicator *E. coli* strain. Thus, the plasmid pUC19-*lacZ* gene complementation in *E. coli* DH5α cells proved to be an important genetic approach for assessment of the mutation spectra of NPs.

Conflict of interest

The authors declare that there is no conflict of interest.

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References

[1] G. Oberdorster, E. Oberdorster, J. Oberdorster, Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles, Environ. Health Perspect. 113 (2005) 823–839.
[2] R.D. Handy, T.B. Henry, T.M. Scown, B.D. Johnston, C.R. Tyler, Manufactured nanoparticles: their uptake and effects on fish – a mechanistic analysis, Ecotoxicology 17 (2008) 396–409.
[3] Y.L. Hu, J.Q. Gao, Potential neurotoxicity of nanoparticles, Int. J. Pharm. 394 (2010) 115–121.
[4] P.J. Borm, D. Robbins, S. Haubold, T. Kuhlbusch, H. Fissan, K. Donaldson, R. Schins, V. Stone, W. Kreyling, J. Lademann, J. Krutmann, D. Warheit, E. Oberdorster, The potential risks of nanomaterials: a review carried out for ECETOC, Part. Fibre Toxicol. 3 (2006) 11.
[5] A. Nel, T. Xia, L. Madler, L. Ning, Toxic potential of materials at the nano level, Science 311 (2006) 622–627.
[6] I. Lynch, K.A. Dawson, S. Linse, Detecting cryptic epitopes created by nanoparticles, Sci. STKE 14 (2006) 14.
[7] M. Geiser, B. Rothen-Rutishauser, N. Kapp, S. Schurch, W. Kreyling, H. Schulz, et al., Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells, Environ. Health Perspect. 113 (2005) 1555–1560.
[8] L. Liu, T. Takenaka, A.A. Zinchenko, N. Chen, S. Inagaki, H. Asada, Cationic silica nanoparticles are efficiently transferred into mammalian cells, Int. Symp. Micro-Nano Mechatron. Hum. Sci. 2 (2007) 281–285.
[9] M. Chen, M.A. Von, Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO_2 nanoparticles, Exp. Cell Res. 305 (2005) 51–62.
[10] E. Bermudez, J.B. Mangum, B.A. Wong, B. Asgharian, P.M. Hext, D.B. Warheit, J.I. Everitt, Pulmonary responses of mice, rats, and hamsters to subchronic inhalation of ultrafine titanium dioxide particles, Toxicol. Sci. 77 (2004) 347–357.
[11] Q. Rahman, M. Lohani, E. Dopp, H. Pemsel, L. Jonas, D.G. Weiss, D. Schiffmann, Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts, Environ. Health Perspect. 110 (2002) 797–800.
[12] C.M. Sayes, R. Wahi, P. Kurian, Y. Liu, J.L. West, K.D. Ausman, D.B. Warheit, V.L. Colvin, Correlating nanoscale titania structure with toxicity: a cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells, Toxicol. Sci. 92 (2006) 174–185.
[13] D.B. Warheit, T.R. Webb, C.M. Sayes, V.L. Colvin, K.L. Reed, Pulmonary instillation studies with nanoscale TiO_2 rods and dots in rats: toxicity is not dependent upon particle size and surface area, Toxicol. Sci. 91 (2006) 227–236.
[14] J.R. Gurr, A.S. Wang, C.H. Chen, K.Y. Jan, Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells, Toxicology 213 (2005) 66–73.
[15] S. Quaiser, A.A. Al-Khedhairy, M.A. Siddiqui, F.M. Abou-Tarboush, A. Azam, J. Musarrat, Titanium dioxide nanoparticles induced cytotoxicity, oxidative stress and DNA damage in human amnion epithelial (WISH) cells, Toxicol. In Vitro 26 (2) (2012) 351–361.
[16] C.I. Vamanu, M.R. Cimpan, P.J. Høl, S. Sornes, S.A. Lie, N.R. Gjerdet, Induction of cell death by TiO_2 NPs: studies on a human monoblastoid cell line, Toxicol. In Vitro 22 (2008) 1689–1696.
[17] R.C. Gopalan, I.F. Osman, A. Amani, M. DeMatas, D. Anderson, The effect of zinc oxide and titanium dioxide nanoparticles in the Comet assay with UVA photoactivation of human sperm and lymphocytes, Nanotoxicology 3 (2009) 33–39.
[18] S. Barillet, A. Simon-Deckers, N. Herlin-Boime, M. Mayne-L'Hermite, C. Reynaud, D. Cassio, B. Gouget, M. Carrière, Toxicological consequences of TiO_2, SiC nanoparticles and multi-walled carbon nanotubes exposure in several mammalian cell types: an *in vitro* study, J. Nanopart. Res. 12 (2010) 61–73.
[19] R.K. Shukla, V. Sharma, A.K. Pandey, S. Singh, S. Sultana, A. Dhawan, ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells, Toxicol. In Vitro 25 (2011) 231–241.
[20] C. Wei, W.Y. Lin, Z. Zainal, N.E. Williams, K. Zhu, A.P. Kruzic, R.L. Smith, K. Rajeshwar, Bactericidal activity of TiO_2 photocatalyst in aqueous media: toward a solar-assisted water disinfection system, Environ. Sci. Technol. 28 (1994) 934–938.
[21] P.C. Maness, S. Smolinski, D.M. Blake, Z. Huang, E.J. Wolfrum, W.A. Jacoby, Bactericidal activity of photocatalytic TiO_2 reaction: toward an understanding of its killing mechanism, Appl. Environ. Microbiol. 65 (1999) 4094–4098.
[22] J.J. Wang, B.J.S. Sanderson, H. Wang, Cyto- and genotoxicity of ultrafine TiO_2 particles in cultured human lymphoblastoid cells, Mutat. Res. 628 (2007) 99–106.
[23] S.J. Kang, B.M. Kim, Y.J. Lee, H.W. Chung, Titanium dioxide nanoparticles trigger p53-mediated damage response in peripheral blood lymphocytes, Environ. Mol. Mutagen. 4 (2008) 399–405.
[24] H.L. Karlsson, P. Cronholm, J. Gustafsson, L. Moller, Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes, Chem. Res. Toxicol. 21 (2008) 726–1732.
[25] D.B. Warheit, R.A. Hoke, C. Finlay, E.M. Donner, K.L. Reed, C.M. Sayes, Development of a base set of toxicity tests using ultrafine TiO_2 particles as a component of nanoparticle risk management, Toxicol. Lett. 171 (2007) 99–110.
[26] X. Pan, J.E. Redding, P.A. Wiley, L. Wen, J.S. McConnell, B. Zhang, Mutagenicity evaluation of metal oxide nanoparticles by the bacterial reverse mutation assay, Chemosphere 79 (2010) 113–116.
[27] A. Kumar, A.K. Pandey, S.S. Singh, R. Shanker, A. Dhawan, Cellular uptake and mutagenic potential of metal oxide nanoparticles in bacterial cells, Chemosphere 83 (2011) 1124–1132.
[28] H. Ogawa, T. Ohyama, E. Katsura, Y. Katoh, Characterization of the pUC19–*lacZ*C^{141} reversion system for assaying chemical mutagenesis, Mutat. Res. 394 (1997) 141–151.
[29] R. Wolf, H. Matz, E. Orion, J. Lipozencic, Sunscreens the ultimate cosmetics, Acta Dermatovenerol. Croat. 11 (2003) 158–162.
[30] T. Kaida, K. Kobayashi, M. Adachi, F. Suzuki, Optical characteristics of titanium oxide interference film and the film laminated with oxides and their applications for cosmetics, J. Cosmet. Sci. 55 (2004) 219–220.
[31] R. Landsiedel, M.D. Kapp, M. Schulz, K. Wiench, F. Oesch, Genotoxicity investigations on nanomaterials: methods, preparation and characterization of test material, potential artifacts and limitations – many questions, some answers, Mutat. Res. 681 (2009) 241–258.
[32] N. Khandoudi, P. Porte, S. Chtourou, F. Nesslany, D. Marzin, F. Le Curieux, The presence of arginine may be a source of false positive results in the Ames test, Mutat. Res. 679 (2009) 65–71.
[33] J.M. Short, J.M. Fernandez, J.A. Sorge, W.D. Huse, Lambda ZAP: a bacteriophage lambda expression vector with *in vivo* excision properties, Nucleic Acids Res. 16 (1988) 7583–7600.
[34] J.D. Thompson, D.G. Higgins, T.J. Gibson, CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, Nucleic Acids Res. 22 (1994) 4673–4680.
[35] A.G. Rincon, C. Pulgarin, Use of coaxial photocatalytic reactor (CAPHORE) in the TiO_2 photo-assisted treatment of mixed *Escherichia coli* and *Bacillus subtilis* and the bacterial community present in wastewater, Catal. Today 101 (2005) 331–344.
[36] J. Musarrat, M. Ahmad, *In vitro* studies on mild alkali induced lesions in DNA, Biochem. Int. 25 (1991) 249–259.
[37] A.M. Chakrabarty, J.R. Mylroie, D.A. Friello, J.G. Vacca, Transformation of *Pseudomonas putida* and *Escherichia coli* with plasmid-linked drug-resistance factor DNA, PNAS 72 (1975) 3647–3651.
[38] A. Ahmad, F.A. Syed, S. Singh, S.M. Hadi, Prooxidant activity of resveratrol in the presence of copper ions: mutagenicity in plasmid DNA, Toxicol. Lett. 159 (2005) 1–12.

