Silver nanoparticles embedded in zeolite membranes: release of silver ions and mechanism of antibacterial action

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Background: The focus of this study is on the antibacterial properties of silver nanoparticles embedded within a zeolite membrane (AgNP-ZM).

Methods and Results: These membranes were effective in killing Escherichia coli and were bacteriostatic against methicillin-resistant Staphylococcus aureus. E. coli suspended in Luria Bertani (LB) broth and isolated from physical contact with the membrane were also killed. Elemental analysis indicated slow release of Ag+ from the AgNP-ZM into the LB broth. The E. coli killing efficiency of AgNP-ZM was found to decrease with repeated use, and this was correlated with decreased release of silver ions with each use of the support. Gene expression microarrays revealed upregulation of several antioxidant genes as well as genes coding for metal transport, metal reduction, and ATPase pumps in response to silver ions released from AgNP-ZM. Gene expression of iron transporters was reduced, and increased expression of ferrochelatase was observed. In addition, upregulation of multiple antibiotic resistance genes was demonstrated. The expression levels of multicopper oxidase, glutaredoxin, and thioredoxin decreased with each support use, reflecting the lower amounts of Ag+ released from the membrane. The antibacterial mechanism of AgNP-ZM is proposed to be related to the exhaustion of antioxidant capacity.

Conclusion: These results indicate that AgNP-ZM provide a novel matrix for gradual release of Ag+.

Keywords: silver nanoparticles, zeolite, antibacterial agent, oxidative stress

Introduction

Given that we are in an era where antibiotic resistance is a growing concern, there is a renewed interest in developing products containing silver for use as antimicrobials. For thousands of years, silver has been used for food and beverage preservation, and in medicines.1 The use of silver as an antibacterial agent declined with the discovery of antibiotics, but the evolution of antibiotic-resistant pathogens has brought a revival in silver-based applications. Silver is now an additive in consumer products including bandages, socks, shirts, water filters, antiperspirants, combs, paints, and washing machines.2

The antibacterial mechanism of silver nanoparticles (AgNP) and Ag+ has been explored extensively. Baker et al3 found that complete bacterial cell death could be achieved at 8 µg/cm² AgNP and that smaller particles were more efficient antibacterials. Others have supported this finding, and found that the amount of chemisorbed Ag+ and aggregation status of AgNP influences antibacterial efficacy.4 The formation of reactive oxygen species has been implicated in bacterial toxicity,3 and these are
thought to damage DNA and proteins, as well as perturb cell membrane integrity.\(^6\)

However, there is growing concern surrounding the increasing use of AgNP and their impact on the environment. The spread of AgNP into wastewater is an environmental concern, in that researchers have found that the numbers of nitrifying bacteria found in sludge are reduced when exposed to large quantities of AgNP,\(^7\) which has severe implications on waste water treatment.

This motivated us to develop a method to immobilize AgNP into lithographically patterned zeolite membranes, and we have already reported that such membranes are effective in killing \textit{Escherichia coli} upon contact.\(^8\) Other research in this area has focused on Ag\(^+\)-zeolite powders as antibacterial agents. Ag\(^+\) ions are ion-exchanged out of the zeolite powder into media and are sufficient to cause bacterial cell death in both \textit{E. coli} and \textit{Staphylococcus aureus}.\(^9,10\) In the case of Ag\(^+\)-zeolite, the release of Ag\(^+\) into solution is primarily determined by the ionic strength of the medium, because this is an ion-exchange process and is media-dependent. Recently, there has also been a report of AgNP in zeolite powders and their activity towards Gram-positive and Gram-negative bacteria.\(^11\)

In this study, we investigated the antibacterial capacity of AgNP embedded in zeolite membranes (AgNP-ZM) and found that their bactericidal properties stem from the gradual release of Ag\(^+\) into the media. From a materials perspective, zeolite membranes are more attractive as supports than powders, since macroscopic membranes can be grown on ceramics, metals, and polymeric and cellulose supports,\(^12\) thus allowing for diverse applications, including use in the hospital setting. The mechanism of \textit{E. coli} death was investigated using viability assays, gene expression arrays, and quantitative reverse transcriptase polymerase chain reaction (PCR). The biological studies suggest that exhaustion of antioxidant capacity is related to antibacterial function.

**Materials and methods**

**Materials**

Silver nitrate (99%), potassium nitrate, trypan blue, polyethylene glycol, Ludox SM-30, poly(methyl methacrylate), and hydrazine were purchased from Sigma Aldrich (St. Louis, MO). PEG-600 (Fluka, Buchs, Switzerland), Darvan (RT Vanderbilt Co Inc, Norwalk, CT), aluminum hydroxide (Alfa Aesar Ward Hill, MA, 80.5%), sodium hydroxide (Mallinckrodt Hazlewood, MO, 98.8%), 25 wt% tetramethyl ammonium hydroxide aqueous solution (Sachem, Austin, TX), AKP30 high-purity alumina powder (Sumitomo Chemical Co Ltd, Tokyo, Japan), with an average particle size of 300 nm, silastic T-2 polydimethylsiloxane (Dow Corning, Midland, MI), 200 proof ethyl alcohol (Pharmco, Brookfiled, CT), and 1-octanol (Puriss, Fluka, Buchs, Switzerland) were also purchased and used without further purification. Luria Bertani (LB) broth powder agar, brain heart-infusion broth, 100 mm sterile Petri dishes, and chloroform were obtained from Fisher Scientific (Pittsburgh, USA) and 0.4 \(\mu\)m pore transwell plates and six-well plates were obtained from Corning (Lowell, MA). Qiagen (Valencia, CA) supplied the Puregene DNA purification kit, the RNeasy RNA isolation kit, DNase, and QuantiTect SYBR Green reverse transcriptase PCR kit. Primers were purchased from Integrated DNA Technologies (San Diego, CA). The \textit{E. coli} strain, XL-1 blue, which was derived from the K-12 strain, was a kind gift from Dr Joanne Trgovcich (Department of Surgery, The Ohio State University Medical Center). Bioanalyzer Lab-On-A-Chip Agilent 6000 Series II chips and \textit{E. Coli} 8x15K Microarrays were purchased from Agilent (Santa Clara, CA).

**Synthesis of AgNP-ZM**

Macroporous alumina oxide supports were used as the substrate for zeolite membrane growth, and their preparation is described in detail in earlier studies.\(^13\) Briefly, nanometer-sized zeolites are deposited on the alumina support and grown into a continuous membrane by hydrothermal synthesis. The zeolite membranes were then ion-exchanged with 0.005 M AgNO\(_3\) solution, washed, and then reduced by hydrazine, as described earlier.\(^8\) After washing, the AgNP-ZM were extensively ion-exchanged with 1 M NaCl to remove unreacted silver ions from the zeolite. A schematic of AgNP-ZM fabrication is provided in Supplemental Figure 1.

**Chemical characterization of AgNP-ZM**

Supernatants were collected from AgNP-ZM suspended in LB broth for various times and used for elemental analysis. Similar experiments were done with AgNP-ZM that were repeatedly exposed to LB broth. Silver content was measured using inductively coupled plasma optical emission spectroscopy at Galbraith Laboratories, Knoxville, TN.

**Biological characterization**

Cultures of XL-1 blue \textit{E. coli} were incubated with the AgNP-ZM or zeolite membrane controls and assessed for viability using traditional colony counts. LB broth solution was prepared using a concentration of 25 g/L of LB. LB agar plates were prepared with 1.5% agar. Individual
clones were inoculated in 3 mL of LB broth and shaken at 225 rpm overnight at 37°C. Prior to exposing bacteria to the zeolite membranes, bacterial cultures were adjusted to obtain an initial optical density between 0.2 and 0.8, with viable colony counts ranging between about $1 \times 10^5$ and $1 \times 10^6$ colony-forming units (cfu)/mL. For the initial viability experiment, one zeolite membrane and one freshly prepared AgNP-ZM were tested three times. Membranes were placed into six-well tissue culture plates and 5 mL (approximately $1 \times 10^7$ cells/mL) of bacterial suspension was added to each well. Experimental plates were then incubated at 37°C and continuously shaken. For each experiment, samples were removed at 0, 30, 60, 120, and 180 minutes, where 100 µL was taken from wells containing zeolite controls or AgNP-ZM and added to a tube containing 0.9 mL of LB broth. Samples were further diluted in LB broth by 10-fold six more times. To obtain colony counts, 100 µL of samples were plated from each dilution. LB plates were incubated at 37°C overnight and cfu were counted to determine bacteria viability. Optical densities of culture supernatants were measured after exposure to two separate AgNP-ZM for 60, 120, or 180 minutes using a Shimadzu spectrophotometer at an absorbance wavelength of 600 nm.

