Electronic supplementary information

Is sulfidation a true detoxification process for silver nanoparticles? From the perspective of chronic exposure

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Materials and methods

Measurement of lipid peroxidation by MDA content

Cells (both wild-type *E. coli* and $E_{s\text{-}AgNP}$) were re-grown from freezer stocks in 50 mL LB medium (37 °C, 160 rpm) and collected at mid-log phase via centrifugation at 8,000 ×g for 8 min at 4 °C, washed three times with saline (0.9% NaCl) and resuspended in saline at a concentration of 10^7-10^8 CFU/mL. One milliliter of cell suspension was exposed to various concentrations of s-AgNPs (0, 0.02 and 1.20 mg/L) in 49 ml LB medium for 5 h (37 °C, 160 rpm) in the dark. The level of lipid peroxidation in cells were analyzed by the thiobarbituricacid (TBA) method,[1] which determines malondialdehyde (MDA) as the end-product of lipid peroxidation after reacted with TBA. The MDA content was measured using a micro-MDA assay reagent kit (Keygen, China). Experiments were carried out at least in triplicate and results were given as the mean ± standard deviation (SD).

Table S1 The primers used in RT-qPCR analysis and their target classification. [a]

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')	Gene classification
16S rRNA	TCCTACGGGAGGCAGCAG	GGACTACCAGGGTATCTAATCCTGTT	
cusA	GGCACCAGCGTGGCATTCTC	CAGCGTCATCGGCACCATCAG	Copper/silver export system
cusB	CCGATGCTGCTCATTCCGTCAC	TTCACCTTCCGCCAGACCAGAG	
cusC	GCCGCAGCAGTTCTCACTCAG	ATTCACCAACGCCTCGCTAATCAG	
cusF	ATGAGCGAAGCACAACCACAGG	AGTTCACGGCAGCAATCGGATC	
silS	ATTGAGCTGGAGCAACTGGT	GATGGGCGTTCTGATCTCAT	Silver efflux system
silE	ATATCCATGAGCGGGTCAAC	CCTGTTCATGCTGGTCCATA	
silA	ATTAACACGCCGGTAGATGC	GGTGGTAAGCGGATAGGTGA	
silP	CCTGGGTTTACAGCGTCATT	CAGCAGAACAAGCACCGTAA	
silR	AGGGTTCGTAGTGGATCACG	ATGATATCCCAGCCGTTCAC	
soxS	GCCAACGCCGCCTGTTACTG	GAAGGTCTGCTGCGAGACATAACC	Oxidative response
marR	CTGCGCGGCGTGTATTACTCC	AGACCAGGCGATCCAGCATACG	
marC	GCAAGTCGGAAGAGCTGGAAGATG	AAGGTTGAACTCTGACGCACTGTG	
oxyR	CAACATGGTGGCGGCAGGTAG	CCAATAGTGCGGCGTGGTTCC	
sodB	CGCTGAAGCTATCGCCGCATC	TCGCATCGGTGGTCAGTGGAG	Detoxification gene
sodC	CGCTGATGGTCCACGTTGGC	TTACACCACAGGCATAGCGTTCAC	
katE	TTCCGCCTGATTAATGCCGAAGG	ATCGCCTGCTTCAATCGCTTCC	
marA	GGAATCGCCACTGTCACTGGAG	GGACTCGAAGCCATATCGTTCTGC	Multidrug resistance
acrA	GCTGAAGTACGTCCGCAGGTTG	CAGTGCCTGTGCTTGTTGTAATGC	
acrB	AACCTGCGTGCCACACTGATTC	GCATCGTCCACCAGCAAGCC	
tolC	AGGATCTGCTGGCACTGAACAATG	GGTGGTAGTGCGTGCGGATG	
ompF	GGTGCTTATGGTGCCGCTGAC	TTCGCTGCCAGGTAGATGTTGTTC	
ompC	ATGCTCAGAACACCGCTGCTTAC	GCGAGTTGCGTTGTAGGTCTGG	

