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Pseudomonas aeruginosa resistance to tetracycline and triclosan

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ABSTRACT

P. aeruginosa is a gram-negative rod bacterium which is widespread in nature and causes dangerous infections in humans. Tetracycline is a common antibiotic which is sometimes used to combat these infections. Triclosan is a chemical widely used in consumer products as an antibacterial agent. Studies have shown *P. aeruginosa* to be highly resistant to triclosan. The purpose of this study was to determine whether the bacterium would develop more resistance to triclosan and tetracycline after exposure to these biocides. *P. aeruginosa* was exposed to different concentrations of the two biocides, then bacteria growing in the highest concentrations were transferred to the next highest concentration to determine whether growth would be observed. In the tetracycline experiment, bacteria in the 50.0ug/ml concentration seemed to have developed resistance, because when they were moved to new sterile plates containing 5.0ug/ml solution, they grew, although bacteria had not grown there originally. The limit solubility for triclosan is 0.01g/L of water and tetracycline is 1g in 10ml of water.

Keywords: *triclosan, Pseudomonas aeruginosa, tetracycline, resistance, FabI*

INTRODUCTION

Pseudomonas aeruginosa is a gram-negative rod-shaped bacterium. These pathogens are widespread in nature, inhabiting soil, water, plants, and animals including humans. They are known to cause nosocomial infections such as pneumonia, urinary tract infections, respiratory system infections and infections of severe burns (Geyik, et al., 2003). This study used *Pseudomonas* because it has been used in related research projects dealing with antibiotic and triclosan resistance.

Only 4 structures of at least 12 resistance nodulation type efflux systems of the *P.* aeruginosa genome have been characterized. Example of structural genes are MexAB-OprM. MexCD-OprJ, MexEF-OprN and MexXY. (Karkhoff-haeizer, 2000). With or without this efflux pump *P. aeruginosa* will still be resistant because of the strains, that maybe the same as other bacteria that has some resistant to triclosan and tetracycline. During recent biochemical genetic studies it has been shown the triclosan acts on defined bacterial targets and nonspecific one as previously thought as it pertains fatty acid biosynthetic pathway, ACP reductase. Possessing both triclosan-sensitive and resistant enzymes, *P. aeruginosa* is unique.

Antibiotic resistance of bacteria is acquired by two genetic processes. One process is mutation. Sometimes bacterial DNA will spontaneously mutate in such a way that the efflux pump will expel antibiotics (Poole, 2002). When a bacterial colony is spread with antibiotic, most of the bacteria will be destroyed, but bacteria that survive have a mutation that allowed them to resist the drug (Poole, 2002).These resistant bacteria then multiply and create a resistant colony (O'Donnell). In the other process, acquired resistance occurs by the exchange of genes between bacterial strains through floating pieces of DNA known as plasmids. These plasmids carry information from one cell to another (O'Donnell, 2003).

P. aeruginosa has been isolated from soil and water, and seems to cause disease in humans. Among human pathogens, *P. aeruginosa* is known for its multidrug resistance. Because of the efflux pump, *P. aeruginosa* can be resistant to antibiotics such as penicillin, cephalosporin, tetracycline and more, even without the R plasmid that is usually responsible for antibiotic resistance among bacteria. Resistance in *P. aeruginosa* is caused by the outer membrane of the bacterium, because it is not very permeable (Livermore et al., 1994). The efflux pump is located in the cell membrane. The pump transports the antibiotics to the outer membrane of the bacterial cell. *P. aeruginosa* is known for possessing metabolic versatility. These bacteria are chemoorganotrophic (Madigan et al., 2002); they are able to grow up to 43 degree celcius and in neutral pH. *P. aeruginosa* does show a prediposition for growth in moist environments (Kwon et al., 2003).

It can be possible that antibiotic resistant bacteria occur not only in humans but also in animals. Antibiotics are mostly used in animals to enhance growth (O' Donnell, 2003). It seems that animals get more antibiotics than humans in a year. This has become a major problem because these antibiotics are used to treat animals for infections. So when treated with antibiotics, the bacteria have often become resistant to antibiotics because they are used in the feed. We fertilize our crops with animal manure which may contain already resistant bacteria; then the bacteria might get in our soil and water and when the animals eat the plants and we eat the animals, the bacteria might get in our food, which might infect humans with resistant bacteria (O'Donnell, 2003).