We also tested the preliminary antibacterial activity of AgNP-ZM against a methicillin-resistant strain of *S. aureus* (MRSA, a kind gift from Dr Vijay Pancholi, Department of Pathology, The Ohio State University). Here, a clone of MRSA was grown overnight in brain-heart infusion broth. A fresh stock was inoculated into brain-heart-infusion broth from the overnight culture and grown at 37°C for 24 hours at 37°C. From the apical and basal chambers, 100 µL samples were taken after 30, 60, and 120 minutes, diluted using the serial dilution scheme described above, plated, and incubated overnight at 37°C.

The efficacy of AgNP-ZM was tested by exposing the same AgNP-ZM to approximately $1 \times 10^7$ cells/mL of *E. coli* in 5 mL of LB broth a total of six times. LB broth samples (100 µL) were collected at 30, 60, and 120 minutes during each of the six experimental exposures. Colony counts were performed in the same manner stated earlier in the Materials and Methods section, where a series of 10-fold dilutions were prepared from the samples, and 100 µL from each dilution was plated on LB agar. Plates were incubated for 24 hours at 37°C prior to counting. In between each use, the AgNP-ZM were sterilized by steam autoclave, much like surgical instruments found in a hospital setting.

**DNA and RNA extraction**

*E. coli* genomic DNA was isolated using a Puregene DNA purification kit according to the manufacturer’s instructions. Bacterial RNA was extracted using standard procedures from *E. coli* exposed to zeolite membranes or exposed to AgNP-ZM for 30–45 minutes (see Supplemental Methods). The quality of RNA was examined using an Agilent 2100 Bioanalyzer Lab-On-A-Chip Agilent 6000 Series II chip. RNA samples were checked for DNA contamination by running PCR using samples with primers with or without reverse transcriptase. The presence of PCR products was determined by gel electrophoresis using 1% agarose gel.

**Gene expression microarrays**

Four individual zeolite supports containing AgNPs and four zeolite support controls were exposed to approximately $1 \times 10^4$ cfu/mL of bacteria for 30 minutes prior to RNA
isolation, and bacterial viability was determined via colony counts (Supplemental Figure 2). RNA samples were stored at −80°C until processing. Sample labeling and hybridizations were performed by the Nationwide Children’s Hospital’s Biomedical Genomics Facility (http://genomics.nchresearch.org/index.html). The RNA was of high quality and all samples passed the standard quality control cutoff. Sample labeling and hybridization was performed according to the manufacturer’s protocols. Samples were hybridized to the *E. coli* 8 × 15 K Microarray (AMADID 020097). Parameters regarding gene array methodology, quality control, and statistical analyses are included in the Supplemental Materials section.

**Quantitative reverse transcriptase PCR**

*E. coli* at a concentration of 1 × 10⁸ cfu/mL were exposed to zeolite membranes alone or containing AgNPs for 45 minutes prior to RNA isolation. Quantitative reverse transcriptase PCR analyses were completed using a Quantitect SYBR Green reverse transcriptase PCR kit under the following conditions. Master mixes were prepared using 12.5 µL 2x QuantiTect SYBR Green Master Mix, 0.25 µL QuantiTect reverse transcriptase mix, and 10.25 µL RNase-free water per reaction. Forward and reverse primers were added using 0.5 µL from 25 µM stocks, and primer sequences are listed in Supplemental Table 1. Master mixes were aliquoted into 96-well plates (24 µL/well) along with 1 µL of RNA at a concentration of 1 ng/reaction. Reactions were cycled under the following conditions. Reverse transcription was performed for 30 minutes at 50°C. PCR activation was performed at 95°C for 15 minutes. Denaturation occurred at 94°C for 15 seconds, annealing occurred for 30 seconds at 58°C and extension occurred at 72°C for one minute. Cycling conditions were repeated 35 times. The ABI Prism 7500 sequence detection system was used to quantify gene expression using a gene-specific standard curve generated with bacterial DNA.

**Results**

**AgNP-ZM synthesis and characterization**

Synthesis of AgNP-ZM starts with the synthesis of porous alumina supports, on which a seeded layer of zeolite is deposited. The zeolite membrane is then grown on the seeded side via secondary hydrothermal growth. Figure 1A shows the powder x-ray pattern of a typical membrane. The three strong peaks (marked with an asterisk) arise from the alumina support. All of the other peaks are from the zeolite, the major phase being zeolite Y with minor quantities of zeolite A (strongest peak 7° 2θ). Figure 1B shows a scanning electron microscopic cross-sectional image of the membrane with the dense 2–3 µm layer of zeolite on the porous alumina (2 mm). In order to make the AgNP, the zeolite membrane is ion-exchanged with Ag⁺, reduced with hydrazine to make Ag nanoclusters on the zeolite membrane, and extensively ion-exchanged with Na⁺ to remove any unreacted Ag⁺. Figure 2A shows a scanning electron microscopic top view of the AgNP-ZM, and Figure 2B is a magnified image that clearly shows the presence of Ag clusters on the zeolite (<50 nm). Figure 2C is the elemental analysis of the surface of the membrane showing the presence of Ag, Si, and Al.
The AgNP-ZM were incubated in LB broth (5 mL), and the amount of Ag⁺ released over time was analyzed by elemental analysis. In order to estimate an upper limit of the amount of Ag⁺ that can be released from the membrane, freshly prepared (autoclaved) AgNP-ZM were exposed to LB broth for 48 hours under gentle shaking and the amount of Ag⁺ was found to be approximately 20 ppm. When this same AgNP-ZM was exposed to media for 30 minutes for a second time, 5.7 ppm Ag⁺ was released. After a third and fourth exposure (sample autoclaved prior to each exposure) of the same AgNP-ZM for 30-minutes and 60-minutes, contact with media released 1.9 and 1.7 ppm of Ag⁺, respectively. The goal of these elemental analysis studies was to evaluate an upper limit upon single exposure for an extended time period (48 hours), and to demonstrate that the AgNP-ZM have a large reservoir of Ag (primarily due to the large internal surface area of the zeolite) so that even a second, third, and fourth exposure leads to release of Ag⁺ at ppm levels.

Interaction of AgNP-ZM with *E. coli*

Antibacterial activity

*E. coli* was incubated alone, with a control zeolite membrane, and with a freshly prepared AgNP-ZM to determine and

![Graph](https://www.dovepress.com/)

**Figure 3 (A)** Turbidity analyses of supernatant samples from unexposed *Escherichia coli* and *E. coli* exposed to zeolite controls or zeolite-supported silver nanoparticles over time. Values are expressed as the mean and standard deviation of two experiments. (B) Enumeration of viable *E. coli* over time upon incubation with zeolite supports containing silver nanoparticles and controls.

**Notes:** *Significant differences versus zeolite membrane controls, n = 3, freshly-prepared zeolite supports containing silver nanoparticles, P < 0.05.

**Abbreviation:** cfu/mL, colony forming units per milliliter.
compare the antibacterial activity of AgNP-ZM. Turbidity measurements performed on two separate occasions using freshly prepared AgNP-ZM revealed that bacterial growth was completely inhibited over a three-hour incubation period when E. coli was exposed to AgNP-ZM (optical density about 0.4), whereas with bacteria only and zeolite supports, proliferation increased over time, as shown in Figure 3A. Figure 3B shows the number of colonies after incubation with controls or AgNP-ZM, and a significant decrease in E. coli viability was observed over a three-hour period, although a decrease in bacterial viability was noted with AgNP-ZM after 30 minutes. All of the data in Figure 3B were obtained with one freshly prepared AgNP-ZM on three separate occasions.

To determine if antibacterial action was contact-dependent, a zeolite membrane control and two AgNP-ZM were incubated with 5 mL of LB broth for three hours. The membranes were then removed, and the conditioned LB broth (5 mL) was collected. Bacterial cultures which had been adjusted to about \(1 \times 10^5\) cells/mL in 5 mL of LB broth were pelleted by centrifugation. The pellet was vortexed, and the conditioned broth (5 mL) was then applied to the culture. As Figure 4A shows, all E. coli were nonviable within 60 minutes in medium conditioned with AgNP-ZM. Medium-conditioned with zeolite membranes alone had no observable effect on bacterial viability. Figure 4B illustrates that for bacteria separated from the zeolite membranes using transwell plates containing inserts with a pore size of 0.4 µm, death was evident within 120 minutes when AgNP-ZM were used, while controls grew normally. To ensure that bacteria were unable to traverse the transwell membrane, media from the bottom chamber were sampled from all experimental treatments and plated for growth on LB agar, and no colonies were observed (data not shown).

The antibacterial activity of a single AgNP-ZM was tested a total of six times in order to evaluate the feasibility of repeated use of a membrane. The support was sterilized by steam autoclave between each use. After the AgNP-ZM had been used three or four times, the viability of E. coli cultures seeded at a concentration of approximately \(1 \times 10^6\) cfu/mL was reduced to 0 cfu/mL within two hours (Figure 5). However, after four or more uses, complete death was apparent after three hours of incubation.