tdcE	GCAGTGGCTCTACTTCGCTTACC	TTCCTGTGCCTGCTGCTCATTG	Carbon metabolism
tdcB	TCCGTGTTATTGGCGTACAGTCTG	GTGCCAGTAGTTCGGTGCGTAG	
tdcG	ACAGTCCGCAGGATGTTGTCATTG	CTTCCAGGCAGTGATCCGCATTC	
tktA	TCGACCACTACACCTACGCCTTC	AACCAGCCTTCAACGTGACCATC	
nrfA	CCGATGGCGTGCTGGAGTTG	TGAGGCGGTGTTATGGCAATCG	Electron transport
nrfB	TGCCGCCTGTCTGGACTGTC	CGCTGTTCTGCTCTCCAACCTTG	
ykgF	GGCACGTTGATGGTCCTGAAGAG	ACGAATACAGCGCAGCACATCC	
napG	AGTGTGCGTGCTGGAACAACC	CCGAAGCGGTAATGGTGACCTAAC	
napH	GACCTCAATCAGTCTGCGACGATC	CCAGATGAGCGTGCCAGTTAAGG	
napF	AGTTGAATGCCGCCGCTGTC	CTCCGCAGTTATGGCTGATACCG	
ylaC	AGGCTCTGTCTGGCTACTGGTTG	CGGAGAGTTCAGGATGGCTTCAAC	
hybA	CGCTGCCGTCTGTCAGTCATG	CGTTGTTCGACCAGGTCTGTTCC	
ygfM	TGGTATACGAATTGCGCTGGATGG	GTTGGCAGTCGGCATACAGGTC	
sdhD	TCGTGCTACCGCTATCGTCCTG	GGTCAACACCTGCCACATGCC	
yhjA	TGGTTGCGTGGAGATGAGAATGC	GGAACGTCCGCCGAGAATAATACC	

[a] Target gene description was found by BLAST on the National Center for Biotechnology Information (NCBI) databases.

Table S2 Hydrodynamic diameters and polydispersity index (PDI) values of nanoparticles in DI water and LB medium over time.

Time (h)	DI Water		LB Medium	
	Hydrodynamic diameter (nm)	PDI	Hydrodynamic diameter (nm)	PDI
0	46.44 ± 1.60	0.41 ± 0.03	164.18 ± 3.13	0.43 ± 0.03
5	58.77 ± 1.17	0.46 ± 0.17	341.99 ± 8.39	0.55 ± 0.09
48	78.82 ± 2.46	0.51 ± 0.17	423.65 ± 15.14	0.63 ± 0.04

Table S3 Sequencing and assembly statistics for the transcriptomic data.

Sample	Raw Reads	Clean Reads	Total mapped [a]	Uniquely mapped [b]
E. coli	13095809	12427362	23808436 (95.79%)	22156967 (89.15%)
E. coli + s-AgNPs	10271682	9823730	18710734 (95.23%)	17170215 (87.39%)
$E_{s\text{-}AgNP}$ + s-AgNPs[1] [c]	10896420	10798333	7020038 (89.43%)	6289858 (80.13%)
$E_{s\text{-}AgNP}$ + s-AgNPs[2] [c]	7728643	6843057	12749947 (93.16%)	11647223 (85.1%)

[a] Total mapped is the number of clean reads that mapped to the reference genome.
[b] Uniquely mapped is the number of clean reads that mapped to the reference genome only at one site.
[c] $E_{s\text{-}AgNP}$ + s-AgNPs[1] and $E_{s\text{-}AgNP}$ + s-AgNPs[2] refer to two biological replicates. Cells were exposed to 1.20 mg/L s-AgNPs in LB medium for 5 h, and untreated *E. coli* was used as a biotic control.

Table S4 The distribution of genes in differentially expressed levels.

FPKM[a] Interval	*E. coli*	*E. coli* + s-AgNPs	$E_{s\text{-}AgNP}$ + s-AgNPs [1 b]	$E_{s\text{-}AgNP}$ + s-AgNPs [2 b]
0~1	905 (19.47%)	909 (19.55%)	942 (20.26%)	882 (18.97%)
1~3	335 (7.21%)	332 (7.14%)	269 (5.79%)	297 (6.39%)
3~15	846 (18.20%)	828 (17.81%)	785 (16.89%)	750 (16.13%)
15~60	1020 (21.94%)	1062 (22.84%)	1037 (22.31%)	1069 (22.99%)
>60	1543 (33.19%)	1518 (32.65%)	1616 (34.76%)	1651 (35.51%)

[a] FPKM is the expected number of Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced.

