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Short Communication

ELUCIDATING THE GENETIC BASIS FOR *ESCHERICHIA COLI* DEFENSE AGAINST SILVER TOXICITY USING MUTANT ARRAYS

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Abstract: Bacterial adaptation and defense mechanisms against silver are poorly understood at the genetic level. A library of *Escherichia coli* gene-deletion mutants was used to show that clones lacking *sodB* (coding for oxidative stress protection), *lon* (protein damage repair), or *cusR* (metal efflux pump) are quite sensitive to silver (with $7.3 \pm 9.1\%$, $5.3 \pm 1.8\%$, and $0.4 \pm 0.1\%$ of cells surviving, respectively, compared with $90.1 \pm 5.4\%$ survival for wild-type *E. coli*, after 6-h exposure to 8 mg/L $AgNO_3$), suggesting the importance of the coded functions as defense mechanisms. Mutants lacking *pgaB* or *wcaD*, which code for production of extracellular polymeric substances (EPS), also showed significant ($p < 0.05$) sensitivity to silver exposure ($23.4 \pm 16.2\%$ and $23.1 \pm 32.6\%$ survival, respectively). Transmission electron microscopy (TEM) with scanning TEM/energy-dispersive X-ray spectroscopy analysis showed accumulation of silver nanoparticles within EPS, suggesting that EPS serve as a protective barrier that also immobilizes dissolved silver as silver nanoparticles. *Environ Toxicol Chem* 2014;9999:1–5. © 2014 SETAC

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INTRODUCTION

Various forms of silver have been used as antimicrobial agents since antiquity, including silver nitrate ($AgNO_3$) to prevent gonorrheal eye infections in newborns and Ag foils to prevent infection of surgical wounds [1,2]. In recent decades, with the burgeoning threat of multidrug-resistant bacteria and the worldwide production and application of silver nanoparticles (AgNPs), silver has generated renewed interest as an antimicrobial agent.

Among the various forms of silver (e.g., salts, colloidal, nanoparticles), released silver ions (Ag^+) are proposed to be the principal bactericidal agent. Silver nanoparticles were recently demonstrated to exert no antimicrobial activity in the absence of Ag^+ release, implying that Ag^+ is the critical effector of the antibacterial activity of AgNPs (although nanoparticles may serve as a more effective delivery vehicle of Ag^+ to cells [3]). Much research has been conducted on the antibacterial activity of silver, and several mechanisms have been proposed, including the following: protein damage, in which Ag^+ binds with thiol groups [4] (e.g., in cysteine) and disrupts protein function [5,6]; generation of reactive oxygen species (ROS), during which harmful ROS are generated (perhaps as an immune response) in the presence of silver [7–9]; and deoxyribonucleic acid (DNA) damage [10], in which Ag^+ has a strong affinity to nucleic acids [11] and forms complexes with DNA by binding with guanine or adenine [12], although the extent to which Ag^+ reaches the nucleoid is not clear.

In contrast to advances in our understanding of the antibacterial mechanisms of silver, bacterial adaptation to silver and the associated defense mechanisms are poorly understood at the genetic level. However, identifying the genes responsible for bacterial adaptation and resistance to silver is important for the development of effective silver-based antimicrobials and for mitigating potential unintended impacts of silver releases on microbial ecosystem services. In the present study, we address the relative importance of different genes potentially conferring resistance by using a library of *Escherichia coli* mutants that lack open reading frame (ORF) clones (the Keio collection) and a library of *E. coli* ORF clones (the ASKA collection) that overexpress a specific functional gene and thus a corresponding physiological or metabolic trait.

MATERIALS AND METHODS

Knockout (Keio) library and ORF clone (ASKA) library

The Keio library [13] (systematic single-gene knockout mutants of *E. coli* K-12 BW25113) and the ASKA library [14] (ORF clones library of *E. coli* K-12 AG1 ME5305) were purchased from the Japan National BioResource Project—*E. coli* at the Japanese National Institute of Genetics. Each Keio mutant carries a deletion of a single gene, with a kanamycin resistance gene serving as the replacement. Each ASKA clone carries a plasmid of a single gene with a chloramphenicol resistance gene on it.