**Gene expression by E. coli exposed to AgNP-ZM**

Gene expression arrays revealed significant differences between E. coli exposed to AgNP-ZM versus zeolite membranes. A total of 145 genes were upregulated greater than two-fold, while 170 genes were downregulated (Supplemental Tables 2 and 3, respectively). Selected genes which were upregulated or downregulated by at least three-fold are included in Tables 1 and 2, respectively. Both copper transporter gene copA and magnesium transporter gene mgtA were upregulated over
n-fold. The genes which encode the antioxidants thioredoxin and glutaredoxin were also upregulated greater than about 7.5-fold compared with zeolite controls. In addition, several genes encoding proteins involved with sulfur transport, ie, cysW, cysA, cysD, and cysU, were upregulated greater than nine-fold (Table 1). Genes coding for multiple antibiotic resistance (marA and marR) were increased approximately eight-fold and seven-fold, respectively (Table 1). Several genes associated with iron transport, including fepG, fecR, fecP, fepA, fhuE, and fhuC, were downregulated (Table 2), although the gene expression of hemH (coding for ferrochelatase) was upregulated about 10-fold (Table 1) in E. coli exposed to AgNP-ZM compared with zeolite membranes.

**Gene expression in E. coli changes with AgNP-ZM use**

Quantitative reverse transcriptase PCR was used to confirm gene expression microarray data. Selected genes representing those involved with metal transport, resistance, and oxidative stress were analyzed in order to identify possible mechanisms of toxicity induced by exposure to AgNP-ZM. Table 3 reports quantitative reverse transcriptase PCR data for genes encoding copper-silver efflux, glutaredoxin, multicopper oxidase, and thioredoxin, and their expression levels after each support use. With progressive use of AgNP-ZM, expression of the gene that encodes glutaredoxin decreased (fold-change of approximately 14 and 2, and virtually no change for trials 1, 2, and 3, respectively). The expression of thioredoxin was increased by approximately five-fold after each support use. With progressive use of AgNP-ZM, expression of genes encoding copper-silver efflux, glutaredoxin, multicopper oxidase, and thioredoxin, and their expression levels after each support use. With progressive use of AgNP-ZM, expression of the gene that encodes glutaredoxin decreased (fold-change of approximately 14 and 2, and virtually no change for trials 1, 2, and 3, respectively). The expression of thioredoxin was increased by approximately five-fold after each support use.

### Table 1: Increases in *Escherichia coli* gene expression in response to 30-minute exposures to four independent zeolite supports containing silver nanoparticles versus *E. coli* exposed to four independent zeolite controls

| Gene name | Gene product | Fold change AgNP-ZM vs zeolite | Description |
|-----------|--------------|--------------------------------|-------------|
| cysP | Thiosulfate transporter subunit | 14.9 | Thiosulfate binding protein |
| cysW | Sulfate/thiosulfate transporter subunit | 11.9 | Sulfate transport system permease W protein |
| copA | Copper transporter | 10.8 | Putative ATPase |
| cysA | Sulfate/thiosulfate transporter subunit | 10.3 | ATP-binding component of sulfate permease A protein; chromate resistance |
| hemH | Ferrochelatase | 9.5 | Ferrochelatase: final enzyme of heme biosynthesis |
| magA | Magnesium transporter | 9.0 | Mg2+ transport ATPase, P-type I |
| cysD | Sulfate adenylyltransferase, subunit 2 | 8.7 | ATP:sulfurylase |
| cysU | Sulfate/thiosulfate transporter subunit | 8.7 | Sulfate, thiosulfate transport system permease T protein |
| cysJ | Sulfite reductase, alpha subunit, flavoprotein | 8.1 | Sulfite reductase |
| marA | DNA-binding transcriptional dual activator of multiple antibiotic resistance | 7.9 | Multiple antibiotic resistance; transcriptional activator of defense systems |
| zraP | Zn-binding periplasmic protein | 7.8 | Orf, hypothetical protein |
| trxC | Thioredoxin 2 | 7.6 | Putative thioredoxin-like protein |
| grxA | Glutaredoxin 1, redox coenzyme for ribonucleotide reductase (RNR1a) | 6.8 | Glutaredoxin1 redox coenzyme for glutathione-dependent ribonucleotide reductase |
| marR | DNA-binding transcriptional repressor of multiple antibiotic resistance | 6.7 | Multiple antibiotic resistance protein; repressor of mar operon |
| cueO | Multicopper oxidase (lacase) | 6.1 | Orf, hypothetical protein |
| cysL | Sulfite reductase, beta subunit, NAD(P)-binding, heme-binding | 5.9 | Sulfite reductase, alpha subunit |
| cysF | Periplasmic copper-binding protein | 5.7 | Orf, hypothetical protein |
| cysH | 3-phosphoadenosine 5′-phosphosulfate reductase | 5.6 | 3-phosphoadenosine 5-phosphosulfate reductase |
| arsR | DNA-binding transcriptional repressor | 4.2 | Transcriptional repressor of chromosomal ars operon |
| arsB | Arsenite/antimonite transporter | 4.2 | Arsenical pump membrane protein |
| soxR | DNA-binding transcriptional dual regulator, Fe-S center for redox-sensing | 3.8 | Redox-sensing activator of soxS |
| cyaC | Arsenate reductase | 3.1 | Orf, hypothetical protein |
| cuaB | Copper/silver efflux system, membrane fusion protein | 3.0 | Putative resistance protein |
| cuaC | Copper/silver efflux system, outer membrane component | 2.7 | Putative resistance protein |

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Table 2 Decreases in Escherichia coli gene expression in response to 30-minute exposures to four independent zeolite supports containing silver nanoparticles versus E. coli exposed to four independent zeolite controls

| Gene Name | Gene product | Fold change AgNP-ZM vs zeolite | Description |
|-----------|--------------|-------------------------------|-------------|
| fepG      | Iron-enterobactin transporter subunit | −12.6 | Ferric enterobactin transport protein |
| fes       | Enterobactin/ferric enterobactin esterase | −8.4 | Enterochelin esterase |
| cirA      | Ferric iron-catecholate outer membrane transporter | −7.1 | Outer membrane receptor for iron-regulated colicin I receptor; porin; requires tonB gene product |
| fecR      | KpLE2 phage-like element; transmembrane signal transducer for ferric citrate transport | −5.9 | Regulator for fec operon, periplasmic |
| fepC      | Iron-enterobactin transporter subunit | −5.6 | ATP-binding component of ferric enterobactin transport |
| fes       | Enterobactin/ferric enterobactin esterase | −5.6 | Enterochelin esterase |
| fepA      | Enterobactin outer membrane transporter | −5.4 | Outer membrane receptor for ferric enterobactin |
| fepD      | Iron-enterobactin transporter subunit | −4.7 | Ferric enterobactin |
| fhuE      | Ferric-rhodotorulic acid outer membrane transporter | −4.0 | Outer membrane receptor for ferric iron uptake |
| fepB      | Iron-enterobactin transporter subunit | −3.7 | Ferric enterobactin |
| sodA      | Superoxide dismutase, Mn | −3.1 | Superoxide dismutase, manganese |
| fhuC      | Iron-hydroxamate transporter subunit | −3.0 | ATP-binding component of hydroxamate-dependent iron transport |

half with latter uses (44-fold, 33-fold, and 16-fold). Gene expression of cusC, which encodes copper-silver efflux, fluctuated slightly with each use (approximately 2-fold, 4-fold, and 2.5-fold for trials 1, 2, and 3, respectively). These experiments were done with a single AgNP-ZM on three separate occasions.

Interaction of AgNP-ZM with other bacteria

The primary goal of this paper was to understand the interaction of AgNP-ZM with E. coli XL-blue, which is a laboratory strain. We also did some preliminary work using MRSA. Exposure to AgNP-ZM resulted in reduced replication, as interpreted by the lack of increase in supernatant turbidity compared with controls, although the killing efficiency of the AgNP-ZM was found to be less potent compared with E. coli over the same exposure period (180 minutes). These data are shown in Figure 6. The gene expression studies were performed with E. coli, although further experimentation is underway using other clinically relevant bacterial strains.

Discussion

The discussion primarily focuses on the properties of the AgNP-ZM and its effect on E. coli.

Release of Ag+ from AgNP-ZM

In our previous study, we did not address the issue of the interactions between bacteria and AgNP-ZM. Our preliminary data indicate that AgNP-ZM were bacteriostatic against MRSA, a Gram-positive clinically relevant strain of S. aureus. However, the bactericidal properties against this bacterium were less potent compared with Gram-negative E. coli. We hypothesize that cell wall differences between these two bacteria may account for the differences in bacterial interactions.