[b] $E_{s\text{-}AgNP}$ + s-AgNPs[1] and $E_{s\text{-}AgNP}$ + s-AgNPs[2] refer to two biological replicates. Cells were exposed to 1.20 mg/L s-AgNPs in LB medium for 5 h, and untreated *E. coli* was used as a biotic control.

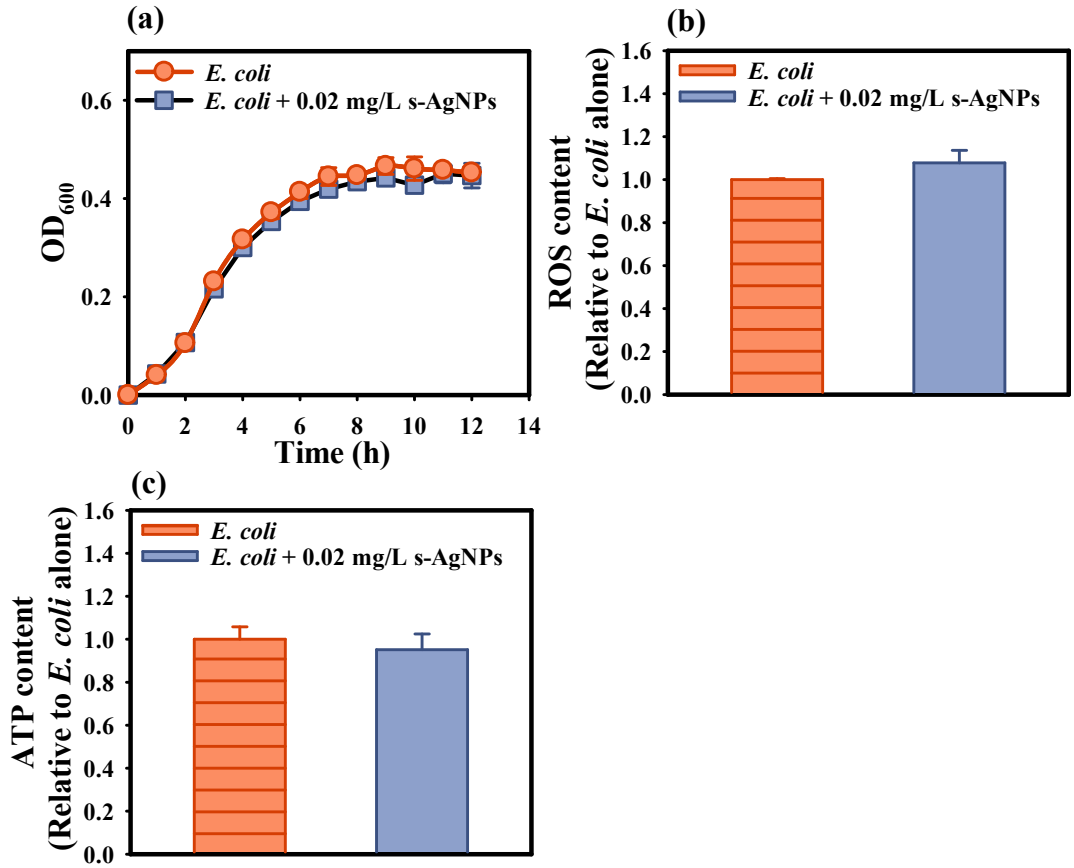

Fig. S1 No obviously adverse effect of 0.02 mg/L s-AgNPs on (a) bacterial growth, (b) intracellular ROS production and (c) ATP production. The wild-type *E. coli* was incubated in LB medium in the presence or absence of 0.02 mg/L s-AgNPs at 37 ºC for 12 h or 5 h, respectively. Error bars, in some cases smaller than the symbols, represent the SD of triplicate.

