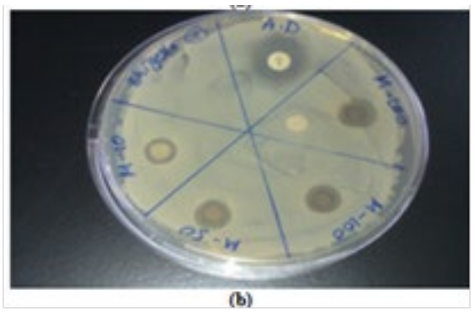
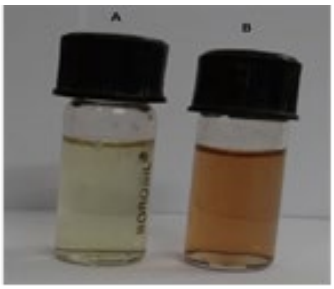
Student Worksheet

Using Biosynthesized Silver Nanoparticles to Kill Antibiotic-resistant *E. Coli*





Introduction: Silver nanoparticles (SNPs) have been used in medicine for hundreds of years. Uses have included "cure-all tonics", topical ointments used on burn patients, and coatings for heart stents to ward off infection. Scientists have shown evidence that silver nanoparticles can even kill off the most stubborn antibiotic resistant bacteria!

Manufacturing of silver nanoparticles has increased over the last two decades to staggering volumes due to the booming field of nanotechnology. Commercially manufactured silver nanoparticles pose numerous risks and are extremely expensive to create. Manufacturing of SNPs involves toxic and hazardous chemicals which pose environmental and biological risks. What if eco-friendly production methods existed? What if harmful antibiotic resistant bacteria can be destroyed by creating biosynthesized silver nanoparticles cheaply?

Recent research has shown that medicinal silver nanoparticles can be created from common plants that already exhibit medicinal properties. These methods are less expensive, safer and can be done in one step. Plants such as peppermint, aloe vera, and geranium are just a few examples of ones that show promise in the making of biosynthesized silver nanoparticles (bSNPs).

During part I of this lab you will be creating antibiotic resistant *E. coli* that will be resistant to two commonly used antibiotics: ampicillin and streptomycin.

Parts II-IV of this lab will consist of you creating biosynthesized silver nanoparticles (bSNP's) from one of three plants. These particles will be absorbed into disks and placed on *E. coli* cultures along with ampicillin and streptomycin disks. You will test the efficacy of inhibiting growth of bacteria using ampicillin, streptomycin and bSNP's.

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Prelab: Read the following and answer the questions in your lab notebook. You may have to research 3 and 4.

Synthesis of silver nanoparticles using leaves of Cathatanthus roseus and their antiplasmodial activities, Ponarulselvam S et al., Asian Pac J Trop Biomed 2012; 2(7): 574-580. On web at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3609350 or obtain from your instructor.

Biosynthesis of Self-Dispersed Colloidal Particals Using the Aqueous Extract of P. peruviana for sensing d;-Alanine, Mohd Rashid and Suhail Sabir, ISRN Nanotechnology, Vol 2014, Article ID 670780. On web at: dx.doi.org/10.1155/2014/670780 or obtain from your instructor.

1. What are metallic nanoparticles? List 3 ways in which the medical field uses them.

2. What can silver nanoparticles be used for? What properties make them a great candidate for nanomedicine?

3. What does it mean for bacteria to be antibiotic resistant? Why are antibiotic bacteria becoming more prevalent?

4. What is bacterial conjugation?

Materials:

Part I: Making antibiotic resistant E. coli

- Introductory Bacterial Conjugation Kit
- Hot plate/stirrers
- Incubator
- Shaking water bath

Parts II-IV: Making and testing bSNP

- $AgNO_3$- Silver Nitrate
- Aloe Vera, Geranium, or Peppermint plant leaves
- Modified *E. coli*
- Ampicillin, streptomycin, ruler and bSNP disks
- Glass beads
- Razorblade
- Eye dropper (1 mL capacity)
- Filter paper
- Beakers and Erlenmeyer flasks
- Distilled water
- Sterile petri dish with agar
- ruler

Procedure for the Activity:

Part I: Making ampicillin and streptomycin resistant *E. coli*.

1. Obtain a copy of procedures to "Introductory Bacterial Conjugation Kit" from your teacher, who will instruct you on which steps you will be completing.
2. Upon completion of bacterial conjugation, store bacteria and LB broth in refrigerator. This sample will be used in parts II-VI.

Part II: Making Biosynthesized Silver Nanoparticles

1. Work in groups of 3-5. Your group will be assigned to work with one of three plants: aloe vera, geranium, or peppermint. At least two groups work on the same plant to compare data.
2. Cut leaves from your assigned plant and weigh until you have approximately 20 grams.
3. Thoroughly wash ~20g of leaves from your assigned plant.
4. **Carefully**, finely cut your leaves from your assigned plant and put it into an Erlenmeyer flask containing 100ml of distilled water.
5. Boil the mixture for 1.5 minutes and let it cool down to a temp that you can safely handle.
6. Pour the supernatant liquid without disturbing the sediment through a Whatman #1 filter paper into a clean 50ml Erlenmeyer flask. Note: If you run out of time this solution may be frozen for up to one week or be stored until the next lab period.
7. Add 1ml of pure plant broth to 20ml of 0.001M $AgNO_3$.
8. Let the mixture sit for 24 hours or until next lab period. Soak two circular pieces of paper from a three hole punch in the mixture and let dry to create a bSNP disk.

Part III: Testing bSNP's, ampicillin and streptomycin.

1. Obtain an ampicillin, streptomycin and bSNP disk from your teacher.
2. Obtain a sterile petri dish that contains agar. Be sure no growth has already occurred.
3. Using a permanent marker, label three locations in the dish where the disks will be placed. Amp, Str and bSNP

4. Use an eyedropper and place 1ml of *E.coli*/LB broth into petri dish. Use 3-4 glass beads and swirl bacteria around evenly. Allow liquid to absorb into agar.
5. Carefully place each disk in its labeled location. Gently push down on the disk to make sure it adheres. Seal the petri dish with parafilm and place in incubator for 24 hrs at 37°C.

Record your observations

Group #	**Plant type**	**Zone of Inhibition (mm)-amp,str,bSNP**	**Conclusion**

Part IV: Analyze the Results.

1. You should notice growth on your petri dish. Measure the zone of inhibition for each disk and record it in you data table.
2. After you are finished with the lab, wash all equipment in Unicide or bleach, or place in an autoclave. Follow you teacher's instruction for clean up.

Draw Conclusions:

1. Why was it important to treat the silver particles with plant broth? How would your results have been different if we hadn't used any plants?

2. Why did we run two groups per plant? Why use ampicillin and streptomycin disks if we know then bacteria to be resistant?

3. Which disks showed the greatest zones of inhibition (amp,str, bSNP)? Which plants showed the greatest zones of inhibition (aloe, geranium, peppermint? Why do you think we saw these results?

4. In your assigned group, research and create a 2-3 minute presentation to share with the class. Research other organsims that are currently being used to create nanoparticles. (What type of nanoparticles? What are they being used for? etc.) Are there other green methods under development for nanoparticles? Present your findings to the class with a slide presentation.

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