Table 3 Quantitative reverse transcriptase polymerase chain reaction analyses of select genes after three independent trials of zeolite support-containing silver nanoparticles. Escherichia coli at a concentration of 1 × 10⁸ colony forming units/mL was exposed to silver nanoparticles embedded in zeolite membranes or zeolite membrane controls for 45 minutes prior to RNA isolation and analyses. Values are fold-change ± standard deviation

| Gene product (gene name) | Gene expression fold increase |
|---------------------------|-------------------------------|
| Zeolite supported AgNP versus zeolite control |
| Trial 1 | Trial 2 | Trial 3 |
| Copper-silver efflux (cusC) | 2.11 ± 0.02 | 3.93 ± 1.12 | 2.68 ± 0.55 |
| Glutaredoxin (grxA) | 14.64 ± 1.16 | 2.29 ± 0.26 | 1.22 ± 0.34 |
| Multicopper oxidase (cueO) | 43.90 ± 5.92 | 33.60 ± 0.89 | 16.41 ± 0.58 |
| Thioredoxin (trxC) | 5.30 ± 0.47 | 1.35 ± 0.15 | 0.99 ± 0.18 |
viability upon exposure to AgNP-ZM, and studies are underway to investigate these findings. It is clear from the present study (Figure 4) that reduction of *E. coli* growth and viability does not require contact with the membrane. This is in contrast with the findings of Su et al., who found that Ag/clay-conditioned supernatants did not have appreciable antibacterial activity. With freshly prepared AgNP-ZM, we found that the release of Ag$^+$ into the broth can be as high as 20 ppm after 48 hours. Since the zeolite membranes were extensively ion-exchanged with Na$^+$ prior to these experiments, any Ag$^+$ in solution would have to occur by AgNP oxidation and release. Slow release of Ag$^+$ from the AgNP-ZM is also supported by the observation that bacteria sequestered in the transwell plates were not killed as quickly as those incubated with supernatants conditioned for three hours (Figure 4). It is known that solutions of Ag$^+$ at 0.05 ppm result in complete reduction of *E. coli* viability within two hours.\(^4\) Oxidation of AgNP over time and release of Ag$^+$ in less than 30 minutes has been noted.\(^4\) With each AgNP-ZM, efficacy of the membranes decreased as well upon repeated use (Figure 5). Ag$^+$ released into media after the second, third, and fourth use was 5.7, 1.9, and 1.7 ppm, for 30-, 30-, and 60-minute exposures. There is clearly a decrease at the 30-minute exposure level between the second and third use, and it took twice as long (60 minutes) for the fourth use to match the release of the third use. Thus, we hypothesize that the bactericidal effects of AgNP-ZM are delayed due to reduction in the amount of Ag$^+$ released from AgNP-ZM with repeated use. As is evident from the scanning electron microscopy images (Figure 2B), there is a distribution of sizes of AgNP. Smaller AgNP are expected to undergo faster dissolution than larger particles, thus after each use, the Ag$^+$ release should decrease. In addition, autoclaving could alter the surface of the AgNP, such as the formation of insoluble hydroxides or oxides. Interestingly, it has been reported that nanosilver bandages exposed to temperatures >90°C have diminished bactericidal activity.\(^15\) Other sterilization measures, such as ultraviolet light, are also problematic, because of the photochemistry of silver. We also used ethanol for decontamination, as reported above.

Disruption of oxidative balance

Several research groups studying AgNP have proposed that their antibacterial activity is due to the formation of reactive oxygen species. The mechanism of bacterial death was found to be a result of persistent surface free radicals found on AgNP, and that the antibacterial activity of both AgNP and Ag$^+$ could be reversed by n-acetylcysteine.\(^16\) The mechanism of toxicity of AgNP in clay was proposed to be cell membrane disruption caused by the generation of reactive oxygen species, and when incubated with the antioxidant, glutathione, their viability was restored.\(^6\) When bacterial reporter strains specifically responding to superoxide radicals were incubated with 100–300 ppm Ag$^+$, it was apparent that the mechanism of antibacterial activity was via reactive oxygen species, specifically superoxide, which formed after perturbation of the electron transport chain.\(^17\) However, gene expression microarrays in the current study revealed downregulation of *sodA*, encoding a superoxide dismutase (3.1-fold decrease), which suggests that superoxide may not be the predominant reactive oxygen species driving bacterial oxidative stress. On the other hand, upregulation of expression of thioredoxin and glutaredoxin, which are crucial to maintaining oxidative balance, was noted after *E. coli* was exposed to AgNP-ZM (Table 3). Several genes associated with sulfur species trans-
port and reduction were correlated with the multiple use of the AgNP-ZM. In the presence of silver (Ag\(^+\)), bacteria may adjust sulfur pools to accommodate the synthesis of sulfur-containing antioxidants.

Genes associated with iron transport (fep genes) were downregulated, while hemH, which codes for ferrochelatase and plays an important role in heme synthesis, was upregulated. A possible response mechanism to the increased presence of Ag\(^+\) is that the bacteria decrease the available pools of intracellular iron by increasing expression of ferrochelatase, which tightly binds Fe\(^{2+}\), to combat and reduce oxidative stress.\(^\text{18,19}\) The downregulation of genes with a role in iron reduction and iron release from proteins further suggests that the bacteria are attempting to control the intracellular levels of Fe\(^{2+}\), thereby reducing the amount of iron available for Fenton reactions. Attempts by bacteria to regulate and restore oxidative balance are also suggested by the upregulation of saxR, which is involved in redox sensing and controlling expression of superoxide dismutase and other antioxidants.\(^\text{21,22}\)

Several genes encoding for metal ion influx and intracellular metal transport and efflux were upregulated. \(E.\ coli\) exposed to AgNP-ZM upregulated mgtA, which encodes for proteins used for Mg\(^{2+}\) influx, and also upregulated arsR and arsB, which encode genes involved in arsenic resistance, but currently have no known role in silver toxicity. The gene zraP, which encodes a protein used for zinc homeostasis and copA, which in turn encodes for an ATPase intracellular copper transporter, were both upregulated. Others have found that copA is induced in the presence of silver salts,\(^\text{23}\) but does not appear to be involved in silver resistance.\(^\text{24}\) Thus, it is likely that the mechanisms of AgNP toxicity are similar to those for copper toxicity. Slawson et al\(^\text{25}\) reported that Ag\(^+\) toxicity was reduced when Cu\(^{2+}\) is also present, indicating that silver may compete with copper for entry into the cell. There appears to be some promiscuity of bacterial metal transport proteins, the functions of which have not been fully elucidated.

Developing resistance to silver

The genes known to encode silver resistance in \(E.\ coli\) are ybdE, ylcD, ylcC, ylcB, ylcA, and ybcZ.\(^\text{26}\) Most silver-resistant bacterial strains have developed tolerance by utilizing Ag\(^+\) ATPase efflux pumps and antiporters rather than chemical detoxification mechanisms.\(^\text{27}\) However, in the current study, only \(cucC\) (\(ybcB\)) and \(cucF\) (\(ylcC\)), the genes encoding for copper-silver efflux outer membrane protein and periplasmic copper and silver binding proteins, respectively, were upregulated when the bacteria were exposed to AgNP-ZM as compared with zeolite membrane controls. Further, we were able to confirm the upregulation of copper-silver efflux outer membrane protein (\(cucC\)) gene transcripts using quantitative reverse transcriptase PCR, although expression levels fluctuated with AgNP-ZM use, which may be a result of variations in Ag\(^+\) release. The increase in gene transcription of multiple antibiotic resistant genes \(marA\) and \(marR\) is remarkable. The increased expression of \(mar\) genes is associated with antibiotic resistance (including tetracycline and ampicillin resistance).\(^\text{28}\) This work indicates that multiple antibiotic resistance genes may also play a role in the evolution of silver resistance in \(E.\ coli\), and warrant further investigation.

**Conclusion**

The concept of using intrazeolitic space for storage of specific molecules and their slow release has found many applications. For example, controlled release of the preservative, cresol, from zeolite was successfully demonstrated by Eriksson\(^\text{29}\) where \(E.\ coli\) and \(S.\ aureus\) viability was reduced. Another study demonstrated the feasibility of zeolites as a vehicle for drug delivery by releasing ketoprofen under different physiological conditions.\(^\text{30}\) We have shown that surface-modified zeolites can release paraquat under controlled conditions.\(^\text{31}\) However, this study is the first to demonstrate that zeolite membranes can serve as supports, and we demonstrate this functionality using AgNP for antibacterial use. The impact of using membranes is that such membranes can be grown on various supports, including plastics, cellulose, and metals.\(^\text{12}\) Possible uses of this technology could include antibacterial coatings for a wide variety of applications. Further, these membranes have the propensity to be tailored for controlled release, thus dictating the amount of cargo released into the environment. Gene expression studies suggest that the mechanism for the antibacterial activity of AgNP-ZM is centered around the depletion of cellular antioxidant capacity by gradual release of Ag\(^+\) from zeolite membranes.

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Disclosure

The authors report no conflicts of interest in this work.