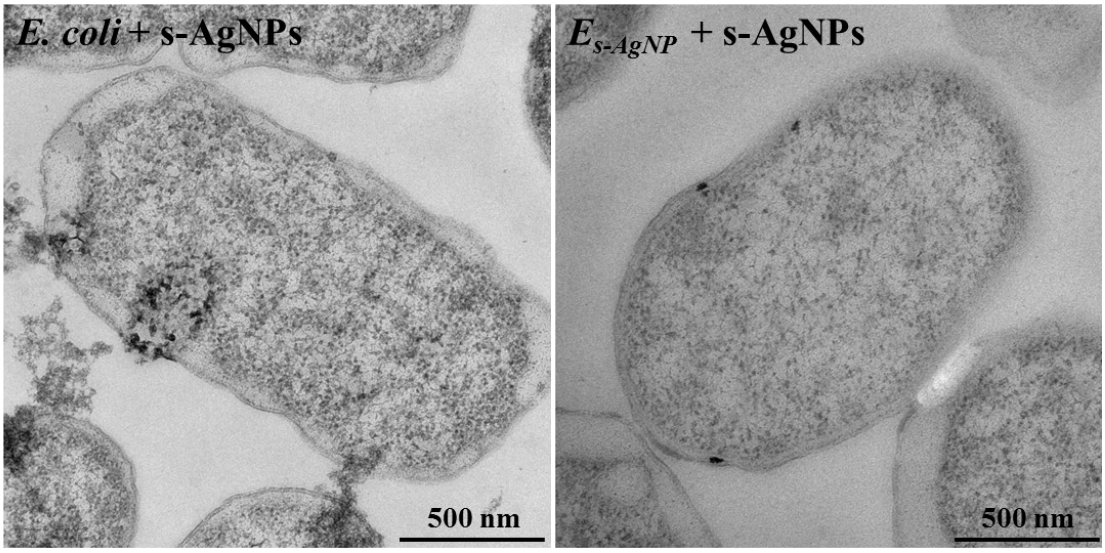

Fig. S2 Transmission electron microscopy image of *E. coli* and $E_{s\text{-}AgNP}$. Cells were exposed to 1.20 mg/L s-AgNPs in LB medium at 37 ºC for 5 h, shaken at 160 rpm.

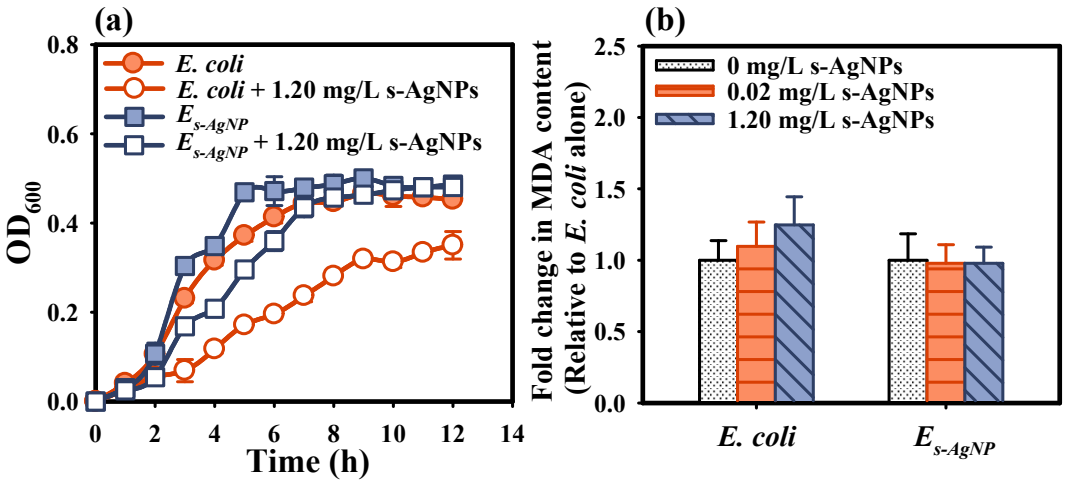

Fig. S3 a) No growth inhibition of $E_{s\text{-}AgNP}$ in the presence of 1.2 mg/L s-AgNPs. b) Fold change of MDA content in *E. coli* and $E_{s\text{-}AgNP}$ upon s-AgNPs exposure. Bacteria were incubated in LB medium under different s-AgNPs concentrations (0, 0.02 and 1.20 mg/L) at 37 °C for 5 h. Experiments were carried out at least in triplicate and results were given as the mean values ± SD.

