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Anti silver nanoparticle bacteria

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1. Introduction

Nanoparticles (NPs) are in the 1- to 100-nm size range and can be composed of many different base materials. Particles in the nanometer size range do occur both in nature and as a result of industrial processes. NPs have been widely used today, especially in the latest developed science materials. Silver nanoparticles (Ag-NPs), one of the most popular antimicrobial materials had been generally utilized in the textile industry and medical engineering (7, 13). Different Ag-NPs fabricating methods will make different physical or chemical property. Besides from silver nanofibers and plasma nanoparticles, most textile processing methods involve the dipping method that produce a lot of Ag-NPs containing waste water. The entire industrial Ag-NPs containing waste water still has not any proper efficient method to grip them out from the waste before it was drained into the environment (1, 6). A novel bacteria mutant was isolated from the ordinary laboratory analysis, and was revealed and identified. This mutant strain could degrade 5 different trade mark Ag-NPs, directly. Under the textile NPs operation concentration, which exhibit a complete antimicrobial activity, all these five different products of commercial Ag-NPs will be degraded into none antimicrobial activity by this mutant within 4 hours. Atomic absorbance analysis observed that the Ag-NPs were absorbed by the cell directly.

Nanotechnology was expected to have a revolutionary impact on life science. A variety of material production processes occur at nanometer length scales (4). Such as, textile Ag-NPs antimicrobial activity, gold nanoparticles super catalase activity and most NPs offer some unique advantages as sensing and drug delivery (2). Due to different fabrication methods, such many varieties of NPs were available: polymeric nanoparticles, dendrimers, metal nanoparticles, liposomes and other types of nano-assemblies (11). They are multipurpose agents with a diversity of biomedical applications including high sensitive diagnostic assays and radiotherapy enhancement (2), as well as drug and gene delivery analysis (5). Since Ag-NPs excellent antimicrobial activity had been applied onto textile industry, cosmetic products and food preservation followed, but the nanoparticle safety problems were confirmed by less positive reports when some others status on the contrary side (3, 4).

Most NPs were never been presented in human life. When more and more NPs have been developed and applied into the environment, how could we estimate the environmental impact (9)? Anyway, today NPs have to be used incessantly and enormously; here we isolate a novel *Klebsiella* mutant strain, an opportunistic pathogen found in the environment

and on mammalian mucosal surfaces, which could degrade many different kinds of Ag-NPs, directly, within 2~4 hours. After this *Klebsiella* mutant had precipitate down the NPs, the solution turn to none antimicrobial activity, at all, and the waste could be drained out into the aquatic environment. It means that, this *Klebsiella* mutant could be served as a powerful bioremediation tool for industrial Ag-NPs containing waste management.

2. Experiments

2.1. Bacteria strains

Bacteria strains used in this study were *Staphylococcus aureus*（ATCC6538P, *Escherichia coli*（BCRC 16081） and *Salmonella enterotidis*（BCRC10744）. These strains were obtained from American Type Culture Collection (ATCC, Rockville, MD, U.S.A.); Culture Collection and Research Center (CCRC, Hsin-Chu, Taiwan).

2.2. PCR primers design

The primer sequences were chosen from the conserved regions of the bacteria 16S rDNA. The sequences of the PCR primers designed were as follows: forward primer, 16S-350F (5′-CCTACGGGAGGCAGCAGT-3′), corresponding to *E. coli* 16S rDNA (accession number J01859), and reverse primer, 16S-820R (5′-CGTTTACGGCGTGGACTAC-3′). Primers were synthesized with a model 394 DNA-RNA synthesizer (Applied Biosystems, Foster City, Calif.).

2.3. DNA isolation and PCR amplification

Genomic DNA from *Klebsiella* sp. was extracted and purified using Genomic Tips (Qiagen, Valencia, Calif.) with Genomic DNA Buffer Set (Qiagen). The concentration of DNA was determined by UV spectrophotometer (Sigma, St. Louis, Mo.). DNA fragment was amplified by using the primer set 16S-350F and 16S-820R that were targeted to universally conserved regions. The amplification reactions were performed with a GeneAmp 2400 PCR thermocycler (Perkin-Elmer, Norwalk, Conn.). The reaction mixtures contained 15 pmol of primers, 200 μM each deoxyribonucleoside triphosphate, 10 mM Tris-HCl (pH 8.0), 1.5 mM $MgCl_2$, 1 U of DynaZyme DNA polymerase (Finnzymes Oy, Espoo, Finland) and 3 μl of the cell-free DNA, in a final volume of 50 μl. The PCR mixtures were then subjected to 95℃ for 5 min followed by 35 cycles of 95℃ for 30 s, 56℃for 30 s, and 72℃ for 30 s, and after amplification an extended step of 5 min at 72℃. The PCR products were confirmed by 2% agarose gel electrophoresis, and proceeded for sequence analysis by the Sequencer (Perkin-Elmer, Norwalk, Conn.). All these sequences were alignment and blasted in the NCBI databank.