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**Supplemental Methods**

**RNA isolation**

Bacteria were collected from each well and pelleted at 4°C in 15 mL centrifuge tubes at 3250 × g for 15 minutes. Supernatants were discarded and the pellets were homogenized in 5 mL Trizol for five minutes. Each tube was shaken vigorously for 30 seconds after the addition of 1 mL of chloroform. The tubes were incubated at room temperature for three minutes prior to centrifugation at 4°C and 3250 × g for 15 minutes. The organic layer was then removed and placed into clean RNase free microfuge tubes. Equal amounts of 100% ethanol were added to each tube and mixed by pipetting. RNA purification was then performed using RNeasy mini kits as per the manufacturer’s instructions, during which DNase was added to remove contaminating DNA. At the final elution step, RNA was resuspended in 20 µL of RNase-free H₂O and stored at −80°C until further use in gene expression arrays and quantitative reverse transcription polymerase chain reaction experiments. The concentration of the samples provided was determined using the NanoDrop® ND-1000 ultraviolet-visible spectrophotometer.

**Gene arrays**

Microarray slides were hybridized overnight, washed, and then scanned with an Agilent G2505C microarray scanner. This high-resolution scanner features an industry-leading extended dynamic range of 10⁶ (20-bits) for high sensitivity scanning without saturation, low-level detection resulting from optimized precision optics, broad dynamic range, and minimal spectral cross talk that enables detection of weak features. The information about each probe on the array was extracted from the image data using Agilent Feature Extraction 10.9. This data was stored in Feature Extraction “.txt” files. The raw intensity values from these files were imported into the mathematical software package “R”, which is used for all data input, diagnostic plots, normalization, and quality checking steps of the analysis process using scripts developed inhouse specifically for this analysis. These scripts call on several Bioconductor packages (http://www.bioconductor.org/). Bioconductor is an open source and open development software project that provides tools for the analysis and comprehension of genomic data.1 Significance analyses of microarrays (SAM) is a powerful tool for analyzing microarray gene expression data useful for identifying differentially expressed genes between two conditions.2 SAM was used to calculate a test.

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**Figure S1** Schematic of fabrication of zeolite support containing silver nanoparticles. Alumina supports were used as the substrate for zeolite membrane synthesis. Zeolite was grown into a continuous membrane by hydrothermal synthesis. Zeolite membranes were then ion-exchanged with 0.005 M AgNO₃ solution/reduction using hydrazine.

**Figure S2** Viability of Escherichia coli after exposure to zeolite support containing silver nanoparticles for 30 minutes. The viability of E. coli was determined after exposure to zeolite support containing silver nanoparticles for 30 minutes. RNA was harvested from these experiments and used for the gene expression microarray analyses. Viability was significantly reduced after incubation with zeolite support containing silver nanoparticles for 30 minutes, compared with zeolite controls. Statistical significance was determined using the Student’s t-test ( n = 4 for zeolite controls and zeolite support containing silver nanoparticles, P < 0.02).
Table S1 E. coli primer sequences for quantitative real-time PCR (QRT-PCR)

| Gene product (gene name) | Sequence | Tm (°C) | Product size |
|--------------------------|----------|---------|--------------|
| Multi copper oxidase (cueO) F | TCCGTATCTTCGCTAGAAAATGGAAC | 58.7 | 195 bp |
| Multi copper oxidase (cueO) R | TACCGTAAACCTAAACTCATCC | 58.2 | |
| Thioredoxin (trxC) F | ACCCTCGAACACTTGTGAGATG | 58.3 | 164 bp |
| Thioredoxin (trxC) R | AATTCACTTTCAGGGTATTAC | 58.7 | |
| Glutaredoxin (grxA) F | TCGCCCTTACTGTTTGCTGTCG | 58.4 | 151 bp |
| Glutaredoxin (grxA) R | CGGCACGGTTCTTCAAGGTT | 58.5 | |
| Copper/silver efflux (cusC) F | TATGAAACGAGATTCAGAAGCGCCT | 58.3 | 228 bp |
| Copper/silver efflux (cusC) R | TAATTCAGATCAAGAATTTTTCGGGT | 58 | |

Abbreviations: F = Forward sequence; R = Reverse sequence; bp = base pairs.

Table S2 Increases in E. coli gene expression in response to 30-minute exposures to four independent zeolite supports containing AgNPs versus E. coli exposed to four independent zeolite controls

| Gene product | Fold change | Description |
|--------------|-------------|-------------|
| Thiosulfate transporter subunit | 14.9 | Thiosulfate binding protein |
| Predicted protein | 14.6 | Multiple antibiotic resistance protein |
| Predicted transcriptional regulator | 13 | orf, hypothetical protein |
| Sulfate/thiosulfate transporter subunit | 11.9 | Sulfate transport system permease W protein |
| Copper transporter | 10.8 | Putative ATPase |
| Sulfate/thiosulfate transporter subunit | 10.3 | ATP-binding component of sulfate permease A protein; chromate resistance |
| Ferrochelatase | 9.5 | Ferrochelatase: final enzyme of heme biosynthesis |
| Magnesium transporter | 9 | Mg2+ transport ATPase, P-type 1 |
| Sulfate adenyllytransferase, subunit 2 | 9 | ATP:sulfurylase |
| Sulfate/thiosulfate transporter subunit | 9 | Sulfate, thiosulfate transport system permease T protein |
| Predicted DNA-binding transcriptional regulator | 8.8 | orf, hypothetical protein |
| Sulfite reductase, alpha subunit, flavoprotein | 8.7 | Sulfite reductase |
| DNA-binding transcriptional dual activator of multiple antibiotic resistance | 8.1 | Multiple antibiotic resistance; transcriptional activator of defense systems |
| Predicted inner membrane protein | 8.1 | Putative transport system permease protein |
| Conserved protein | 8 | orf, hypothetical protein |
| Predicted protein | 7.9 | orf, hypothetical protein |
| Zn-binding periplasmic protein | 7.9 | orf, hypothetical protein |
| Pseudo | 7.8 | Attaching and effacing protein, pathogenesis factor |
| Thioredoxin 2 | 7.8 | Putative thioredoxin-like protein |

(Continued)
Table S2 (Continued)

| Gene product | Fold change AgNP-ZM vs zeolite | Description |
|--------------|--------------------------------|-------------|
| Glutaredoxin I, redox coenzyme for ribonucleotide reductase (RRN1a) | 7.6 | Glutaredoxin I redox coenzyme for glutathione-dependent ribonucleotide reductase |
| Sulfate transporter subunit | 7.6 | Periplasmic sulfate-binding protein |
| Predicted oxidoreductase with NAD(P)-binding | 7.2 | Putative oxidoreductase |
| Rossman-fold domain | | |
| Predicted cyanide hydratase | 7.1 | orf, hypothetical protein |
| 5,10-methylenetetrahydrofolate reductase | 7.1 | 5,10-methylene tetrahydrofolate reductase |
| DNA-binding transcriptional repressor of multiple antibiotic resistance | 6.8 | Multiple antibiotic resistance protein; repressor of mar operon |
| Multicopper oxidase (laccase) | 6.7 | orf, hypothetical protein |
| Sulfite reductase, beta subunit, NAD(P)-binding, heme-binding | 6.1 | Sulfite reductase, alpha subunit |
| Predicted quinol oxidase subunit | 6 | orf, hypothetical protein |
| Predicted protein | 5.9 | orf, hypothetical protein |
| Periplasmic copper-binding protein | 5.9 | orf, hypothetical protein |
| Alkyl hydroperoxide reductase, F52a subunit, FAD/NAD(P)-binding | 5.8 | Alkyl hydroperoxide reductase, F52a subunit; detoxification of hydroperoxides |
| Predicted DNA-binding transcriptional regulator | 5.8 | orf, hypothetical protein |
| DNA-binding transcriptional activator, homocysteine-binding 3'-phosphoadenosine 5'-phosphosulfate reductase | 5.7 | 3'-phosphoadenosine 5'-phosphosulfate reductase |
| DNA-binding transcriptional repressor | 5.6 | Transcriptional repressor of chromosomal ars operon |
| IS1 transposase InsAB' | 5.6 | IS1 protein InsB |
| Conserved protein | 5.4 | orf, hypothetical protein |
| Conserved protein | 5. | orf, hypothetical protein |
| Predicted membrane protein | 4.9 | orf, hypothetical protein |
| Sulfate adenylyltransferase, subunit I | 4.9 | ATP-sulfurylase |
| Predicted protein | 4.4 | orf, hypothetical protein |
| Alcohol dehydrogenase class III/glutathione-dependent formaldehyde dehydrogenase | 4.4 | Alcohol dehydrogenase class III; formaldehyde dehydrogenase |
| N-ethylmaleimide reductase, FMN-linked | 4.3 | N-ethylmaleimide reductase |
| Conserved protein | 4.3 | orf, hypothetical protein |
| RNA polymerase, sigma 32 (sigma H) factor | 4.3 | RNA polymerase, sigma |
| Nitrate reductase I, beta (Fe-S) subunit | 4.2 | Nitrate reductase I, beta subunit |
| Arsenite/antimonite transporter | 4.2 | Arsenical pump membrane protein |
| DNA-binding transcriptional dual regulator, Fe-S center for redox-sensing | 4.2 | Redox-sensing activator of soxs |
| Respiratory NADH dehydrogenase 2/cupric reductase | 3.9 | Respiratory NADH dehydrogenase |
| Predicted inner membrane protein, part of terminus | 3.9 | Putative transport protein |
| Envelope stress induced periplasmic protein | 3.8 | Periplasmic protein related to spheroblast formation |
| Fused fructose-specific PTS enzymes: IIA component/HPr component | 3.8 | PTS system, fructose-specific IIA/fpr component |
| Arsenate reductase | 3.8 | Arsenate reductase |
| Fructose-1-phosphate kinase | 3.6 | Fructose-1-phosphate kinase |
| DNA-binding transcriptional repressor | 3.6 | orf, hypothetical protein |
| Predicted transporter | 3.6 | Part of a kinase |
| Nitrate reductase I, alpha subunit | 3.6 | Nitrate reductase I, alpha subunit |
| Predicted esterase | 3.5 | Putative esterase |
| Molybdenum-cofactor-assembly chaperone subunit of nitrate reductase I | 3.5 | Nitrate reductase I, delta subunit, assembly function |
| 3-oxoacyl-[acyl-carrier-protein] synthase II | 3.4 | 3-oxoacyl- |
| DL-methionine transporter subunit | 3.3 | ATP-binding component of a transporter |
| Predicted (D)-galactarate transporter | 3.3 | Putative transport protein |
| Fused chorismate mutase T/prephenate dehydrogenase | 3.3 | Chorismate mutase-T and prephenate dehydrogenase |
| Nitrate reductase I, gamma (cytochrome b(NR)) subunit | 3.2 | Nitrate reductase I, cytochrome b |
| Fumarate hydratase (fumarase C), aerobic Class II | 3.2 | Fumarase C=fumarate hydratase Class II; isozyme |
| Copper/silver efflux system, membrane fusion protein | 3.1 | Putative resistance protein |