Survival tests

Wild-type *E. coli* (BW25113) and selected mutants were inoculated in 10 mL Luria–Bertani (LB) broth and incubated at 37 °C on a 200-rpm shaking incubator for 15 h. The optical density (OD) values of each mutant culture were measured with an ultraviolet-visible spectrometer and diluted to 0.8 (OD_{600}) using deionized water to normalize the starting bacteria concentration, which is a critical factor for the test. For the Keio mutants, the *E. coli* wild type was selected as a control; it was exposed to a series of $AgNO_3$ concentrations to determine an appropriate sublethal concentration for testing (8 mg/L,

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which caused ~10% inhibition of wild-type *E. coli* growth and 10%–90% inhibition of mutant growth) to facilitate discernment of how specific genes may affect tolerance to silver.

To assess bacterial survival, each mutant (1-mL culture) was exposed to 8 mg/L $AgNO_3$ and incubated in the dark for 6 h. The surviving cells were quantified by serial dilution and plate counting [15]. This concentration was normalized to its respective control (without $AgNO_3$) to account for variability of initial cell concentrations. Wild-type *E. coli* BW25113 served as control for the Keio mutants, and *E. coli* wild-type AG1 (ME5305) with an inserted empty plasmid (vector *pCA24N*) served as a control for ASKA clones. All tests were conducted in triplicate and repeated 3 times to ensure reproducibility.

Data analysis

Colony-forming units of each gene deletion mutant (Keio collection) were counted after exposure to Ag^+ and normalized to the wild-type control to determine the percentage of surviving cells. If the deletion of a certain gene is crucial for *E. coli* survival, the mutant should be more sensitive than the control. Whether survival differences were statistically significant was determined using Student's *t* test at the 95% confidence level. All measurements are reported as mean ± 1 standard deviation with 3 independent replicates.

TEM sample preparation

To assess the role of extracellular polymeric substances (EPS), wild-type *E. coli* (BW25113) and a *ΔpgaB* mutant were also grown overnight at 37 °C. Samples (1 mL) were taken from each culture and centrifuged at 1×10^4 rpm for 1 min. The supernatants were discarded, and the cells were resuspended using 1 mL deionized water. This process was repeated 4 times to remove nutrients and salts in the LB medium. Both cell suspensions were diluted 10-fold with deionized water, and 10 μL were dropped on copper grids (400 mesh, Ted Pella). The samples were dried under ambient condition (25 °C) for 2 d, and imaged using a JEOL1230 transmission electronic microscope (TEM).

Resuspended cells were mixed with $AgNO_3$ (8 mg/L) and incubated for 6 h in the dark. Cells were then diluted 10-fold with deionized water, and TEM samples were prepared and imaged similarly. The elemental composition of nano-sized particles in *E. coli* EPS was analyzed using energy-dispersive X-ray spectroscopy (EDS) on a JEOL 2100 field emission gun TEM (EF-TEM) under scanning transmission electron microscopy (STEM) mode.

RESULTS AND DISCUSSION

Silver-induced oxidative stress is attenuated by sodB

Various specific superoxide dismutase (SOD) genes (e.g., *sodA*, *sodB*, and *sodC*) encode the enzymes responsible for the dismutation of superoxide that protect *E. coli* against oxidative stress [16] (Table 1). Deletion mutants lacking different *sod* genes responded differently to silver exposure (Figure 1a). Mutants lacking *sodA* or *sodC* did not display greater sensitivity to silver than the wild-type control, whereas the *ΔsodB* mutant was significantly more sensitive (with 7.3% ± 9.1% survival after 6-h $AgNO_3$ treatment) than the wild type (90.1% ± 5.4% survival). In *E. coli*, *sodA* and *sodB* code for manganese- and iron-bound SOD, respectively, and the transcription of both is regulated by the intracellular iron concentration [17,18]. Specifically, *sodA* is repressed [18] while *sodB* is induced [19] in the presence of Fe(II). In the present study, all mutants were tested in LB medium containing approximately 30 mM of iron [20], which favors *sodA* repression and *sodB* induction. We also assessed deletion of *sodC*, which encodes a periplasmic SOD, but this gene does not appear to be important for defense against silver.

