Journal of Hazardous Materials 287 (2015) 51–58

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

# Use of bioreporters and deletion mutants reveals ionic silver and ROS to be equally important in silver nanotoxicity

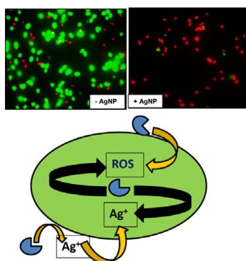
Nimisha Joshi [a,c,*], Bryne T. Ngwenya [a,**], Ian B. Butler [a], Chris E. French [b]

[a] School of Geosciences, Microbial Geochemistry Laboratory, University of Edinburgh, West Mains Road, Edinburgh EH9 3FE, United Kingdom
[b] School of Biological Sciences, Institute of Cell Biology, Darwin Building, University of Edinburgh, Edinburgh EH9 3JR, United Kingdom
[c] School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Oxford Road, Manchester M13 9PL, United Kingdom

## HIGHLIGHTS

- Novel bioreporter was developed and employed to investigate silver nanotoxicity.
- Response of deletion mutant strains for oxidative stress and metal efflux gene to AgNp suggest that both ROS and ionic silver are important.
- Oxidative stress plays an important role in causing cell death.
- Presence of chloride ions does not completely eliminate silver nanotoxicity.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 5 October 2014
Received in revised form
29 December 2014
Accepted 31 December 2014
Available online 5 January 2015

*Keywords:*
Nanotoxicity
Mechanism
Environment
Ionic
Oxidative stress
Nanomaterials
Biosensor
Speciation

## ABSTRACT

The mechanism of antibacterial action of silver nanoparticles (AgNp) was investigated by employing a combination of microbiology and geochemical approaches to contribute to the realistic assessment of nanotoxicity. Our studies showed that suspending AgNp in media with different levels of chloride relevant to environmental conditions produced low levels of ionic silver thereby suggesting that dissolution of silver ions from nanoparticulate surface could not be the sole mechanism of toxicity. An *Escherichia coli* based bioreporter strain responsive to silver ions together with mutant strains of *E. coli* lacking specific protective systems were tested against AgNp. Deletion mutants lacking silver ion efflux systems and resistance mechanisms against oxidative stress showed an increased sensitivity to AgNp. However, the bioreporter did not respond to silver nanoparticles. Our results suggest that oxidative stress is a major toxicity mechanism and that this is at least partially associated with ionic silver, but that bulk dissolution of silver into the medium is not sufficient to account for the observed effects. Chloride ions do not appear to offer significant protection, indicating that chloride in receiving waters will not necessarily protect environmental bacteria from the toxic effects of nanoparticles in effluents.

© 2015 Elsevier B.V. All rights reserved.

## 1. Introduction

Engineered metal-based nanoparticles (NP) such as silver, zinc oxide and titanium dioxide are toxic to bacteria and other microorganisms because of the enhanced reactivity arising from their small size [1,2]. In addition to their chemical properties, variables including the presence of capping agents, shape of the nanoparticles, oxidation state and pH affect toxicity [3–5]. Recent studies indicate that oxidative stress and dissolved ionic species play an important

*Abbreviations:* NP, nanoparticle; ROS, reactive oxygen species; FSU, relative fluorescence unit; M9RC, M9 medium with reduced chlorides; OD600, optical density at 600 nm; EYP, enhanced yellow fluorescent protein; CFU, colony forming unit; ATP, adenosine triphosphate.

* Corresponding author at: School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Oxford Road, Manchester M13 9PL, United Kingdom. Tel.: +1 612 750 760.
** Corresponding author.
*E-mail addresses:* nimisha.joshi@manchester.ac.uk (N. Joshi), Ngwenya@ed.ac.uk (B.T. Ngwenya).

http://dx.doi.org/10.1016/j.jhazmat.2014.12.066
0304-3894/© 2015 Elsevier B.V. All rights reserved.

role in nanoparticle toxicity, which may also involve membrane damage and protein oxidation [6–11]. However, the overall picture remains ambiguous, especially while attempting to extrapolate from laboratory experiments to the natural environment where physicochemical transformations of nanoparticles can take place [12,13]. The extent and nature of the damage varies with the type of nanomaterial and exposure conditions. For instance, the presence of ligands such as chloride and phosphate and changes in pH, can influence the speciation of dissolved nanoparticles and hence further complicate mechanistic interpretations [14,15].

Toxicity studies using silver nanoparticles have generated much debate about the toxicity mechanisms in the recent past. Some studies suggested that AgNp operated via particle specific effects [16,17], including oxidative stress [7] and ionic toxicity. Xiu et al. [18] questioned particle-specific effects and by extension that oxidative stress may also arise from ionic silver [19,20]. However, this view is being increasingly challenged in more recent studies as we develop a better understanding of silver speciation in different media [14]. Existing studies have largely relied on assessing cell viability, with oxidative stress being detected using fluorescent probes that at times provide unreliable data [21]. Microarray studies conducted on *Escherichia coli* using silver and polystyrene nanoparticles indicate that multiple genetic pathways are up-regulated on exposure to nanoparticles [22,23]. A systematic investigation of the interaction of bacteria with engineered nanoparticles using stress responsive and metal ion responsive genes in the bacteria under investigation could provide better insight into the toxicity mechanisms.

*E. coli* is a model bacterium with well-understood stress resistance mechanisms. All of the non-essential genes in *E. coli* have been individually mutated, and mutant strains are available from the KEIO collection [24]. Furthermore, bioreporter strains can be developed in which known stress-responsive promoters are linked to reporter genes.