(Continued)
Table S2 (Continued)

| Gene product                                                                 | Fold change AgNP-ZM vs zeolite | Description                                                                                     |
|------------------------------------------------------------------------------|---------------------------------|-----------------------------------------------------------------------------------------------|
| Membrane protein of efflux system                                            | 3.1                             | orf, hypothetical protein                                                                      |
| Copper/silver efflux system, outer membrane component                        | 3                               | Putative resistance protein                                                                    |
| Regulator protein that represses frmRAB operon                               | 3                               | Putative alpha helix chain                                                                      |
| Homoserine O-transsuccinylase                                                | 3                               | Homoserine transsuccinylase                                                                     |
| Predicted pirin-related protein                                              | 3                               | orf, hypothetical protein                                                                      |
| Fused nitric oxide dioxygenase/dihydropteridine reductase 2                 | 3                               | Dihydropteridine reductase, ferrisiderophore reductase activity                               |
| Nitrite reductase, large subunit, NAD(P)H-binding                            | 2.9                             | Nitrite reductase                                                                               |
| Predicted inner membrane protein                                             | 2.9                             | Putative transport protein                                                                     |
| Adenosine 5'-phosphosulfate kinase                                           | 2.8                             | Adenosine 5-phosphosulfate kinase                                                               |
| Maltose regulon periplasmic protein                                          | 2.8                             | Periplasmic protein of mal regulon                                                              |
| Conserved protein                                                            | 2.8                             | orf, hypothetical protein                                                                      |
| Carbon-phosphorus lyase complex subunit                                      | 2.8                             | Phosphonate metabolism                                                                         |
| Maltose transporter subunit                                                  | 2.7                             | Periplasmic maltose-binding protein; substrate recognition for transport and chemotaxis         |
| Fructuronate transporter                                                     | 2.7                             | Gluconate transport system permease 3                                                           |
| Predicted regulator of cell morphogenesis and cell wall metabolism           | 2.6                             | orf, hypothetical protein                                                                      |
| DNA-binding transcriptional dual regulator, glycolate-binding                | 2.6                             | Transcriptional activator for glc operon                                                        |
| Predicted endopeptidase                                                      | 2.6                             | Heat shock protein, integral membrane protein                                                    |
| Conserved protein                                                            | 2.6                             | orf, hypothetical protein                                                                      |
| Formate dehydrogenase-N, Fe-S (beta) subunit, nitrate-inducible             | 2.6                             | Formate dehydrogenase-N, nitrate-inducible, iron-sulfur beta subunit                            |
| Predicted glucarate dehydratase                                             | 2.6                             | Putative glucarate dehydratase                                                                 |
| Calcium/sodium/proton antiporter                                            | 2.5                             | Sodium-calcium/proton antiporter                                                                |
| Formate dehydrogenase-N, alpha subunit, nitrate-inducible                   | 2.5                             | Formate dehydrogenase-N, nitrate-inducible, alpha subunit                                      |
| Gluconate transporter, high-affinity GNT 1 system                            | 2.5                             | High-affinity transport of gluconate/gluconate permease                                          |
| Predicted peptidoglycan peptidase                                            | 2.5                             | orf, hypothetical protein                                                                      |
| Formate dehydrogenase-N, cytochrome B556 (gamma) subunit, nitrate-inducible | 2.4                             | Formate dehydrogenase-N, nitrate-inducible, cytochrome B556                                   |
| e14 prophage; predicted DNA-binding transcriptional regulator               | 2.4                             | orf, hypothetical protein                                                                      |
| Nitrite reductase, NAD(P)H-binding, small subunit                           | 2.4                             | Nitrite reductase                                                                               |
| Predicted zinc-dependent peptidase                                           | 2.4                             | orf, hypothetical protein                                                                      |
| Conserved protein required for cell growth                                   | 2.4                             | orf, hypothetical protein                                                                      |
| Cystathionine gamma-synthase, PLP-dependent                                  | 2.4                             | Cystathionine gamma-synthase                                                                    |
| Conserved protein                                                            | 2.4                             | orf, hypothetical protein                                                                      |
| L-serine deaminase I                                                        | 2.4                             | L-serine deaminase                                                                             |
| Predicted reductase                                                          | 2.4                             | Putative reductase                                                                             |
| Alpha-dehydro-beta-deoxy-D-glucarate aldolase                               | 2.4                             | orf, hypothetical protein                                                                      |
| D-serine ammonia-lyase                                                       | 2.4                             | D-serine dehydratase                                                                           |
| Serine endoprotease (protease Do), membrane-associated                      | 2.3                             | Periplasmic serine protease Do; heat shock protein HtrA                                         |
| NADH-azoreductase, FMN-dependent                                             | 2.3                             | Acyl carrier protein phosphodiesterase                                                         |
| Isopentenyl diphosphate isomerase                                            | 2.3                             | Putative enzyme                                                                                |
| Nitrite reductase, NAD(P)H-binding, small subunit                           | 2.3                             | Nitrite reductase                                                                               |
| Porphobilinogen synthase                                                    | 2.3                             | S-aminolevulinate dehydratase = porphobilinogen synthase                                        |
| Fusion predicted pyruvate-flavodoxin oxidoreductase                         | 2.3                             | Putative oxidoreductase, Fe-S subunit                                                           |
| Nitrite reductase, NAD(P)H-binding, small subunit                           | 2.3                             | Nitrite reductase                                                                               |
| Sorbitol-6-phosphate dehydrogenyan                                        | 2.3                             | Glucitol                                                                                       |
| e14 prophage; predicted DNA-binding transcriptional regulator               | 2.3                             | orf, hypothetical protein                                                                      |
| Inhibitor of replication at Ter, DNA-binding protein                        | 2.2                             | DNA-binding protein; inhibition of replication at Ter sites                                     |
| Glycerol-3-phosphate O-acyltransfer                                          | 2.2                             | Glycerol-3-phosphate acyltransfer                                                               |
| Predicted DNA-binding transcriptional regulator                             | 2.2                             | orf, hypothetical protein                                                                      |
| (D)-glucarate dehydratase                                                   | 2.2                             | Putative glucarate dehydratase                                                                 |
| Sodium:serine/threonine symporter                                           | 2.2                             | Putative transport protein                                                                      |
| Conserved protein                                                           | 2.2                             | orf, hypothetical protein                                                                      |

