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Tellurite biotransformation and detoxification by *Shewanella baltica* with simultaneous synthesis of tellurium nanorods exhibiting photo-catalytic and anti-biofilm activity

Diviya Chandrakant Vaigankar[a], Santosh Kumar Dubey[b,*], Sajiya Yusuf Mujawar[a], Avelyno D'Costa[c], Shyama S.K.[c]

[a] Laboratory of Bacterial Genetics and Environmental Biotechnology, Department of Microbiology, Goa University, Taleigao Plateau, Goa 403206, India
[b] Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi, Uttar Pradesh 221005, India
[c] Genetic Toxicology Laboratory, Department of Zoology, Goa University, Taleigao Plateau, Goa 403206, India

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ABSTRACT

Tellurite reducing bacterial strain was isolated from Zuari estuary, Goa India which could tolerate 5.5 mM potassium tellurite with a minimum inhibitory concentration of 6 mM. This strain was designated as GUSDZ9 and was identified as *Shewanella baltica* (accession number: MF350629) based on 16S rRNA gene sequencing and BLAST analysis. The Diethyl-dithiocarbamate based colorimetric analysis clearly demonstrated a complete reduction of 2 mM tellurite to elemental tellurium during the late stationary phase. Te Nanoparticles (TeNPs) biosynthesis which initiated at early log phase (i.e. 4 h) was evidently monitored through colour change and a peak due to surface plasmon resonance at 210 nm using UV–Vis spectroscopic analysis. X-ray crystallographic studies and transmission electron microscopy revealed unique nano-rods with a diameter ranging from 8 to 75 nm. Energy dispersive X-ray analysis further confirmed the presence of pure tellurium. The biogenic TeNPs at 10 and 5 μg/mL evidently demonstrated 90% degradation of methylene blue dye and anti-biofilm activity against potential Gram-positive and Gram-negative human pathogens respectively. The alkaline comet assay revealed time and dose-dependent genotoxicity at concentrations higher than 15 μg/mL of TeNPs. This study clearly demonstrated the potential of *Shewanella baltica* strain GUSDZ9 in bioremediation of toxic tellurite through bio-reduction into elemental tellurium and involvement of biogenic TeNPs in the photo-catalytic reduction of methylene blue and anti-biofilm activity. This is the first report of its kind on the synthesis of biogenic TeNPs from *Shewanella baltica* demonstrating photo-catalytic, anti-biofilm activity as well as genotoxicity.

1. Introduction

Estuarine environment is the most common dumping site for industrial, electronic and mining wastes. Consequently, estuaries are heavily contaminated with various persistent toxic metals viz. Cu, Hg, Cd, Pb and metalloids viz. Se, Te, As posing a serious threat to aquatic biota including microorganisms (Tchounwou et al., 2012). During the last several decades, metal and metalloid bioremediation of polluted sites using metal/metalloid resistant microorganisms have been studied extensively (Satyanarayana et al., 2012; Khalilian et al., 2015; Gupta et al., 2016). Tellurium (Te) is a metalloid present at 0.027 ppm concentration in the earth crust. It occurs in the environment as inorganic, unstable telluride [Te^{2-}], water-soluble, toxic tellurate [TeO_4^{2-}] and tellurite [TeO_3^{2-}]; organic form as dimethyl telluride (CH_3TeCH_3) and elemental tellurium (Te°). Industrially Te and its compounds find applications in solar panels, glasses, rubber, photocopying machine, metal alloys, rechargeable batteries, semiconductors in electronics, protein crystallographic analysis and as catalysts in various chemical processes (Chasteen et al., 2009; Naumov, 2010).