2.4. Silver nanoparticle antimicrobial activity analysis

The antimicrobial activity of silver nanoparticle was tested quantitatively by a viable cell count method, depending on JIS-L-1902-2000 standard method.

S. aureus was grown in BHI broth (Difco) and incubated aerobically for 16h at 37 °C. Another two Gram-negative bacteria, *S. typhimurium* and *E. coli* , were separately cultivated in TS broth (Difco) at 37 °C for 16 h. Each 30 mL tube of bacterial cell culture was then

centrifuged for 5 min at 4 °C and 7000 x*g*, decanted, washed with 0.1% peptone (Difco), centrifuged for 5 min, and decanted. The cell pellet was placed into 100 mL of BHI or TS broth and diluted to 10% of the original broth concentration with 900 mL of sterile distilled water to obtain an inoculum of (1.0~2.5) $\times 10^6$ colony-forming units (CFU)/ mL. Then, 0.5 mL of the inoculum was aseptically added to each of the microtube containing 0.5 mL Ag-NPs solution, and keep agitation at 50 rpm in 37 °C for 18 hours. For each analyzed bacteria, an inoculum of cell suspension in a microtube without Ag-NPs solution was used as a control. Aliquots of 0.1 mL of cell suspension were periodically taken from the microtube, and plated in duplicate on BHI agar for *S. aureus* cells or on TS agar for *S. typhimurium* and *E. coli* cells. The plates were incubated in an aerobic chamber for 16 hours at 37 °C. The number of colonies on each plate was counted and calculated to the inhibit activity.

2.5. Antagonism analysis of the mutant with silver nanoparticle

Klebsiella sp. mutant was cultured at 37 °C overnight from a single colony. Aspirate 0.5 ml cultured broth (1.0~2.0X10^9 cfu/ml) mixed with different Ag-NPs to the final concentration of 0.5, 1.0, 1.5, 2.0 and 2.5 %. After incubate at 37 °C for different period of time (1~4 hours), the mixtures were boiled for 10 minutes to inactive the bacteria, and centrifuged in 10,000 xg for 10 minutes, followed by aspirate 0.5ml, cell free, clear supernatant for antimicrobial activity analysis.

3. Conclusion

Today, nanotechnology is a major innovative scientific and economic growth area. However nanomaterial residues may have a detrimental effect on human health and the environment. To date there is a lack of quantitative ecotoxicity data, and recently there has been great scientific concern about the possible adverse effects that may be associated with manufactured nanomaterials (6, 8, 10). Nanomaterial properties were different from the parent compounds, more than 50% of the atoms in NPs are on the surface, resulting in greater activity than bulk materials this make the NPs have a complete dissimilar chemical, biological activity than parent compounds (12).

Ag-NPs have been known for it's inhibit and bactericidal activity and more than 85% major pathogens were inhibited by this new material (7). Unfortunately, in our laboratory routine analysis, we isolate a mutant strain which could resistant to Ag-NPs bactericidal activity. The occurrence was seems like to the bacterial multidrug resistance revolution.

This *Klebsiella* mutant is a gram negative rod bacilli, and all the biochemical identification analysis of API 40H exhibit that this novel mutant was *Klebsiella* sp.. By the bacteria conserved 16S rRNA sequence analysis and alignment in the GeneBank, we could make sure that this mutant was *Klebsiella pneumoniae*.

All these analyzed Ag-NPs (NTX-205, 305, LG-05, 06, VY707) were purchased from the cooperative companies products (Table 1), and renumbered. The antimicrobial activity analysis was preceded by the modified standard method of JIS-L-1902-2000, and all these five Ag-NPs exhibit a strong antimicrobial activity with less than 1.0 % concentration against gram positive or gram negative bacteria (Table 1). After this *Klebsiella* mutant had inactive Ag-NPs containing solutions, the cell free supernatant exhibits no antimicrobial

activity anymore, then the analyzed bacteria could grow up on the plate again rather than the control group without any colony on the plate. It means that, this *Klebsiella* mutant could degrade these five different kinds of Ag-NPs, properly, and make the Ag-NPs containing solution turned to harmless and safety normal waste water.