(Continued)
Table S3 Decreases in Escherichia coli gene expression in response to 30-minute exposures to four independent zeolite supports containing silver nanoparticles versus E. coli exposed to four independent zeolite controls

| Gene product | Fold change AgNP-ZM vs zeolite | Description |
|--------------|-------------------------------|-------------|
| fliQ Flagellar biosynthesis protein | −2 | Flagellar biosynthesis |
| mdtQ Pseudo | −2 | orf, hypothetical protein |
| yhdE Conserved protein | −2 | orf, hypothetical protein |
| ydT Predicted DNA-binding transcriptional regulator | −2 | Putative DEOR-type transcriptional regulator |
| yycC Predicted signal transduction protein (EAL domain containing protein) | −2 | orf, hypothetical protein |
| yehE Predicted protein | −2 | orf, hypothetical protein |
| sdiA DNA-binding transcriptional activator | −2 | Transcriptional regulator of ftsQAZ gene cluster |
| yeeA Conserved inner membrane protein | −2 | orf, hypothetical protein |
| yncH Predicted protein | −2 | orf, hypothetical protein |
| ndk Multifunctional nucleoside diphosphate kinase | −2 | Nucleoside diphosphate kinase |
| yebW Predicted protein | −2 | orf, hypothetical protein |
| ycjW Predicted DNA-binding transcriptional regulator | −2 | Putative LACI-type transcriptional regulator |
| Gene name | Gene product | Fold change AgNP-ZM vs zeolite | Description |
|-----------|--------------|-------------------------------|-------------|
| ycgN      | Conserved protein | −2.1 | orf, hypothetical protein |
| alsA      | Fused D-allose transporter subunits of ABC superfamily; ATP-binding components | −2.1 | Putative ATP-binding component of a transport system |
| sugE      | Multidrug efflux system protein | −2.1 | Suppresses groEL, may be chaperone |
| ybdD      | Pseudo | −2.1 | orf, hypothetical protein |
| yhlI      | KpLE2 phage-like element; predicted DNA-binding transcriptional regulator | −2.1 | Putative regulator |
| gkF       | Glycolate oxidase-iron-sulfur subunit | −2.1 | Glycolate oxidase iron-sulfur subunit |
| ybdO      | Predicted DNA-binding transcriptional regulator | −2.1 | Putative transcriptional regulator LYSR-type |
| mcrA      | e14 prophage; 5-methylcytosine-specific restriction endonuclease B | −2.1 | Restriction of DNA at 5-methylcytosine residues; at locus of e14 element |
| efsB      | Conserved protein | −2.1 | orf, hypothetical protein |
| yobP      | Pseudo | −2.1 | orf, hypothetical protein |
| amT       | 4-amino-4-deoxy-L-arabinose transferase | −2.1 | orf, hypothetical protein |
| ais       | Conserved protein | −2.1 | Protein induced by aluminum |
| rscC      | Fused predicted 4Fe-4S ferredoxin-type protein; conserved protein | −2.1 | Putative membrane protein |
| yagU      | Conserved inner membrane protein | −2.1 | orf, hypothetical protein |
| yciF      | Conserved protein | −2.1 | Putative structural proteins |
| yifK      | Predicted transporter | −2.1 | Putative amino acid/amine transport protein |
| yncD      | Predicted iron outer membrane transporter | −2.1 | Putative outer membrane receptor for iron transport |
| yhcO      | Predicted barnase inhibitor | −2.1 | orf, hypothetical protein |
| elsD      | Predicted enzyme | −2.1 | Putative sulfatase/phosphatase |
| emmA      | Cysteine and O-acetyl-L-serine efflux system | −2.1 | orf, hypothetical protein |
| gkD       | Glycolate oxidase subunit, FAD-linked | −2.1 | Glycolate oxidase subunit D |
| ydgC      | Conserved inner membrane protein associated with alginate biosynthesis | −2.2 | orf, hypothetical protein |
| sugE      | Multidrug efflux system protein | −2.2 | Suppresses groEL, may be chaperone |
| alsC      | D-allose transporter subunit | −2.2 | Putative transport system permease protein |
| yifK      | Predicted transporter | −2.2 | Putative amino acid/amine transport protein |
| yhlY      | Predicted acetyltransferase | −2.2 | orf, hypothetical protein |
| fhuB      | Fused iron-hydroxamate transporter subunits of ABC superfamily; membrane components | −2.2 | Hydroxamate-dependent iron uptake, cytoplasmic membrane component |
| hdeB      | Acid-resistance protein | −2.2 | orf, hypothetical protein |
| yfhA      | Predicted DNA-binding response regulator in two-component system | −2.2 | Putative 2-component transcriptional regulator |
| glgS      | Predicted glycogen synthesis protein | −2.2 | Glycogen biosynthesis, rpoS dependent |
| ydoQ      | Rac prophage; conserved protein | −2.2 | orf, hypothetical protein |
| yblH      | Pseudo | −2.2 | orf, hypothetical protein |
| rfaP      | Kinase that phosphorylates core heptose of lipopolysaccharide | −2.2 | Lipopolysaccharide core biosynthesis; phosphorylation of core heptose |
| stpA      | DNA binding protein, nucleoid-associated | −2.2 | DNA-binding protein; H-NS-like protein; chaperone activity |
| lpxT      | Undecaprenyl pyrophosphate phosphatase | −2.2 | orf, hypothetical protein |
| ydiY      | Predicted 4Fe-4S ferredoxin-type protein | −2.2 | Putative oxidoreductase, Fe-S subunit |
| yifK      | Predicted transporter | −2.2 | Putative amino acid/amine transport protein |
| hisP      | Histidine/lysine/arginine/ornithine transporter subunit | −2.2 | ATP-binding component of histidine transport |
| yhgE      | Predicted inner membrane protein | −2.2 | Putative transport |
| thiQ      | Thiamin transporter subunit | −2.2 | Putative ATP-binding component of a transport system |
| ycgZ      | Predicted protein | −2.2 | orf, hypothetical protein |
| gkG       | Conserved protein | −2.2 | orf, hypothetical protein |

(Continued)
Table S3 (Continued)

| Gene name | Gene product | Fold change AgNP-ZM vs zeolite | Description |
|-----------|--------------|---------------------------------|-------------|
| fliP      | Flagellar biosynthesis protein | −2.2 | Flagellar biosynthesis |
| ydbF      | Predicted glutathione S-transferase | −2.2 | Putative S-transferase |
| rpiR      | DNA-binding transcriptional repressor | −2.4 | Transcriptional repressor of rpiB expression |
| yojI      | Fused predicted multidrug transport subunits of ABC superfamily | −2.4 | Putative ATP-binding component of a transport system |
| hsdS      | Specificity determinant for hsdM and hsdR | −2.4 | Specificity determinant for hsdM and hsdR |
| racR      | Rac prophage; predicted DNA-binding transcriptional regulator | −2.4 | orf, hypothetical protein |
| ycel      | Predicted protein | −2.4 | orf, hypothetical protein |
| tonB      | Membrane spanning protein in TonB-ExbB-ExbD complex | −2.4 | Energy transducer; uptake of iron, cyanocobalimin; sensitivity to phages |
| rpoS      | Lipopolysaccharide core biosynthesis protein | −2.5 | Lipopolysaccharide core biosynthesis |
| rutB      | Predicted enzyme | −2.5 | Putative synthetase |
| yclI      | Predicted enzyme | −2.5 | orf, hypothetical protein |
| wzyE      | Predicted Wzy protein involved in ECA polysaccharide chain elongation | −2.5 | TDP-Fuc4NAc/lipidII transferase; synthesis of enterobacterial common Ag |
| nth       | DNA glycosylase and apyrimidinic (AP) lyase (endonuclease III) | −2.5 | Endonuclease III; specific for apurinic and/or apyrimidinic sites |
| yncC      | Predicted DNA-binding transcriptional regulator | −2.5 | orf, hypothetical protein |
| yifK      | Predicted transporter | −2.5 | Putative amino acid/amine transport protein |
| yhiB      | KpLE2 phase-like element; predicted transporter | −2.5 | Putative transport protein |
| yhiR      | Conserved protein | −2.5 | orf, hypothetical protein |
| ydiL      | CPS-53 (KpLE1) prophage; predicted inner membrane protein | −2.5 | putative ligase |
| yfn       | Pseudo | −2.6 | orf, hypothetical protein |
| caiF      | DNA-binding transcriptional activator | −2.6 | Transcriptional regulator of cai operon |
| fliA      | assembly protein for flagellar basal-body periplasmic P ring | −2.6 | Flagellar biosynthesis; assembly of basal-body periplasmic P ring |
| serA      | D-3-phosphoglycerate dehydrogenase | −2.6 | D-3-phosphoglycerate dehydrogenase |
| can       | Carbonic anhydrase | −2.7 | Putative carbonic anhydrase |
| yoaC      | Predicted protein | −2.7 | orf, hypothetical protein |
| ydB      | Conserved protein | −2.7 | orf, hypothetical protein |
| efeO      | Conserved protein | −2.7 | orf, hypothetical protein |
| fliF      | Flagellar basal-body MS-ring and collar protein | −2.7 | Flagellar biosynthesis; basal-body MS |
| fliC      | Flagellar component of cell-proximal portion of basal-body rod | −2.7 | Flagellar biosynthesis, cell-proximal portion of basal-body rod |
| can       | Carbonic anhydrase | −2.7 | Putative carbonic anhydrase |
| rsiD      | Predicted inner membrane oxidoreductase | −2.7 | orf, hypothetical protein |
| fim       | Flagellar motor switching and energizing component | −2.7 | Flagellar biosynthesis, component of motor switch and energizing |
| ybaN      | Conserved inner membrane protein | −2.8 | Putative gene 58 |
| proV      | Glycine betaine transporter subunit | −2.8 | ATP-binding component of transport system for glycine, betaine and proline |
| fluD      | Iron-hydroxamate transporter subunit | −2.8 | Hydroxamate-dependent iron uptake, cytoplasmic membrane component |
| can       | Carbonic anhydrase | −2.8 | Putative carbonic anhydrase |
| suE       | Sulfur acceptor protein | −2.8 | orf, hypothetical protein |
| suS       | Selenocysteine lyase, PLP-dependent | −2.8 | orf, hypothetical protein |
| arR       | Predicted protein | −2.8 | orf, hypothetical protein |
| wcaM      | Predicted colanic acid biosynthesis protein | −2.8 | orf, hypothetical protein |
| ymgA      | Predicted protein | −2.9 | orf, hypothetical protein |