Tellurite is highly toxic to microorganisms at concentrations as low as 1 μg/mL (Taylor, 1999). The toxicity of tellurite is of great concern to prokaryotes as well as eukaryotes since its lethal concentration is several folds lower than that of other metals viz. Fe, Hg, Cd, Cu, Cr, Zn, Co and Se which is a metalloid (Chasteen et al., 2009; Presentato et al., 2016). Some microorganisms have evolved resistance mechanisms such as reduction of tellurite to black elemental tellurium, intracellular and extracellular accumulation of reduced tellurium and volatilization by methylation (Trutko et al., 2000; Basnayake et al., 2001; Fuentes et al.,

* Corresponding author.
E-mail address: santosh.dubey@bhu.ac.in (S.K. Dubey).

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2007; Chasteen et al., 2009). Few tellurite resistant marine bacteria have already been reported for their possible role in tellurite bioremediation (Rathgeber et al., 2002; Csotonyi et al., 2006; Amoozegar et al., 2008; Ollivier et al., 2008; Kim et al., 2012; Arenas et al., 2014; Borghese et al., 2014; Soda et al., 2018; Valdivia-González et al., 2018).

Bioreduction of soluble tellurite to insoluble elemental tellurium by microorganisms can occur with the formation of nanostructured particles. Since reductive biotransformation and synthesis of nanostructures proceed contextually, the use of estuarine microbes for simultaneous tellurite bioreduction in polluted environments and biogenesis of nanomaterials appears highly promising and economically attractive. Microbially-mediated strategies for nanoparticle synthesis are environment-friendly because they occur in mild reaction conditions avoiding energy-intensive procedures as well as the use of highly toxic stabilizing reagents, which are usually associated with physical and chemical approaches (Xi et al., 2005; Kaushik et al., 2010). There are few strains of bacteria which have been reported to synthesize TeNPs and include *Bacillus* sp., *Rhodococcus aetherivorans, Rhodobacter capsulatus, Bacillus selenitireducens, Sulfurospirillum barnesii* and *Shewanella oneidensis* (Klonowska et al., 2005; Baesman et al., 2007; Kim et al., 2012; Zare et al., 2012; Borghese et al., 2014; Presentato et al., 2016).

Nanoparticles are in high demand in various fields viz. medicine, electronic, catalyst, biosensors, paint, glass, alloy and battery industries (Li et al., 2011). Te in nano-dimensions possesses unique properties such as high surface to volume ratio, piezo-thermoelectrical, photoconductivity, catalytic and non-linear optical characteristics which have attracted the attention of several researchers around the world (Liu et al., 2003; Kurimella et al., 2013). More recently, Te and Cd quantum dots have been reported to have great potential in solar cells and imaging (Liu et al., 2003; Li et al., 2014). Application of nanoparticles in photo-catalytic degradation of toxic and hazardous effluents containing dyes, phenols and pesticides from textile, paper and agro-industries has drawn a lot of attention from environmental scientists. Since current methods employed for the degradation of organic pollutants are laborious and expensive, there is a pressing need for safe, efficient and eco-friendly methods to treat these organic pollutants. Thus, the use of nanoparticles in photo-catalytic degradation of organic pollutants may prove to be a better alternative.

Nanoparticles also find applications in medicine as antimicrobial agents to treat bacterial infections resistant to multiple antibiotics. Over the last few decades, the effectiveness of antibiotic treatment has decreased significantly due to the emergence of bacterial resistance to multiple antibiotics in hospital and community settings. The problem is particularly more serious in the treatment of biofilm-associated microbial infections. Therefore, there is an urgent need to develop novel nanomaterial-based antimicrobials possessing high bactericidal activity against biofilm forming pathogenic microorganisms.

However, with the profound use of nanoparticles in biomedical applications viz. antibacterial therapy and drug delivery, along with enhanced exposure to nanomaterials in everyday life, it is mandatory to investigate the toxicity of these nanoparticles. Under these circumstances, the genotoxicity of nanomaterials is a burgeoning issue in the area of nanotechnology. Although the genotoxicity of chemically-synthesized nanoparticles has been studied extensively, the genotoxicity of biologically-synthesized nanomaterials is scarcely reported (Foldbjerg et al., 2011; Ghosh et al., 2012; De Lima et al., 2013; Lebedová et al., 2017). Moreover, there are no reports on the genotoxicity of TeNPs even though they have been already studied for their antimicrobial and anti-biofilm applications (Lin et al., 2012; Zare et al., 2012; Mohanty et al., 2014; Pugin et al., 2014; Srivastava et al., 2015; Zonaro et al., 2015). Thus, it is highly imperative to study the genotoxic effect of biogenic TeNPs, intended for biomedical and environmental applications.