Protein repair genes are critical for resistance to silver

The adenosine triphosphate (ATP)-dependent protease Lon is involved in both general quality control (by degrading abnormal proteins) and specific control of several regulatory proteins [21] (Table 1). Lon also controls toxin/antitoxin systems involved in plasmid maintenance [22]. The *Δlon* mutant was very sensitive to silver, with only 5.3% ± 1.8% of cells surviving after 6-h $AgNO_3$ treatment (Figure 1a). Because Ag^+ is known to bind and damage cellular proteins, *Δlon* mutants lack the ability to degrade or repair the damaged proteins, resulting in the intracellular accumulation of oxidized and damaged proteins. Apparently, Lon protease plays an essential role in silver defense by preventing the accumulation of damaged proteins.

DNA damage repair genes may also endow resistance to silver

Several studies on the interaction and complexation of Ag^+ with nucleic acids have been conducted [23], and DNA has been used as a carrier to complex Ag^+ as an antibacterial

Table 1. Genes considered in this study[a]

Name	Description
sodA	Superoxide dismutase, Mn
sodB	Superoxide dismutase, Fe; response to oxidative stress; chromate resistance; negatively regulated by *ryhB* RNA as part of indirect positive regulation by *Fur*; acid-inducible
sodC	Superoxide dismutase, Cu, Zn, periplasmic; mutants are sensitive to exogenous hydrogen peroxide in early stationary phase
lon	Component of DNA-binding, ATP-dependent protease
recA	General recombination and DNA repair; pairing and strand exchange; role in cleavage of *LexA* repressor, *SOS* mutagenesis
fabR	Transcriptional repressor of *fabA* and *fabB*
cusA	Silver and copper efflux, membrane transporter; confers copper and silver resistance
cusB	Silver and copper efflux, membrane fusion protein; confers copper and silver resistance
cusC	Silver and copper efflux, outer membrane factor (OMF) lipoprotein component; OMF of a tripartate efflux pump; confers copper and silver resistance
cusR	Response regulator of the *cusCFBA-cusRS* divergon; *cusS* sensor and *cusR* mediate copper induction
pgaA	Biofilm adhesin polysaccharide, PGA secretin; outer membrane porin
pgaB	PGA N-deacetylase; deacetylase required for biofilm adhesin polysaccharide PGA export; outer membrane lipoprotein
wcaA	Putative colanic acid biosynthesis glycosyl transferase
wcaD	Putative colanic acid polymerase

[a]From Zhou and Rudd [43].
RNA = ribonucleic acid; DNA = deoxyribonucleic acid; ATP = adenosine triphosphate; PGA = poly-β-1,6-N-acetyl-D-glucosamine.