The industrial scale production and wide variety of applications of manufactured NPs and their possible release in considerable amounts into the natural aquatic environment have produced an increasing concern among the nanotechnology and environmental science community. Assessing the risks of these NPs in the environment requires an understanding of their mobility, reactivity, ecotoxicity and persistency (6, 12).

In addition, release of engineered NPs containing waste into the aquatic environment is largely an unknown. NPs provide surfaces that could bind and transport toxic chemical pollutants, which maybe a mutagen or a nutrition molecular. Industrial NPs was the newest molecular of microorganism who had never been touched within the past few billion years. Unfortunately, microorganism must could faced up the industrial NPs, but the quantity have to be concerned, because that must will make the ecotoxicological problems turned to more complex, and the environment may will be no more predictable.

How much environmental damage could be estimated? Never know. Oberdorster *et al.* investigated the effect of water-soluble fullerene aggregates, nano-C_{60}, on HDF, HepG2, and NHA cells in culture. Nano-C_{60} demonstrated significant toxicity in cell culture studies, while a highly hydroxylated, water-soluble fullerene, $C_{60}(OH)_{24}$ did not (10). It means that each new engineered nanoparticle should be analyzed relative ecotoxicological analysis.

In contrast to environmental harmful effects, NPs could interact with some toxic chemical compounds and alleviate the chemical toxicity. But we almost could make sure that no engineered NPs was produced to treatment the toxic chemical compounds containing waste, today.

Different nanoparticles fabricated from different carrier, cortex, protocol and modification. In these five different Ag-NPs analysis results, different kinds of NPs will not influence this *Klebsiella* mutant degradation activity, even the Ag-NPs concentration was increased to 2.0 %, the mutant still could degrade Ag-NPs containing solution down to none bactericidal activity within 4 hours.

At the cellular level, bacteria may be largely protected against the uptake of many types of NPs since they do not have mechanisms for transport of colloidal particles across the cell wall (7). But quite a lot of results were available for Ag-NPs due to their use as bactericides. The bacterial cell membrane proteins were damaged in the presence of Ag-NPs, finally resulting in death of the organisms (13). Another study reported that C_{60} adsorption onto the gram-negative *E. coli* was 10 times higher than on gram-positive *Bacillus subtilis* (11). The interaction of NPs with the cell is size-dependent and seems to depend on the shape of the NPs (11).

Most of the countries, NPs containing waste had been extensively, directly drained into environment through the industrial manipulation of such many Ag-NPs containing items. Due to the environmental impact of Ag-NPs was under evaluated, most country governments were still uncertain, unknown, how to manage the relative products. People could not withdraw the science development, but we have to evaluate the risk assessment of NPs, carefully, before the bioterrorism was happened on us. We proposed that this *Klebsiella*

mutant could be served as a powerful tool for NPs containing waste management and slow down the environmental impact.

Instead, ecotoxicology in this emerging area must provide a basis for predicting systematically how Ag-NPs biological behavior relates to its structure, composition and morphology. In vitro cell culture experiments are well suited for developing mechanistic models to inform material development. All these results could set as reference data for future efforts to characterize the environmental and health impacts of the other engineered NPs. Ultimately, such positive environmental and toxicological studies will be imperative to ensure the NPs design process yields both effective and safety engineered products.

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Nanoparticles	**NTX-205**	**NTX-305**	**LG-05**	**LG-06**	**VY-707**
Original concentration	6.0%	6.0%	8.0%	8.0%	12.0%
Concentration[1] of 100% inhibit activity[2]	0.5%	0.45%	0.6%	0.65%	0.4%
Production country	Taiwan	Taiwan	Korea	Korea	China

1. the concentration was refer to the original solution by volumn/volumn (v/v).
antimicrobial inhibit activity=[1－sample cfu/control cfu] x 100%

Table 1. The antimicrobial activity of the five different silver nanoparticles.

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The main purpose of this book is to describe important issues in various types of devices ranging from conventional transistors (opening chapters of the book) to molecular electronic devices whose fabrication and operation is discussed in the last few chapters of the book. As such, this book can serve as a guide for identifications of important areas of research in micro, nano and molecular electronics. We deeply acknowledge valuable contributions that each of the authors made in writing these excellent chapters.

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