Interactions of Citrate-Coated Silver Nanoparticles in the Rhizosphere

Michael A. Doody,[1,*] Harsh P. Bais[1,2] and Yan Jin[1]

[1]University of Delaware, Newark, Delaware, USA. [2]Delaware Biotechnology Institute, Newark, Delaware, USA.

*Presenter; Email: mdoody@udel.edu

INTRODUCTION

- The increasing use of silver nanoparticles (AgNPs) in commercial and industrial antimicrobial products presents the opportunity for increased environmental exposures.[1] At risk for increasing loads of AgNPs in the near future is the rhizosphere, the most active portion of soil where biogeochemical processes influence a host of landscape and global scale processes.[2] Our broad goal is to determine the impact citrate-coated AgNPs have on those processes, as well as specific constituents and interactions between those constituents. Our specific goal for this project is to quantify the effects of citrate-coated AgNPs on the growth of two species of bacteria, *Bacillus subtilis* and *Escherichia coli*, to characterize how AgNPs behave in relevant environmental media, and to determine the impact of AgNPs on germination of *Zea mays* seeds.

EXPERIMENTAL METHODS

Particle Characterization

- Particles characterized for several parameters, including diameter by TEM and ImageJ, hydrodynamic radius by laser diffraction, and zeta potential by electrophoretic mobility

Dissolution

- Dissolution of 1.0 mg L^{-1} AgNPs in DI Water, LB-liquid media, and *Z. mays* (strain MO-17) root excretes measured over 7 days
  - Concentration of aqueous silver (Ag_{aq}), differentiated by filtration through 25-nm membrane, and quantified by ICP-MS

Antagonism of Bacteria

- Treatments: 0.1, 0.5, and 1.0 mg L^{-1} AgNPs and 0.015 mg L^{-1} Ag_{aq} ($AgNO_3$) control
- Bacteria Cultures (*B. subtilis*, strain FB17 and *E. coli*, strain OP50)
  - Incubated bacteria in LB-liquid media at 30°C in shaker for 24 h
  - Plated bacteria on LB-solid media after 24 h of incubation
  - Incubated plates at 30°C for 24 h

Germination of MO-17

- Treatments: Surface sterilized with ethanol and bleach, or rinsed with DIW
- Plated on filter paper saturated with DIW, or 0.1, 0.5, or 1.0 mg L^{-1} AgNPs.
  - Placed under constant light at room temperature
  - Observed for germination and/or contamination over 7 d

RESULTS

Particle Characterization

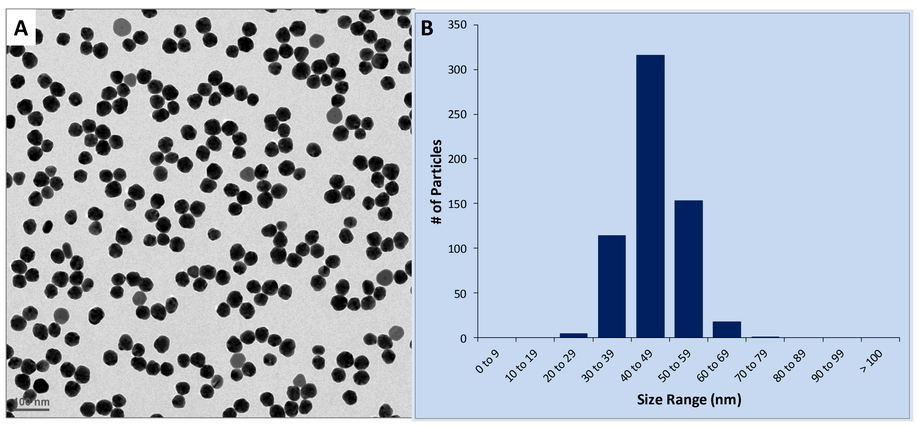

Figure 1: A) TEM Micrograph of AgNPs. Particles are spherical and monodisperse AgNPs. Scale bar is 100 nm. **B)** Particle Size Distribution of AgNPs. Distribution is normal around 40-49 nm, n = 607

- Size and Morphology
  - Particles are spherical and monodisperse; average TEM diameter of 45 +/- 8 nm
- Stability
  - Particles are moderately stable in DIW: Hydrodynamic diameter of 107.7 +/- 2.8 nm and zeta potential of -16.6 +/- 3.3 mV
  - Stability decreases in LB Media: Hydrodynamic diameter greater than 400 nm and zeta potential of -10.1 +/- 1.2 mV
  - Particles appear most stable in MO-17 root excretes: Hydrodynamic diameter of 46.0 +/- 0.1 nm and zeta potential of -17.4 +/- 2.6 mV