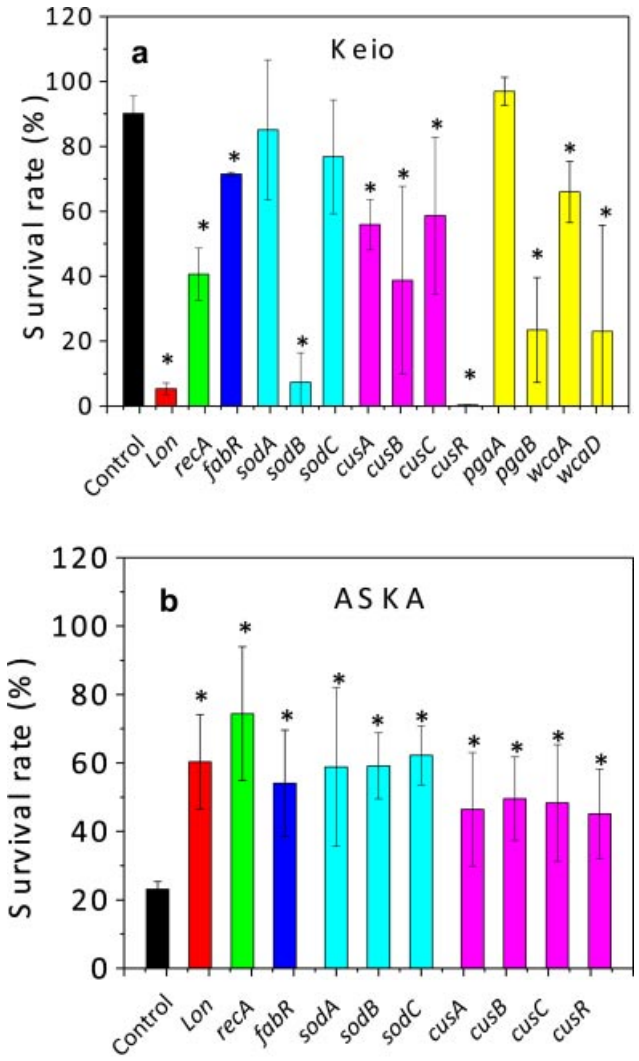

Figure 1. Survival rate of (**a**) Keio gene deletion mutants and (**b**) ASKA gene overexpression clones after exposure to 8 mg/L of $AgNO_3$ for 6 h. Different gene deletion mutants in the Keio collection responded differently in the presence of Ag(I), indicating the essential or nonessential function of certain genes in defending silver toxicity. All tested gene overexpression mutants appeared to be more resistant than the wild-type control, presumably because of higher production of proteins (binding with silver).

material [24,25]. Therefore, it is likely that Ag^+ would bind and damage bacterial DNA if it reaches the cell nucleoid. The present study shows that *ΔrecA* mutants, which lack the ability to repair DNA, are more sensitive to silver exposure ($40.6 \pm 8.0\%$ survival) than the wild-type control ($90.1 \pm 5.4\%$ survival; Figure 1a). Apparently, *recA* plays an essential role in genetic recombination [26] and in repair of various kinds of DNA damage by catalyzing DNA strand exchange reactions [27,28]. The higher sensitivity of *ΔrecA* mutants to Ag^+ implies that DNA damage is an important consequence (if not mechanism) of silver toxicity.

Membrane damage repair gene helps mitigate silver damage

The gene *fabR* encodes a fatty acid biosynthesis regulator, and is essential for the synthesis of monounsaturated fatty acids (Table 1). This transcription factor exclusively regulates expression of type II fatty acid synthase enzymes [29], and directly influences membrane lipid homeostasis, which could be important for silver resistance. Deletion of *fabR* decreases resistance to Ag^+ ($71.5 \pm 0.6\%$ survival) compared with wild-type controls ($90.1 \pm 5.4\%$ survival; Figure 1a), suggesting that membrane repair is an important response to silver exposure. However, resistance to Ag^+ for *ΔfabR* mutants was significantly higher than for the *ΔrecA* and *Δlon* mutants (Figure 1a), suggesting a lesser importance of *fabR* in adaptation to silver.

Metal efflux pump coding genes are critical for silver defense

The expression of metal efflux pump genes is controlled by ultrasensitive regulators that bind metals with *femto*-molar affinities [30,31] (Table 1), and the activity of these transporters may be driven by ATP hydrolysis or chemiosmotic potential [30,32,33]. Deletion of any gene in the *cus* operon (copper and silver efflux pump) significantly decreases the resistance of the resultant mutants to Ag^+ compared with the wild type (Figure 1a), confirming that the loss of metal efflux capacity makes bacteria more vulnerable to Ag^+. Interestingly, *ΔcusR* mutants ($0.4 \pm 0.1\%$ survival) were much more sensitive than *ΔcusA* ($55.9 \pm 7.8\%$), *ΔcusB* ($38.7\% \pm 28.9\%$), or *ΔcusC* ($56.7 \pm 24.1\%$) mutants, possibly because *cusR* is responsible for transcriptional regulation, while the others code for cation efflux system proteins [34,35].

