Does nanoparticle shape influence the evolve ability of resistance to silver nanoparticles in bacteria?

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Abstract

Does the shape of nanoparticles affect the evolution of resistance to silver? In an earlier study we demonstrated that *E. coli* K12 MG1655 could rapidly evolve resistance to spherical nanoparticles. In ongoing work, we showed that this bacterium easily evolved resistance to ionic silver. To test the effect of nanoparticle shape, we established 5 replicate populations exposed to triangular silver nanoparticles (AgNPl); 5 replicates exposed to spherical silver nanoparticles (AgNP), 5 exposed to ionic silver (Ag+) in the form of AgNO3, and 5 controls for a total of 20 replicates. The AgNPl and AgNP were citrate-coated. In the AgNPl replicates, we observed a minimal response to selection over the first 100 generations (~15 days). Minimum inhibitory concentration (MIC) had increased from 12.5 mg/L to 18 mg/L. In both the AgNP and Ag+ replicates, MIC continued to increase as in our past experiments. The measured MIC in the AgNPl-exposed replicates began to decline after 100 generations. By generation 230 (~35 days) all of the AgNPl lines were extinct. We maintained the selection lines at 100 g/L, a concentration that showed positive selection results for both the spherical AgNP-selection and Ag+-selection replicates. We are in the process of repeating this experiment. We are currently maintaining 10 AgNPl-selected replicates at a culture concentration of 40 g/L. We will test these replicates for 24-hour growth at this concentration to determine if we observe superior growth relative to controls. These preliminary results suggest that nanoparticle shape does impact the evolve ability of silver resistance.

Keywords: *E. coli*, Evolution, Resistance, Silver nanoparticles

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Introduction

Bacteria that are exposed to stressful environments have evolved mechanisms that respond to the stress based on their duration (1,2,3). In the case of heat stress, it is known that bacteria will display a transient heat response by upregulating genes encoding heat stress proteins (4). If the stress is maintained for over a long period of time, individual bacterial lineages will accumulate mutations that have been acted upon by natural selection which result in long-term adaptation to the new environment (5). Much is known concerning how bacteria respond to heavy metal stress, particularly silver. Silver has been employed in extensive way since very ancient time (6). It has been used to control spoilage and to fight infections. There have been investigations on antiviral and antibacterial actions of silver, silver compounds and silver ions. Fortunately, silver in the concentrations used to control bacteria is non-toxic to human cells (6). It has been claimed that bacteria will have a difficult time evolving resistance to silver because it impacts multiple physiological and genetic systems simultaneously. Silver (Ag+) interacts with the thiol groups in respiratory enzymes causing them to become inactivated; it binds to cell envelope and inhibits respiration; it inhibits the uptake of phosphorus and causes to release phosphate, mannitol, and glutamine from cell; and it impedes DNA replication by causing it to be condensed (6). In addition Ag+ ions disrupt metabolism, cell signaling, DNA replication, transcription, translation, and cell division through the generation of reactive oxygen species (6). Despite this, several observations and experiments have already demonstrated that bacteria display long-term adaptation to ionic silver and silver nanoparticles. For example, in variety of applications, including dental work, catheters and burn wounds, ionic silver (Ag+) is used to control bacterial growth and silver resistant bacteria have been isolated (6). Our laboratory as well as others has demonstrated that evolving resistance to ionic silver and spherical silver nanoparticles (AgNPs) is relatively easy (1, 2, 7, 8) and that it occurred by few genomic changes. The similarity of response to ionic and spherical AgNPs is supported by observations that the key impact of spherical AgNPs is due to their release of Ag + ions (9). However studies of triangular shaped silver nanoparticles suggests that they may be more lethal to bacteria (10,11). For this reason, we are testing the ability of *E. coli* K12 MG1655 (a naïve bacterium) to evolve resistance to triangular shaped nanoparticles. We predict that if the release of Ag+ ions is the sole mechanism of AgNPl impact on *E. coli* that we will observe no difference in the rate of response and the genomic changes resulting from long-term exposure. However if nanoparticle shape is an important factor in the effect of AgNPls then we predict that the rate of evolution (evolveability) of AgNPl resistance will be lower and that we will observe different genomic mechanisms associated with this phenotype.

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