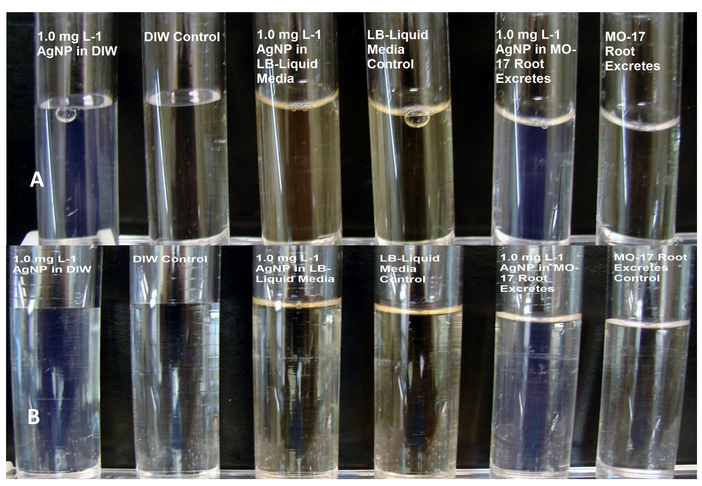

Figure 2: A) Digital Photograph of Particle Suspensions at 0 Days. Particles are stable in all media, as displayed by the "glow" of the suspension compared to the control media. **B)** Digital Photograph of Particle Suspensions at 7 days. Particles suspended in DIW and MO-17 Root Excretes remain stable, but those in LB-Liquid Media have aggregated and fallen out of suspension.

Dissolution

- No detectable Ag_{aq} was released into solution over 7 d in any of the media

Acknowledgements

The authors acknowledge NSF CBET-0932686 and thank Deborah Powell and Shannon Modla of the Delaware Biotechnology Institute, Rovshan Mahmudov of the Civil and Environmental Engineering Department, and Caroline Golt of the Plant and Soil Sciences Department at the University of Delaware.

Antagonism of FB17 and OP50

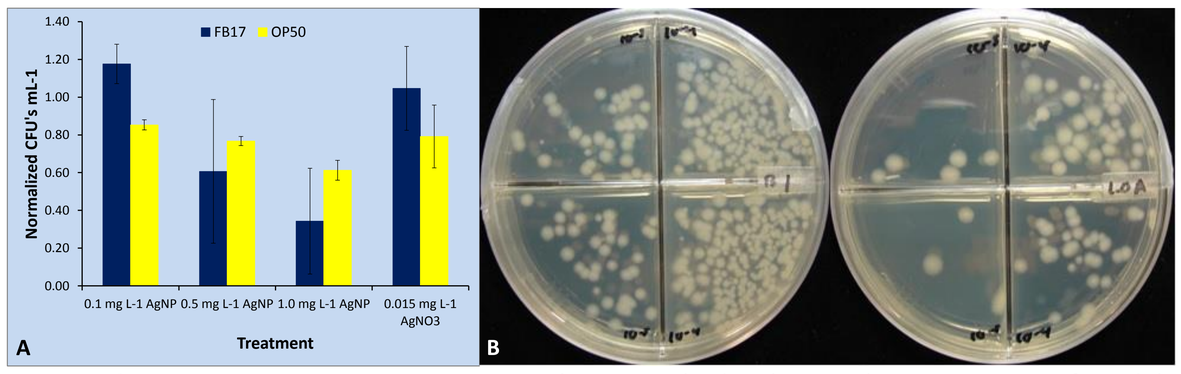

Figure 3: A) 24-H Plate Counts for FB17 and OP50. CFU's are normalized to the control group. FB17 outperformed the control in the 0.1 mg L^{-1} AgNP and 0.015 mg L^{-1} $AgNO_3$ treatments. Dose response appears stronger in FB17 than in OP50; For the 1.0 mg L^{-1} AgNP treatment, 24-h growth of FB17 was 34% of control, while OP50 showed growth of 61% of control. **B)** Images of FB17 on LB-solid media after 24 h of incubation. The control replicate (labeled B1, on the left) has many more colonies than a replicate of the 1.0 mg L^{-1} AgNP treatment (labeled 1.0A, on the right).

