**Exposure to Al2O3 nanoparticles facilitates conjugative transfer of antibiotic resistance genes from *Escherichia coli* to *Streptomyces***

Xiaomei Liu1, Jingchun Tang1,2,3\*, Benru Song1, Meinan Zhen1, Lan Wang1, John P Giesy4,5

1College of Environmental Science and Engineering, Nankai University, Tianjin 300350, China.

2Key Laboratory of Pollution Processes and Environmental Criteria (Ministry of Education), Tianjin 300350, China.

3Tianjin Engineering Research Center of Environmental Diagnosis and Contamination Remediation, Tianjin 300350, China.

4Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

5Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

\*Corresponding author Tel: 86-13682055616, E-mail: tangjch@nankai.edu.cn

**Methods**

***Analysis of concentrations of Al3+ in media***

To estimate dissolution of Al2O3 in the media, the Al3+ concentrations were determined after shaking for 48 h at concentrations of 10, 100 or 1000 mg/L Al2O3. The media were centrifuged at 14,000 ×g for 30 min, and dynamic light scattering was used to prove no particles existed in the supernatant. 4 mL HNO3 and 2 mL H2O2 were used to digest the supernatant in a microwave digestion system (MDS-15, Sineo, China) at 800 W, 120 °C for 10 min and then 800 W, 160 °C for 20 min. All digested samples were adjusted to the final volume of 50 mL with distilled water.The Al3+ concentration was quantified by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) (IRIS Intrepid II XSP, Thermo Elemental, America).

***Scanning electron microscopy (SEM) analysis***

The impacts of NPs on spores’ morphology were assessed by scanning electron microscopy (SEM). After 2.5 h exposure, cells were centrifuged at 4,000 ×g for 10 min, and fixed in 2.5% glutaraldehyde (in 0.1 M phosphate buffer, pH 7.0) overnight, then washed three times with phosphate buffer (pH 7.0) before dehydrating in 30, 50, 70, 80, 90, and 100% methanol successively, after naturl air-drying, sputter-coated with gold, and then observed via a scanning electron microscope (SEM, JEOL, Beijing, China) (Pakrashi *et al.* 2011).

***Transmission electron microscopy (TEM) analysis***

After conjugation, the bacteria were fixed at 4 °C over night with precooled 2.5% glutaraldehyde fixative. The cells were post-fixed in 1% osmium tetroxide for 2 h and washed three times with phosphate buffer (pH 7.0). Subsequently, cells were dehydrated in a graded ethanol series (30%, 50%, 70%, 80%, 90% and 100%); The cells were then embedded in resin, and resin blocks were sectioned using an ultra-microtome (Leica EM UC7, Solms, Germany) with a diamond knife. Ultra-thin sections (70−90 nm) were stained with uranyl acetate (2%, 20 min, 25 °C) for TEM observation.

**Results**

Table S1. List of primers used in this work

|  |  |  |
| --- | --- | --- |
| Gene |  | Primer sequence |
| AMF |  | CTCACGTTAAGGGATTTTG |
| AMR |  | ATGAGCTCAGCCAATCGA |
| *trfA* | F | GAAGCCCATCGCCGTCGCCTGTAG |
|  | R | GCCGACGATGACGAACTGGTGTGG |
| *trbB* | F | CGCGGTCGCCATCTTCACG |
|  | R | TGCCCGAGCCAGTACCGCCAATG |
| *korA* | F | TCGGGCAAGTTCTTGTCC |
|  | R | GCAGCAGACCATCGAGATA |
| *korB* | F | CTGGTCGGCTTCGTTGTA |
|  | R | TGAAGTCACCCATTTCGGT |
| *trbA* | F | TGGAAACTCCCCTACCTCTT |
|  | R | CCACACTGATGCGTTCGTAT |
| *ompC* | F | GTCGGCGGTTCTATCACTTATG |
|  | R | CGAGTTGCGTTGTAGGTCTG |
| *intA* | F | GTTCGAGCCCGACGTAATCC |
|  | R | CCACGCCTGAAGCTCATACC |
| 16sr DNA | F | CCTACGGGAGGCAGCAG |
|  | R | ATTACCGCGGCTGCTGG |

Table S2. Characterization of different size of Al2O3 (n=100)

|  |  |  |  |
| --- | --- | --- | --- |
|  |  30 nm |  80 nm |  BPs |
| Particle size | 34.9±3.7 nm | 83.7±4.3 nm | 2.8±1.0 µm |
| DLSa | 842±56 nm | 897±43 nm | 3.4±0.9 µm |

a: scattering intensity-weighted mean

Table S3 Number of transconjugants after treated with different kinds of materials

|  |  |  |  |
| --- | --- | --- | --- |
| Material | Size | Concentration（mg/L） | Number of transconjugants |
| CuO NPs | 40 nm | 10 | 59.7±11.9 |
| 100 | 15.0±5.6 |
| 100 nm | 10 | 46.3±3.5 |
| 100 | 8.3±3.8 |
| CuO BPs | 2.5 µm | 10 | 2.7±1.5 |
| 100 | 3.3±1.5 |
| Al2O3 NPs | 30 nm | 10 | 182.0±11.0 |
| 100 | 55.0±9.6 |
| 80 nm | 10 | 87.6±11.5 |
| 100 | 66.0±9.5 |
| Al2O3 BPs | 3.2 µm | 10 | 2.3±1.2 |
| 100 | 2.7±1.2 |
| Ball-milled biochar | 100-1000 nm | 10 | 89.7±12.0 |
| 100 | 26.7±5.0 |
| Biochar | ＞10 µm | 10 | 2.3±1.5 |
| 100 | 3.7±1.2 |
| Control |  |  | 3.0±1.0 |

Table S4. The solubility of Al3+ in different sizes (30 nm, 80 nm and BPs) and different concentration (10 mg/L,100 mg/L and 1000 mg/L) Al2O3 NPs in 2×YT medium. The number of transconjugants after spores of *S. coelicolor M145* treated with these concentrations of Al3+ without heat shocking was listed in the table. The number of transconjugants of control without Al3+ and heat shocking was 3.0±1.0 and the numbers of tranconjugants with different sizes and concentration of nanoparticles were all lower than that of control indicating no impact of Al3+ on transconjugation.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | 10 mg/L(mg/L) | Number of transconjugants | 100 mg/L(mg/L) | Number of transconjugants | 1000 mg/L(mg/L) | Number of transconjugants |
| 30 nm | 0.446±0.047 | 3.33±1.52 | 0.527±0.019 | 3.67±1.53 | 0.857±0.035 | 3.00±2.00 |
| 80 nm | 0.192±0.025 | 2.33±0.57 | 0.349±0.046 | 2.67±0.58 | 0.381±0.032 | 2.33±1.15 |
| BPs | 0.116±0.034 | 2.67±1.53 | 0.188±0.021 | 2.67±1.15 | 0.211±0.046 | 2.33±1.53 |
| Control |  3.0±1.0. |



Figure S1: TEM images of Al2O3 particles with different size. (A) 30 nm, (B): 80 nm, (C): Bulk particles



Figure S2: Electrophoretic mobility of different size particles in YBP media.

**References**

Pakrashi, S., Dalai, S., Sabat, D., Singh, S., Chandrasekaran, N. and Mukherjee, A., 2011. Cytotoxicity of Al2O3 nanoparticles at low exposure levels to a freshwater bacterial isolate. *Chemical Research in Toxicology,* *24*(11), 1899.