EPS protects bacteria against silver

Some bacteria upregulate EPS genes in response to heavy metal exposure [36], because EPS contain functional groups capable of binding metal ions. The EPS can trap or precipitate metal ions in the extracellular environment to curtail intracellular accumulation. Metals might bind to, or precipitate on, bacterial cell surfaces [37] through interactions involving cell-associated polysaccharides, such as lipopolysaccharide [38]. In *E. coli*, *pgaA*, *pgaB*, *wcaA*, and *wcaD* are responsible for biofilm and EPS production (Table 1). Deletion of *pgaB* ($23.4 \pm 16.2\%$ survival), *wcaA* ($66.0 \pm 9.4\%$), or *wcaD* ($23.1 \pm 32.6\%$) made *E. coli* more sensitive than wild-type bacteria ($90.1 \pm 5.4\%$; Figure 1a), indicating that EPS production genes are important for silver resistance. However, *ΔpgaA* mutants did not show significant differences in survival ($97.0 \pm 4.3\%$) compared with the wild-type control ($90.1 \pm 5.4\%$). While both *pgaA* and *pgaB* are required for poly-β-1,6-N-acetyl-D-glucosamine (PGA) export from the periplasm to the outer membrane, *pgaB* also catalyzes deacetylation of PGA, whereas *pgaA* acts solely as a porin for PGA translocation to the cell surface [39,40]. These results suggest that periplasm to outer membrane translocation of PGA is not critical for protection against silver toxicity, but PGA deacetylation, which results in the formation of glucosamine residues, appears to play a significant role.

TEM characterization of EPS production and AgNP formation

Compared with the *ΔpgaB* mutant, higher amounts of EPS were produced by wild-type *E. coli* (Figure 2a and b). The EPS may protect bacteria from Ag^+ by reducing it to form AgNPs, which exert negligible direct toxicity to *E. coli* (i.e., Ag^+ is the critical effector of the antibacterial activity of AgNPs) [3]. The potential formation of AgNPs in the presence of EPS was further investigated by incubating wild-type cells with $AgNO_3$ (8 mg/L). The TEM images show that nanoparticles were formed inside the EPS (Supplemental Data, Figure S1a), with sizes ranging from 10 nm to 40 nm (Figure 2c). Analysis by STEM-EDS confirmed that the main constituent of the nanoparticles was silver (Figure 2d; copper signal came from the copper grid), which agrees with recent results reported by Kang et al. [41]. Formation of AgNPs was also corroborated by high-resolution TEM analysis, which showed that the measured interplanar