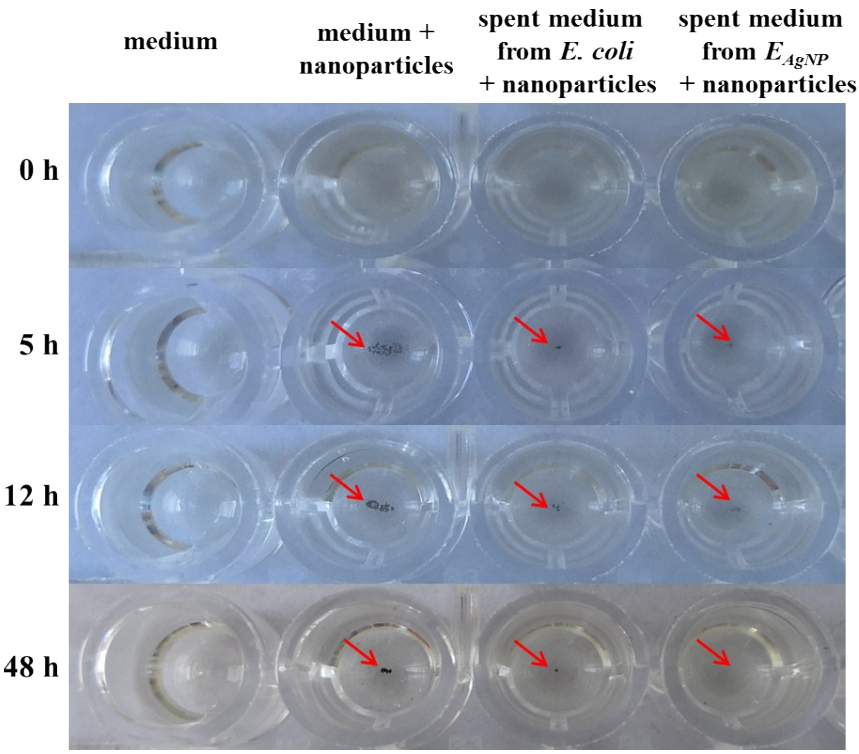

Fig. S4 Photographs of the nanoparticle dispersion in medium or spent medium over time. *E. coli* and $E_{s\text{-}AgNP}$ were cultured in LB medium at 37 ºC for 12 h, shaken at 160 rpm. The respective supernatant (spent medium) was collected by centrifugation at 8000 × g for 8 min. AgNPs (20 mg/L) was dispersed in medium or spent medium and the suspendability was monitored over 48 h. The red arrows pointed out the nanoparticle precipitation. The pictures were taken by smart phone.

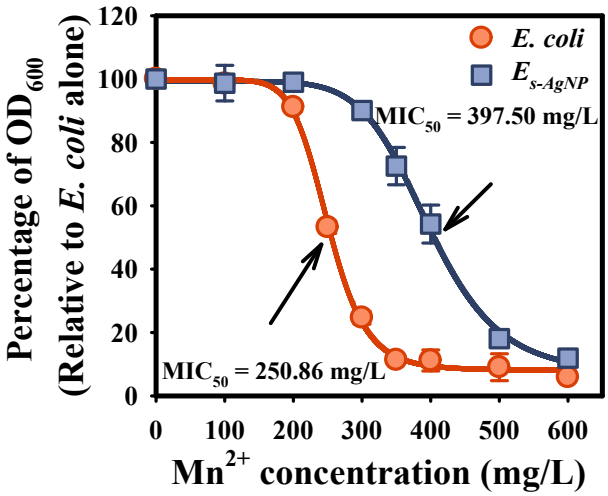

Fig. S5 MIC_{50} determination of *E. coli* and $E_{s\text{-}AgNP}$ towards Mn^{2+}. Cells were exposed to serial concentrations of $MnSO_4$ in LB medium at 37 ºC for 12 h. The MIC_{50} values were calculated by fitting the data using four-parameter logistic curve with Sigma Plot v10.0. Error bars, in some cases smaller than the symbols, represent the standard deviation of triplicate.

References

1 R. L. Heath and L. Packer, Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation, *Arch. Biochem. Biophys.,* 1968, **125**, 189-198.