Investigation of Minimal Media and Chemical Stress on Antibiotic Sensitivity of *Pseudomonas putida* and *Pseudomonas fluorescens*

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**Abstract:**

The genus *Pseudomonas* contains many species that range from harmless commensals to antibiotic resistant pathogens. The most dangerous organism is *Pseudomonas aeruginosa*, a leading cause of concern as the source of many antibiotic resistant infections. *Pseudomonas putida* and *Pseudomonas fluorescens* were used in this study as they exhibit properties similar to those of *P. aeruginosa* without the issue of pathogenicity. Previous studies with non-pathogenic organisms such as *Escherichia coli* and *Staphylococcus epidermidis* have shown an increase in antibiotic sensitivity when grown in the presence of sodium acetate, a known chemical stress agent, on complex media. This project is designed to study the effects of sodium acetate as the sole carbon source on antibiotic sensitivity of *Pseudomonas putida* and *Pseudomonas fluorescens* grown on M9 minimal medium. Antibiotic sensitivity is measured using the standard Kirby-Bauer method.

**Introduction:**

The genus *Pseudomonas* contains over 200 different types of organisms (2). Species of *Pseudomonas* range from harmless commensals to antibiotic resistant pathogens and are commonly found all throughout the environment. The most dangerous of this group is *Pseudomonas aeruginosa*, the source of many antibiotic resistant infections. Both *Pseudomonas putida* and *Pseudomonas fluorescens* are safe nonpathogenic alternatives used in teaching laboratories to demonstrate the genus *Pseudomonas* without using the pathogen *P. aeruginosa*. Recently both organisms were used in colloquium projects to study antibiotic sensitivity and cold and warm stress (3). Another project used the nonpathogenic lab strains of *Staphylococcus epidermidis* and *Escherichia coli* to explore sodium acetate as a chemical stressor that increases antibiotic sensitivity. These studies have used Mueller Hinton (MH) agar, which is a complex medium where the exact chemical composition is unknown (5,7). An improved method to study the effects of sodium acetate on antibiotic sensitivity is to use the chemically defined medium M9 minimal agar (3). M9 minimal agar is a chemically defined media with sodium acetate as the sole carbon source to match the turbidity of a 0.5 McFarland Standard and swabbed onto the appropriate minimal media plates. Antibiotic disks are dispensed using a commercial multidisc dispenser and incubated for an additional 48 hours at the respective temperature. Five antibiotics were used in this activity, all of which are aminoglycosides (Gentamicin, Kanamycin, Neomycin, Tobramycin, and Streptomycin). Zones of inhibition are measured and compared to standardized tables published in the laboratory manual or provided with the antibiotic disks.

**Procedure:**

In order to study the effect of acetate on pseudomonads, M9 minimal media and Mueller Hinton were used. Mueller Hinton is a complex media commonly used for antibiotic sensitivity testing, these plates can be made quickly by mixing the ingredients and autoclaving. M9 minimal agar is a chemically defined media with a sole carbon source. Minimal media requires the individual components to be mixed, filter sterilized and aseptically added to autoclaved water and agar in a laminar flow hood. For this experiment glucose and sodium acetate were the two carbon sources added to the media (separately). Antibiotic sensitivity was determined using the Kirby-Bauer antibiotic susceptibility method using both media. We used the American Type Culture Collection (ATCC) strains of *P. fluorescens* (Strain 13525), and *P. putida* (Strain 49128). *P. fluorescens* is grown at room temperature (20-25°C) on the appropriate media. *P. putida* is grown at 37°C in an incubator. Both *Pseudomonas* strains are grown on minimal media with two passages to ensure there are no reserve carbon sources stored in the organisms. After 48 hours, the cultures are suspended in a minimal broth with no carbon source to match the turbidity of a 0.5 McFarland Standard and swabbed onto the appropriate minimal media plates. Antibiotic disks are dispensed using a commercial multidisc dispenser and incubated for an additional 48 hours at the respective temperature. Five antibiotics were used in this activity, all of which are aminoglycosides (Gentamicin, Kanamycin, Neomycin, Tobramycin, and Streptomycin). Zones of inhibition are measured and compared to standardized tables published in the laboratory manual or provided with the antibiotic disks.

**Results:**

![Graph 1](image1.png)

**Graph 1** and **Graph 2** represent multiple trials of 5 aminoglycosides on Mueller Hinton (MH), Minimal media with Acetate (Min + A), and Minimal Media with Glucose (Min + Glu).

**Conclusion:**

In our study both *Pseudomonas putida* and *fluorescens* ATCC strains can be successfully grown on the chemically defined media with acetate as a sole carbon source (Table 1). When performing the Kirby-Bauer antibiotic sensitivity tests with the various aminoglycosides, an increase in sensitivity was generally observed when sodium acetate was the primary carbon source for both *Pseudomonas* in this study (Graph 1 and 2). Although no "magic bullet", the presence of acetate appears to increase sensitivity significantly with Tobramycin and Gentamycin, two known antibiotics that are particularly effective against *Pseudomonas aeruginosa* species (1). Since this was consistent throughout the trials, we believe that this system should be used to test various strains of this human pathogen in future trials.

**References:**


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