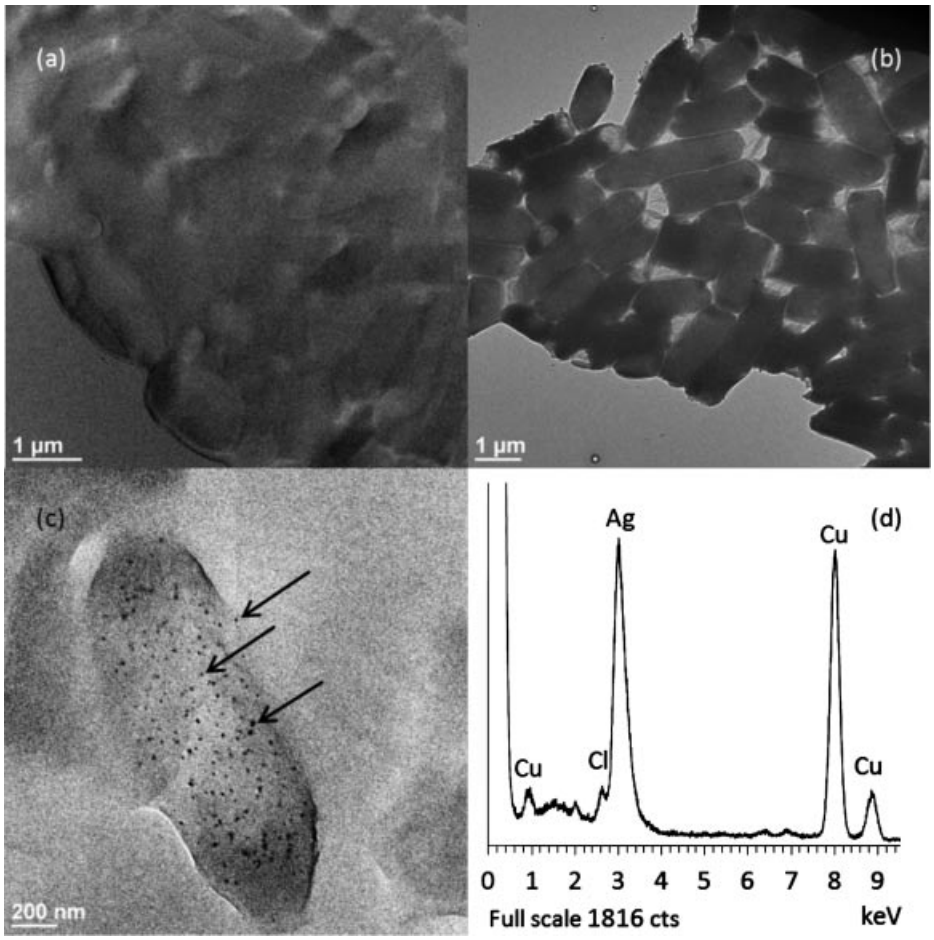

Figure 2. Morphology of the (**a**) wild-type *Escherichia coli*, showing bacterial agglomeration in the extracellular polymeric substances (EPS) matrix; (**b**) *ΔpgaB* mutants, showing cells with less EPS that are easier to visualize under high-resolution transmission electron microscopy; and (**c**) wild-type *E. coli* cells after 16-h growth and 6-h subsequent incubation with $AgNO_3$ (8 mg/L), revealing that black nanoparticles (shown by arrows) were formed in the presence of EPS. (**d**) Scanning transmission electron microscopy/energy-dispersive X-ray spectroscopy spectra collected from nanoparticles in (**c**) confirming that nanoparticles are mainly silver. (Cu signal comes from the copper grid.)

spacing (0.24 nm) of the lattice-fringe fingerprinting of the nanoparticles is consistent with the crystal face of elemental metallic silver (Supplemental Data, Figure S1b).

Note that under silver exposure, bacteria tend to contract their cytoplasm, leaving both ends of the cell transparent (Supplemental Data, Figure S2a). No intact cells were found in the *ΔpgaB* culture, and only some cell debris-like subjects were noted (Supplemental Data, Figure S2b). The absence of cells in the sample could be the result of the higher sensitivity of mutants to Ag^+, as shown in the Ag^+ toxicity assay (Figure 1b). Thus, EPS can be a critical factor in defending against silver ion exposure.

ORF clones exhibit higher resistance to silver because of protein overproduction

Open reading frame clones overexpress specific genes of interest [14], which could be useful in assessing their role in protecting bacteria against silver. However, all tested mutants produced significantly higher amounts of protein than the wild type, as determined by the Bradford method (Supplemental Data, Figure S3), which confounds data interpretation because proteins tend to bind Ag^+, reducing its bioavailability [42]. Consequently, all clones were similarly much more resistant to silver than the wild type (Figure 1b), which precluded discernment of the relative importance of overexpressing different genes for conferring resistance.

CONCLUSIONS

Adaptation and associated defense strategies of *E. coli* against Ag^+ were investigated at the genetic level using the Keio and ASKA libraries. Our research shows that silver toxicity is multifaceted and involves multiple modes of action and associated defense mechanisms. Quenching of ROS, protein damage repair, and metal efflux are important defense mechanisms for *E. coli*. The TEM images also showed that EPS plays an important role as a protective barrier that immobilizes dissolved silver as AgNPs, making it less bioavailable. This information advances our fundamental understanding of silver–microbial interactions relevant to disinfection and to the assessment of potential unintended impacts of silver releases to microbial ecosystem services.

SUPPLEMENTAL DATA

Figures S1–S3. (1.6 MB DOC).