Germination/Contamination of MO-17

Treatment	No. Germinated Seeds	No. Contaminated Seeds
Sterile	6	4
Sterile + 0.1 mg L^{-1} AgNP	6	4
Sterile + 0.5 mg L^{-1} AgNP	7	3
Sterile + 1.0 mg L^{-1} AgNP	9	1
Non-Sterile	0	10
Non-Sterile + 0.1 mg L^{-1} AgNP	2	8
Non-Sterile + 0.5 mg L^{-1} AgNP	4	6
Non-Sterile + 1.0 mg L^{-1} AgNP	3	7

Table 1: Impact of Sterilization and Treatment with AgNPs on Germination of MO-17 Seeds. No sterilized seed germinated before 96 h. Greater than 50% of all sterilized seeds germinated. This percentage increased with increasing concentration of AgNP treatment. All of the non-sterile control seeds contaminated within 36 h before any signs of germination. Non-sterile seeds treated with AgNPs contaminated less frequently and after less time than sterilized seeds.

DISCUSSION

Impact of AgNPs on Growth of FB17 and OP50

- Antagonism of *B. subtilis* strain FB17 is potentially ecologically significant:
  - FB17 is involved in several beneficial plant-microbe relationships, including biocontrol via secretion of antimicrobial compounds, plant growth promotion, and degradation of organic polymers in the soil[3]

AgNP-Bacteria Interactions

- Proposed mechanisms for antagonism include: sorption of NPs on the cell surface, uptake of AgNPs into the cell, and uptake of Ag_{aq}.[4,5] It is likely that surface interaction are controlled by the chelating properties of the citrate coating
- Observed antagonism (Fig. 4A and B) attributed to cell membrane disruption and/or cell wall pitting caused by sorption of AgNPs or small AgNP aggregates onto the cell surface.[5]

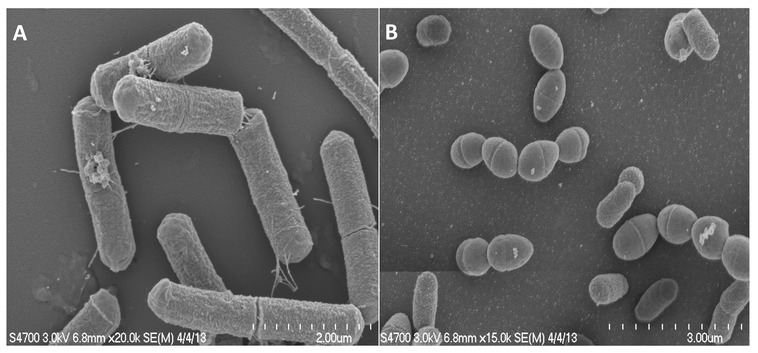

Figure 4: FE-SEM Micrographs of Interaction of AgNPs with Bacteria. **A)** Rod-shaped FB17 with AgNP aggregates and single AgNPs or small aggregates sorbed to the cell surface and on substrate. **B)** Cocci-shaped OP50 with aggregates and single NPs sorbed to cell surface.

Germination and Contamination of MO-17 Seeds

- Surface-sterilized MO-17 seeds plated on AgNP-treated substrates have the greatest frequency of germination and lowest incidence of contamination; this suggests that the contaminating bacteria/fungi are susceptible to exposures to AgNPs
- Impact of treatment with AgNPs on later development of MO-17 currently unknown but under investigation

CONCLUSIONS

- Antagonism of FB17 and OP50 is significant at 1.0 mg L^{-1} AgNPs and likely caused by citrate-controlled sorption of the coated NPs and small aggregates of NPs onto the cell surface
  - This may limit the ability of FB17 to participate in beneficial interactions with various plant species, including Z. mays strain MO-17
- Treating the plating substrate with AgNPs may decrease the incidence of contamination of surface sterilized MO-17 seeds prior to and during germination

FUTURE WORK

- Future work will focus on determining the extent of AgNP influence on *Z. mays* strain MO-17 and its mutualistic relationship with *B. subtilis* strain FB17.

References

[1] Nowack, and Bucheli. (2007). Occurrence, behavior and effects of nanoparticles in the environment. *Journ. Environ. Pollut.,* 150: 5-22.

[2] Morrissey, et al. (2004). Are microbes at the root of a solution to world food production? *EMBO Rept.* 5(10): 922-926.

[3] Bais, et al. (2004). Biocontrol of *Bacillus subtilis* against infection of Arabidopsis roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. *Plant Physiol.,* 134: 307-319.

[4] Choi, et al. (2008). The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Journ. Water. Res.,* 42: 3066-3074.

[5] El Badawy, et al. (2011). Surface charge-dependent toxicity of silver nanoparticles. *Environ. Sci. Technol.,* 42: 283-287.