(Continued)
Table S3 (Continued)

| Gene name | Gene product                                | Fold change | Description                                                                                       |
|-----------|---------------------------------------------|-------------|---------------------------------------------------------------------------------------------------|
| ybiX      | Conserved protein                           | −2.9        | Putative enzyme                                                                                   |
| yacC      | Predicted protein                           | −2.9        | orf, hypothetical protein                                                                        |
| sufC      | Component of SubBCD complex, ATP-binding   | −2.9        | Component of ABC superfamily                                                                      |
|           | component of ABC superfamily               |             | Putative ATP-binding component of a transport system                                             |
| can       | Carbonic anhydrase                          | −2.9        | Putative carbonic anhydrase                                                                       |
| yacC      | Predicted protein                           | −3          | orf, hypothetical protein                                                                        |
| fhuC      | Iron-hydroxamate transporter subunit        | −3          | ATP-binding component of hydroxamate-dependent iron transport                                      |
| sodA      | Superoxide dismutase, Mn                    | −3.1        | Superoxide dismutase, manganese                                                                  |
| can       | Carbonic anhydrase                          | −3.2        | Putative carbonic anhydrase                                                                       |
| cbrB      | Predicted inner membrane protein            | −3.2        | orf, hypothetical protein                                                                        |
| flgB      | Flagellar component of cell-proximal portion of basal-body rod | −3.3 | Flagellar biosynthesis, cell-proximal portion of basal-body rod |
| ompT      | DLP12 prophage; outer membrane protease    | −3.3        | Outer membrane protein 3b                                                                         |
| rsaE      | Predicted inner membrane NADH-quione reductase | −3.3 | orf, hypothetical protein                                                                        |
| pqqL      | Predicted peptidase                         | −3.3        | Putative peptidase                                                                               |
| fltA      | RNA polymerase, sigma 28 (sigma F) factor   | −3.3        | Flaggellar biosynthesis; alternative sigma factor 28                                              |
| yfdH      | CPS-53 (KpLE1) prophage; bactoprenol        | −3.5        | Putative glycan biosynthesis enzyme                                                                |
|           | glucosyl transferase                        |             |                                                                                                   |
| yjlL      | Predicted protein                           | −3.5        | orf, hypothetical protein                                                                        |
| sufD      | Component of SubBCD complex                 | −3.7        | orf, hypothetical protein                                                                        |
| yfdH      | CPS-53 (KpLE1) prophage; bactoprenol        | −3.7        | Putative glycan biosynthesis enzyme                                                                |
|           | glucosyl transferase                        |             |                                                                                                   |
| fepB      | Iron-enterobactin transporter subunit       | −3.7        | Ferric enterobactin                                                                              |
| flIL      | Flagellar biosynthesis                      | −3.7        | Flagellar biosynthesis                                                                           |
| sufB      | Component of SubBCD complex                 | −3.7        | orf, hypothetical protein                                                                        |
| sufA      | Fe-S cluster assembly protein               | −3.8        | orf, hypothetical protein                                                                        |
| ycgF      | Predicted FAD-binding phosphodiesterase    | −3.8        | orf, hypothetical protein                                                                        |
| yfdH      | CPS-53 (KpLE1) prophage; bactoprenol        | −3.8        | Putative glycan biosynthesis enzyme                                                                |
|           | glucosyl transferase                        |             |                                                                                                   |
| sufA      | Fe-S cluster assembly protein               | −3.9        | orf, hypothetical protein                                                                        |
| ybdB      | Conserved protein                           | −4          | orf, hypothetical protein                                                                        |
| fhuE      | Ferric-rhodotorulic acid outer membrane     | −4          | Outer membrane receptor for ferric iron uptake                                                   |
|           | transporter                                 |             |                                                                                                   |
| sufA      | Fe-S cluster assembly protein               | −4.3        | orf, hypothetical protein                                                                        |
| yfdH      | CPS-53 (KpLE1) prophage; bactoprenol        | −4.4        | Putative glycan biosynthesis enzyme                                                                |
|           | glucosyl transferase                        |             |                                                                                                   |
| exbD      | Membrane spanning protein in TonB-ExbB-ExbD complex | −4.5 | Uptake of enterochelin; tonB-dependent uptake of B colicins                                        |
| yncE      | Conserved protein                           | −4.5        | Putative receptor                                                                                |
| fepD      | Iron-enterobactin transporter subunit       | −4.7        | Ferric enterobactin                                                                              |
| sufA      | Fe-S cluster assembly protein               | −4.9        | orf, hypothetical protein                                                                        |
| entB      | Isochorismatase                             | −5          | 2,3-dihydro-2,3-dihydroxybenzoate synthetase, isochroismatase                                   |
| entC      | Isochorismatase synthase I                 | −5.3        | Isochorismatase hydroxymutase 2, enterochelin biosynthesis                                        |
| tdcA      | DNA-binding transcriptional activator       | −5.3        | Transcriptional activator of tdc operon                                                          |
| FepA      | Iron-enterobactin outer membrane transporter | −5.4 | Outer membrane receptor for ferric enterobactin                                                   |
| FepC      | Iron-enterobactin transporter subunit       | −5.6        | ATP-binding component of ferric enterobactin transport                                             |
| Fes       | Enterobactin/ferric enterobactin esterase   | −5.6        | Enterobactin esterase                                                                            |
| ExbB      | Membrane spanning protein in TonB-ExbB-ExbD complex | −5.8 | Uptake of enterochelin; tonB-dependent uptake of B colicins                                        |
Table S3 (Continued)

| Gene name | Gene product | Fold change AgNP-ZM vs zeolite | Description |
|-----------|--------------|-------------------------------|-------------|
| nrdH      | Glutaredoxin-like protein | −5.8 | Glutaredoxin-like protein; hydrogen donor |
| fecR      | KpLE2 phage-like element; transmembrane signal transducer for ferric citrate transport | −5.9 | Regulator for fec operon, periplasmic |
| entS      | Predicted transporter | −6.6 | Putative transport |
| entF      | Enterobactin synthase multienzyme complex component, ATP-dependent | −6.8 | ATP-dependent serine activating enzyme |
| cirA      | Ferric iron-catecholate outer membrane transporter | −7.1 | Outer membrane receptor for iron-regulated colicin I receptor; porin |
| entA      | 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase | −7.2 | 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase |
| entE      | 2,3-dihydroxybenzoate-AMP ligase component of enterobactin synthase multienzyme complex | −7.4 | 2,3-dihydroxybenzoate-AMP ligase |
| yddB      | Predicted porin protein | −7.9 | orf, hypothetical protein |
| fecI      | KpLE2 phage-like element; RNA polymerase, sigma 19 factor | −8.5 | Probable RNA polymerase sigma factor |
| nrdI      | Protein that stimulates ribonucleotide reduction | −8.8 | orf, hypothetical protein |
| ydiE      | Conserved protein | −9.2 | orf, hypothetical protein |
| nrdE      | Ribonucleoside-diphosphate reductase 2, alpha subunit | −10.4 | Ribonucleoside-diphosphate reductase 2, alpha subunit |
| bfd      | Bacteriaferroferricite associated ferredoxin | −10.6 | orf, hypothetical protein |
| yddA      | Fused predicted multidrug transporter subunits of ABC superfamily | −12.4 | Putative ATP-binding component of a transport system |
| fepG      | Iron-enterobactin transporter subunit | −12.6 | Ferric enterobactin transport protein |
| flu      | Predicted iron outer membrane transporter | −13.7 | Putative outer membrane receptor for iron transport |
| nrdF      | Ribonucleoside-diphosphate reductase 2, beta subunit, ferritin-like protein | −13.9 | Ribonucleoside-diphosphate reductase 2, beta chain, frag |
| fhuF      | Ferric iron reductase involved in ferric hydroximate transport | −17.6 | orf, hypothetical protein |

Supplemental References
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