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REFERENCES

1. Silver S, Phung LT, Silver G. 2006. Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. *J Ind Microbiol Biot* 33:627–634.
2. Lemire JA, Harrison JJ, Turner RJ. 2013. Antimicrobial activity of metals: Mechanisms, molecular targets and applications. *Nat Rev Microbiol* 11:371–384.
3. Xiu ZM, Zhang QB, Puppala HL, Colvin VL, Alvarez PJJ. 2012. Negligible particle-specific antibacterial activity of silver nanoparticles. *Nano Lett* 12:4271–4275.
4. Liau SY, Read DC, Pugh WJ, Furr JR, Russell AD. 1997. Interaction of silver nitrate with readily identifiable groups: Relationship to the antibacterial action of silver ions. *Lett Appl Microbiol* 25:279–283.
5. Ratte HT. 1999. Bioaccumulation and toxicity of silver compounds: A review. *Environ Toxicol Chem* 18:89–108.
6. Wang JM, Huang CP, Pirestani D. 2003. Interactions of silver with wastewater constituents. *Water Res* 37:4444–4452.
7. Choi O, Hu ZQ. 2008. Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. *Environ Sci Technol* 42: 4583–4588.
8. Park HJ, Kim JY, Kim J, Lee JH, Hahn JS, Gu MB, Yoon J. 2009. Silver-ion-mediated reactive oxygen species generation affecting bactericidal activity. *Water Res* 43:1027–1032.
9. Kim S, Choi JE, Choi J, Chung K-H, Park K, Yi J, Ryu D-Y. 2009. Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. *Toxicol In Vitro* 23:1076–1084.
10. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. 2000. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J Biomed Mater Res* 52:662–668.
11. Izatt RM, Christen J, Rytting JH. 1971. Sites and thermodynamic quantities associated with proton and metal ion interaction with ribonucleic acid, deoxyribonucleic acid, and their constituent bases, nucleosides, and nucleotides. *Chem Rev* 71:439–481.
12. Arakawa H, Neault JF, Tajmir-Riahi HA. 2001. Silver(I) complexes with DNA and RNA studied by Fourier transform infrared spectroscopy and capillary electrophoresis. *Biophys J* 81:1580–1587.
13. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H. 2006. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: The Keio collection. *Mol Syst Biol* 2.
14. Kitagawa M, Ara T, Arifuzzaman M, Ioka-Nakamichi T, Inamoto E, Toyonaga H, Mori H. 2005. Complete set of ORF clones of *Escherichia coli* ASKA library (a complete set of *E. coli* K-12 ORF archive): Unique resources for biological research. *DNA Res* 12:291–299.
15. Xiu ZM, Ma J, Alvarez PJJ. 2011. Differential effect of common ligands and molecular oxygen on antimicrobial activity of silver nanoparticles versus silver ions. *Environ Sci Technol* 45:9003–9008.
16. Gu MB, Mitchell RJ, Kim BC. 2004. Whole-cell-based biosensors for environmental biomonitoring and application. *Adv Biochem Eng Biotechnol* 87:269–305.
17. Compan I, Touati D. 1993. Interaction of six global transcription regulators in expression of manganese superoxide dismutase in *Escherichia coli* K-12. *J Bacteriol* 175:1687–1696.
18. Niederhoffer EC, Naranjo CM, Bradley KL, Fee JA. 1990. Control of *Escherichia coli* superoxide dismutase (*sodA* and *sodB*) genes by the ferric uptake regulation (*fur*) locus. *J Bacteriol* 172:1930–1938.
19. Massé E, Gottesman S. 2002. A small RNA regulates the expression of genes involved in iron metabolism in *Escherichia coli*. *Proc Natl Acad Sci U S A* 99:4620–4625.
20. Wang ZO, Schmitt MP, Holmes RK. 1994. Characterization of mutations that inactivate the diphtheria-toxin repressor gene (Dtxr). *Infect Immun* 62:1600–1608.
21. Van Melderen L, Aertsen A. 2009. Regulation and quality control by Lon-dependent proteolysis. *Res Microbiol* 160:645–651.