[39] M.N. Routledge, F.J. Mirsky, D.A. Wink, L.K. Keefer, A. Dipple, Nitrite-induced mutations in a forward mutation assay: influence of nitrite concentration and pH, Mutat. Res. 322 (1994) 341–346.
[40] M.J. Juedes, G.N. Wogan, Peroxynitrite-induced mutation spectra of pSP 189 following replication in bacteria and in human cells, Mutat. Res. 349 (1996) 51–61.
[41] M.N. Routledge, Mutations induced by reactive nitrogen oxide species in the *supF* forward mutation assay, Mutat. Res. 450 (2000) 95–105.
[42] J.M. Bishop, Molecular themes in oncogenes, Cell 64 (1991) 235–248.
[43] A. Xu, Y. Chai, T. Nohmi, T.K. Hei, Genotoxic responses to titanium dioxide nanoparticles and fullerene in gpt delta transgenic MEF cells, Part. Fibre Toxicol. 6 (2009) 3.
[44] K. Kino, H. Sugiyama, Possible cause of G-C–>C-G transversion mutation by guanine oxidation product, imidazolone, Chem. Biol. 8 (4) (2001) 369–378.
[45] M. Watanabe-Akanumaa, R. Woodgateb, T. Ohta, Mutat. Res. (Fund. Mol. Mech. Mutagen.) 373 (1) (1997) 61–66.