Developing Resistance to Silver Nanoparticles in Pseudomonas Fluorescens

Purpose

The purpose of the experiment to is make cultures of *Pseudomonas fluorescens* resistant to silver nanoparticles.

Introduction

Bacteria and the diseases they cause are a constant public health concern. While antibiotics are commonly used on bacteria, easily killing them, overuse of antibiotics, halting the treatment when the symptoms first disappear, or not using enough antibiotics can produce bacteria that are resistant to antibiotics (Ventola, 2015). This is a major problem in hospitals, where the rooms are routinely disinfected, and many different patients enter. Because of this, silver nanoparticles are often used in hospitals to deal with antibiotic-resistant bacteria, as the nanoparticles can kill even antibiotic-resistant bacteria with ease (Friar, 2016). These nanoparticles kill bacteria so easily by producing silver ions when oxidized, which are very toxic to bacteria (Williams, 2012). It may seem like silver nanoparticles are a panacea like penicillin was seen as. However, incorrect usage will lead to bacteria that can resist even silver nanoparticles.

This experiment will attempt to illustrate the dangers of incorrect use of silver nanoparticles by making *Pseudomonas fluorescens* resistant to the nanoparticles. *Pseudomonas fluorescens* are resistant to antibiotics, so silver nanoparticles are often the best way to kill them. Therefore, the amount it takes to kill the bacteria is well known, as well as the point where bacteria first become negatively impacted by the particles (Fabrega, 2009). This makes *Pseudomonas fluorescens* an ideal choice for this experiment, as the experiment can begin by using the concentration of particles at which the bacteria starts to suffer negative effects and then use increasingly larger concentrations of the silver nanoparticles to induce greater levels of resistance. If resistance to silver nanoparticles can be induced in *Pseudomonas fluorescens* through this process, this is a grave health issue that needs to be addressed.

Hypothesis

The hypothesis of the experiment is that *Pseudomonas fluorescens* can be made to be resistant to the effects of silver nanoparticles by giving them small but increasing concentrations of silver nanoparticles. Given that only the bacteria that possess sufficient resistance to silver nanoparticles can survive, this experiment should be able to produce bacteria with resistance to silver nanoparticles as long as the concentration of the particles increases slowly enough.

Results

Cultures of *Pseudomonas fluorescens* were able to survive in Petri dishes containing 8.198ml of a .02mg/ml silver nanoparticle solution after the procedure was carried out, showing that this process can produce bacteria with resistance to silver nanoparticles.

Figure 1, showing bacterial growth in experimental plate containing .256ml of nanoparticle solution. Note that all pictures were taken by Ian Szejbach

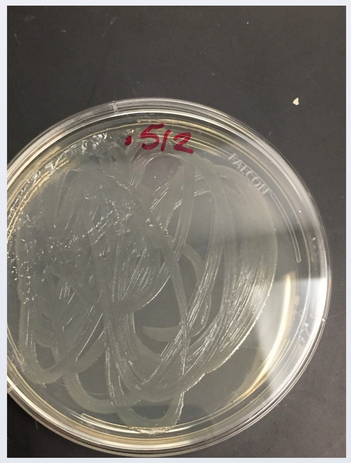

Figure 2, showing bacterial growth in experimental plate containing .512ml of nanoparticle solution.

Figure 3, showing bacterial growth in experimental plate containing 1.025ml of nanoparticle solution.

Figure 4, showing bacterial growth in experimental plate containing 2.050ml of nanoparticle solution.

Figure 5, showing bacterial growth in experimental plate containing 4.099ml of nanoparticle solution.

Figure 6, showing bacterial growth in experimental plate containing 8.198ml of nanoparticle solution.

Methodology

Materials

In order to conduct this experiment, specific materials are needed. For example, 30 Petri dishes that each hold 25ml of agar, a pressure cooker to disinfect the dishes, and agar are needed to produce ideal nurseries for the bacteria. Two 25ml containers with solutions of .02mg/ml concentrations of silver nanoparticles that are ten nanometers in diameter and a delivery pipette are also needed to distribute silver nanoparticles throughout the agar in the Petri dishes. A culture of *Pseudomonas fluorescens* is required so that the bacteria can develop resistance to the silver nanoparticles.

Procedure

In order to complete the experiment, the Petri dishes must first be sterilized by the pressure cooker and then filled with 25ml of agar. Then, the delivery pipette must be used to remove .256ml from three of the Petri dishes, and then .256ml of the silver nanoparticle solution must be added to those three Petri dishes by using another delivery pipette. The delivery pipette that removed the agar will then be used to stir the combined agar and silver nanoparticle solution so that the solution becomes fully mixed with the agar. Then, this process will repeat, but twice the amount of the particle solution will be added and twice the agar will be removed. This process will continue until 8.198ml of solution is added to the Petri dish. Then, a culture of bacteria will be placed into each of the three Petri dishes with the lowest concentration of nanoparticles, and the bacteria will be left in the dish for 48 hours. After 48 hours are finished, a culture of the surviving bacteria will be placed into a dish with twice the concentration of silver nanoparticles, and so on, until the bacteria get placed in the final three Petri dishes. As a control, 12 Petri dishes, prepared in the same manner as the experimental plates, but with two dishes containing each concentration instead of three, will be used. These 12 dishes will all have untreated *Pseudomonas fluorescens* placed into them, showing how untreated *Pseudomonas fluorescens* reacts to the different concentrations of silver nanoparticles. After this, the data will be analyzed and conclusions will be made.

Conclusion

Because *Pseudomonas fluorescens* was able to develop a much greater resistance to silver nanoparticles, the hypothesis was supported. This shows that not using enough silver nanoparticles to sterilize a surface will result in bacteria that have a resistance to silver nanoparticles, showing the dangers of improper usage of silver nanoparticles.