In this study, we have used mutated strains of *E. coli* lacking specific protective mechanisms to re-assess the relative roles of ionic and nanoparticulate silver on toxicity to bacteria. We have also used a bioreporter to test the bioavailability of ionic silver in a medium containing chloride at concentrations in the range found in urban freshwater and upper estuarine bodies (0–10000 mg/L up to the mesohaline) [25], which are the first receptors of NP discharges and always contain some chloride from road run-off [12,26,27]. Based on recent predictions that ionic silver precipitates as silver chloride in freshwaters [28], it was hypothesised that ionic silver would have minimal involvement in nanoparticle toxicity.

## 2. Materials and methods

### 2.1. Bacterial strains and plasmids

Bacterial strains used in this study are listed in Table 1. Deletion strains were obtained from the KEIO collection via the Yale Genetic Stock Centre [24]. The triple deletion strain LE106, lacking *katG*, *katE* and *ahpF*, was kindly provided by Prof. James Imlay, University of Iowa [29]. A biosensor responsive to copper and silver ions was developed by cloning the promoter of *copA* [30–32] in BioBrick RFC10 format [33,34] in vector pSB1C3 (Registry of Standard Biological Parts). The following primers were designed to amplify the promoter:

5′-CCTTGAATTCGCGGCCGCTTCTAGAGTGAAATTGGGTGTAAGC-3′

5′-AAGGCTGCAGCGGCCGCTACTAGTACTGTGATAAAGGTTAAA-CC-3′

A reporter gene, enhanced yellow fluorescent protein (EYFP, BioBrick BBa_E0040), was then inserted downstream of the promoter to generate the final reporter construct, designated pSB1C3/P*copA* + EYFP.

### 2.2. Reagents

Components of the growth medium, catalase, and sodium citrate-stabilized silver nanoparticles were obtained from Sigma–Aldrich. The LIVE–DEAD cell viability kit was procured from Invitrogen. Phosphate buffered saline (PBS) was made by adding 8 g/L NaCl, 0.2 g/L KCl and 1.44 g/L $Na_2HPO_4$ in deionized water. The pH was adjusted to 7.4 and the solution sterilized by autoclaving. Catalase (Sigma catalogue number C9322) at 5 mg/ml was suspended in PBS and added to the cultures to achieve a final concentration of 100 U/ml (30 μg/ml).

### 2.3. Growth conditions and assays

Exposure studies used M9 medium, made by diluting a 4× stock solution containing 64 g/L $Na_2HPO_4{\cdot}7H_2O$, 15 g/L $KH_2PO_4$, 5 g/L $NH_4Cl$ and 2.5 g/L NaCl. This was supplemented with 0.011 g/L calcium chloride, 1.204 g/L magnesium sulphate, 2 g/L Casamino acids, 0.34 g/L thiamine hydrochloride and 0.4% w/v glycerol as a carbon source. This medium contained approximately 1225 mg/L chloride ions. Control tests for silver chloride precipitation were conducted by using M9RC (Reduced Chloride) medium consisting of the same medium modified by replacing sodium chloride with sodium nitrate and ammonium chloride with ammonium sulphate

**Table 1**
Strains of *E. coli* used for exposure assay in this work.

| Bacterial strain | Genotype | Details |
|---|---|---|
| *E.coli* JM109/pSB1C3/P*copA* + EYFP | Lab strain transformed with plasmid pSB1C3-PcopA_YFP. It forms yellow fluorescence protein on induction with silver ions | This work |
| MG1655 | | Wild type *E. coli*, parent strain of LE106 |
| LE106 | | Triple deletion mutant strain Δ*katG*/Δ*katE*/Δ*ahpF* |
| BW25113 | | Parent strain of KEIO strains. |
| JW0473-3 | Δ*copA*767::*kan* | Deletion of copper efflux pump |
| JW0476-1 | Δ*cueR*770::*kan* | Deletion of regulator of *copA* |
| JW0564-1 | Δ*cusA*784::*kan* | Deletion of cation transporter |
| JW0563-2 | Δ*cusB*783::*kan* | Deletion of copper/silver efflux membrane fusion protein |
| JW0562-1 | Δ*cusF*782::*kan* | Deletion of copper/silver efflux system protein |
| JW0560-1 | Δ*cusR*780::*kan* | Deletion of DNA binding transcriptional activator of *cusABF* |
| JW3914-1 | Δ*katG*729::*kan* | Deletion of catalase |
| JW1721-1 | Δ*katE*731::*kan* | Deletion of catalase |
| JW3879-1 | Δ*sodA*768::*kan* | Deletion of superoxide dismutase A |
| JW1648-1 | Δ*sodB*734::*kan* | Deletion of superoxide dismutase B |
| JW0005-1 | Δ*yaaA*726::*kan* | Deletion of peroxidase |
| JW0422-1 | Δ*cyoA*789::*kan* | Deletion of cytochrome bo terminal oxidase subunit |
| JW0421-1 | Δ*cyoB*788::*kan* | Deletion of component of cytochrome bo terminal oxidase |
| JW0723-2 | Δ*cydB*782::*kan* | Deletion of cytochrome *bd*-I terminal oxidase subunit |

Download English Version:

https://daneshyari.com/en/article/576344

Download Persian Version:

https://daneshyari.com/article/576344

Daneshyari.com