22. Van Melderen L, De Bast MS. 2009. Bacterial toxin-antitoxin systems: More than selfish entities? *PloS Genet* 5:e1000437.
23. Jensen RH, Davidson N. 1966. Spectrophotometric potentiometric and density gradient ultracentrifugation studies of binding of silver ion by DNA. *Biopolymers* 4:17–32.
24. Kitamura H, Kondo Y, Sakairi N, Nishi N. 1997. Preparation and characterization of antibacterial alginate film containing DNA as a carrier of silver ion. *Nucleic Acids Symp Ser* 1997:273–274.
25. Yamada M, Kato K, Shindo K, Nomizu M, Sakairi N, Yamamoto H, Nishi N. 1999. Immobilization of DNA by UV irradiation and its utilization as functional materials. *Nucleic Acids Symp Ser* 1999: 103–104.
26. Clark AJ, Margulies AD. 1965. Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K12. *Proc Natl Acad Sci U S A* 53:451–459.
27. Kuzminov A. 1999. Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage λ. *Microbiol Mol Biol Rev* 63: 751–813.
28. Courcelle J, Hanawalt PC. 2003. RecA-dependent recovery of arrested DNA replication forks. *Annu Rev Genet* 37:611–646.
29. Zhang Y-M, Marrakchi H, Rock CO. 2002. The FabR (YijC) transcription factor regulates unsaturated fatty acid biosynthesis in *Escherichia coli*. *J Biol Chem* 277:15558–15565.
30. Ma Z, Jacobsen FE, Giedroc DP. 2009. Coordination chemistry of bacterial metal transport and sensing. *Chem Rev* 109:4644–4681.
31. Outten CE, O'Halloran TV. 2001. Femtomolar sensitivity of metal-loregulatory proteins controlling zinc homeostasis. *Science* 292:2488–2492.
32. Nies DH. 1999. Microbial heavy-metal resistance. *Appl Microbiol Biot* 51:730–750.
33. Silver S, Phung LT. 1996. Bacterial heavy metal resistance: New surprises. *Annu Rev Microbiol* 50:753–789.
34. Mills SD, Lim C-K, Cooksey DA. 1994. Purification and characterization of CopR, a transcriptional activator protein that binds to a conserved domain (*cop* box) in copper-inducible promoters of *Pseudomonas syringae*. *MGG* 244:341–351.
35. Munson GP, Lam DL, Outten FW, O'Halloran TV. 2000. Identification of a copper-responsive two-component system on the chromosome of *Escherichia coli* K-12. *J Bacteriol* 182:5864–5871.
36. Teitzel GM, Parsek MR. 2003. Heavy metal resistance of biofilm and planktonic *Pseudomonas aeruginosa*. *Appl Environ Microb* 69: 2313–2320.
37. Mullen MD, Wolf DC, Ferris FG, Beveridge TJ, Flemming CA, Bailey GW. 1989. Bacterial sorption of heavy-metals. *Appl Environ Microb* 55:3143–3149.
38. Langley S, Beveridge TJ. 1999. Effect of O-side-chain-lipopolysaccharide chemistry on metal binding. *Appl Environ Microb* 65:489–498.
39. Little DJ, Poloczek J, Whitney JC, Robinson H, Nitz M, Howell PL. 2012. The structure- and metal-dependent activity of *Escherichia coli* PgaB provides insight into the partial de-N-acetylation of poly-beta-1, 6-N-acetyl-D-glucosamine. *J Biol Chem* 287:31126–31137.
40. Itoh Y, Rice JD, Goller C, Pannuri A, Taylor J, Meisner J, Beveridge TJ, Preston JF, Romeo T. 2008. Roles of *pgaABCD* genes in synthesis, modification, and export of the *Escherichia coli* biofilm adhesin poly-β-1, 6-N-acetyl-D-glucosamine. *J Bacteriol* 190:3670–3680.
41. Kang F, Alvarez P, Zhu D. 2014. Microbial extracellular polymeric substances reduce Ag^+ to silver nanoparticles and antagonize bactericidal activity. *Environ Sci Technol* 48:316–322.
42. Sedlak RH, Hnilova M, Grosh C, Fong H, Baneyx F, Schwartz D, Sarikaya M, Tamerler C, Traxler B. 2012. Engineered *Escherichia coli* silver-binding periplasmic protein that promotes silver tolerance. *Appl Environ Microb* 78:2289–2296.
43. Zhou J, Rudd KE. 2013. EcoGene 3.0. *Nucl Acids Res* 41:D613–D624.