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

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**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. | | | | | |

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

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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.