Name(s)

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Project Number

S1508

Project Title

Determining the Roles of CuSB, Flagellin, and AcrAB-TolC in Bacterial Responses to Nonlethal Nanosilver Concentrations

Abstract

Objectives

Renowned as potent antimicrobials, silver nanoparticles (AgNPs) have recently been integrated into several industries for a vast array of purposes, from countering HIV strains to treating wastewater. However, prolonged exposure to AgNPs will inevitably induce resistance in bacteria, which possess defense systems such as the AcrAB-TolC pump, CuSB pump, and flagellin. As a solution, this project aims to determine whether bacteria are capable of developing resistance when exposed to nonlethal concentrations of AgNPs, identify the mechanisms involved in resistance, and propose a method of suppressing resistance (a substantial and necessary step in ameliorating the global antibiotics overuse crisis).

Methods

Citrate-coated AgNPs were synthesized using AgNO(3) and trisodium citrate. Resistance to AgNPs was induced in E. Coli by exposing the bacteria to progressively increasing sub-inhibitory concentrations of the nanoparticles in agar media until the bacteria could consistently tolerate >50 mg/L AgNPs. Using an original assay design, UV/Vis spectroscopic characterization was used to determine the effects of inhibiting flagellin production with pomegranate rind extract (PGRE), the AcrAB-TolC pump with 2-chloroquinoline, and the CuSB pump with copper on AgNP stability in resistant bacteria. AgNPs would destabilize when in contact with resistant bacteria unless the defense mechanism involved in resistance was inhibited.

Results

Bacteria tolerated up to 90 mg/L AgNP after 6 sets of AgNP concentration increases (with an original tolerance threshold of 2 mg/L). When solutions of 50 mg/L AgNPs, resistant bacteria, and each inhibitor were characterized, only the solution where flagellin production was inhibited possessed silver that was still in nano-form. Max wavelength values indicating nanoparticle stability lie within 380-430 nm, and the vial with inhibited flagellin expressed a max wavelength of 413 nm an hour after the inhibitor was added.

Conclusions

Results indicated that flagellin production is involved in bacterial resistance to AgNP toxicity. PGRE shows promise as a novel approach to combating the global antibiotics resistance crisis by prolonging or even preventing bacterial resistance to AgNPs. Further research should test for potential carriers that can direct PGRE to harmful bacteria, preventing the inhibition of beneficial bacteria.

Summary Statement

UV/Vis spectroscopy demonstrated that bacteria strengthen flagellin production when developing resistance to silver nanoparticles and that this resistance can be combated by coupling the nanoparticles with pomegranate rind extract.

Help Received

I devised my experimental methodology myself and conducted the project independently. My AP Biology teacher Ms. Chelsey Beck and Biotechnology teacher Dr. Christine Koltermann provided me with materials and space to perform experimentation.