In the present study, the tellurite reduction potential of *Shewanella baltica* strain GUDSZ9 from Zuari estuary Goa, India, is discussed along with the simultaneous synthesis of TeNPs. We have also studied the potential application of these biogenic TeNPs in photo-catalytic degradation of methylene blue dye, anti-biofilm activity and genotoxicity against human lymphocytes.

2. Materials and methods

2.1. Materials

All the chemicals used for the present study were of certified analytical grade and were procured from Himedia (Mumbai, India) unless specified otherwise.

2.2. Enrichment and isolation of tellurite reducing estuarine bacteria from Zuari estuary, Goa, India

Estuarine surface water was collected from the Zuari estuary Goa, India (Latitude: 15°24'31.03"N, Longitude: 73°53'31.02"E and temperature: 27 °C) using a sterile polycarbonate bottle. One mL of water sample was added to 50 mL Zobell Marine Broth (ZMB) supplemented with 0.5 mM potassium tellurite (K_2TeO_3) and was incubated at 28 ± 2 °C on a shaker at 150 rpm for 48 h. Isolation of tellurite reducing bacteria was done by dilution plating of the enriched sample on Zobell marine agar (ZMA) plates amended with 2 mM K_2TeO_3 and plates were incubated at 28 ± 2 °C for 24 h. Discrete black coloured colonies were re-streaked on ZMA plates without K_2TeO_3 in order to ensure that blackening of the colonies was certainly due to the reduction of K_2TeO_3 to elemental tellurium and not because of bacterial pigment. Morphologically distinct tellurite reducing bacterial colonies were selected for further studies.

2.3. Determination of minimum inhibitory concentration (MIC) of tellurite

Total 20 bacterial isolates were selected and spot inoculated on ZMA plates with increasing concentrations of K_2TeO_3 (0–20 mM). These plates were incubated at 28 ± 2 °C for 24 h and were checked for the appearance of metallic black coloured colonies. The minimum concentration of tellurite at which no visible colonies were obtained was designated as MIC. Ten bacterial isolates with the high MIC on ZMA plates were selected for determining the MIC in ZMB. MIC in liquid medium was determined by inoculating the selected bacterial isolates in ZMB with various concentrations of K_2TeO_3 (0–20 mM). The flasks were incubated at 28 ± 2 °C for 24 h and absorbance at 600 nm was recorded. The lowest concentration of tellurite which inhibited growth was considered as MIC. Out of ten isolates, the bacterial strain exhibiting the highest MIC in ZMB for K_2TeO_3 was considered for further characterization.

2.4. Identification of potential tellurite reducing bacterial strain

The selected tellurite-resistant strain was characterized morphologically and biochemically followed by molecular identification. DNA extraction of the tellurite reducing bacterial strain was carried out using Dneasy® Blood & Tissue Kit (Qiagen, Hilden, Germany). The 16S ribosomal RNA gene (16 S rRNA) was amplified with 27 F (5' AGAGTTTG ATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTTACGACTT 3') universal eubacterial primers using Nexus Gradient Mastercycler (Eppendorf, Germany). The PCR amplicon was analysed on 1% agarose gel followed by purification using Wizard SVGel and PCR clean-up system (Promega, USA). The 16S rRNA gene was sequenced at Eurofins Genomics Bangalore, India. The DNA sequence was analysed by BLAST (Altschul et al., 1990) and submitted to GenBank. Neighbor-joining method was used for the construction of a phylogenetic dendrogram using MEGA 7 package (Tamura et al., 2013).