This study will examine the effect of tetracycline and triclosan on *P. aeruginosa*. Triclosan (2, 4, 4-tricloro-

2-hydroxydiphenyl ether) is a chemical widely used as antibacterial agent. Triclosan was introduced in 1972 for hospital use (Jones et al., 2000). It is used in consumer products such as antibacterial soap, detergent soaps, household cleaners and other hygiene products. Triclosan inhibits an enoyl-ACP reductase of bacterial fatty acid biosynthesis. Recent studies showed *P. aeruginosa* contains two enoyl-ACP reductases known as FabI and FabK which are both resistant to triclosan. The enzyme Fabk is resistant to inhibitors that are designed to attack FabI (Heath and Rock, 2000).

Tetracycline is a bacteriostatic antibiotic and used to select mutants of multidrug resistance (Ana *et al.,* 1999) *P. aeruginosa* is resistant to tetracycline due to low permeability of the outer membrane of the bacteria. When cell is contacted by tetracycline, the strains of the bacteria pump the antibiotic out of the cell (Livermore, 1994). Overexpression or high mutation stress the strains of bacteria makes it multidrug resistant (Ana *et al.,*1999)

The objective of this study is to see if exposing an organism to tetracycline and to triclosan could select for resistance to tetracycline and triclosan. I will determine which different concentrations of tetracycline and triclosan can select for resistant strains of the bacteria, the minimum inhibitory concentration (MIC) and how quickly the resistance would develop.

MATERIALS AND METHODS

Triclosan used in the experiment was purchased from Sigma-Aldrich. First 1g of 97% triclosan was dissolved in 17.5ml of ethanol and 82.5ml of distilled water, and filter-sterilized (0.2um). Equal amounts (2.8ml) of each dilution were pipetted into each test tube containing 25ml of tryptic soy agar. Six different concentrations of triclosan were used ([1000.0],[100.0],[10.0], [1.0],[0.10],[0.010] ug/ml). A vortex machine was used to mix the contents. Each test tube was poured into a plate to be solidified. *P. aeruginosa* was purchased from Ward's Biology & Chemistry as freeze dried culture. Inoculation was used to culture bacteria, using 9ml of nutrient broth and a loop of the *P. aeruginosa*, which was then incubated for 24 hours. The spread method was used on plates by adding 0.3ml of *P. aeruginosa* in nutrient broth and the plates were incubated for 72 hours at 37 degrees.

A powder of tetracycline was purchased from Sigma. Then 0.5g was dissolved in 10ml distilled water and filter-sterilized (0.2um). Equal amounts (2.8ml) of each dilution were pipetted into each test tube containing 25ml of tryptic soy agar. Another 7 dilutions ([5000.0], [500.0], [50.0], [5.0], [0.5], [0.05], [0.005]ug/ml) were prepared, using the antibiotic tetracycline. A vortex machine was used to mix the contents. The spread method was used on plates by adding 0.3ml of a *P. aeruginosa* on the plates and the plates were incubated for 72 hours at 37 degrees.

The transferring method was performed. Bacteria from colonies growing on low concentration plates were transferred to new sterile plates whose [500.0ug/ml], in order to determine if any growth would be observed. This procedure was repeated, moving bacteria to plates of higher concentration, to determine at what concentration growth would be prevented.

RESULTS

Maximum concentrations of triclosan that still allowed growth on plates of *P. aeruginosa* were in all plates. There was an error in each plate because of the amount of triclosan that was in each plates was not enough to act on *P. aeruginosa*.

For tetracycline, the highest concentration that had *P. aeruginosa* growth contained 50.0ug/ml of tetracycline. The minimum inhibitory concentration, at which no bacteria were able to grow, was 500.0 ug/ml of the tetracycline.

Table 1. Table of Tetracycline of different concentrations, Growth/No Growth, ug/ml of Tetracycline in the plate of *P. aeruginosa.*

Plates	Growth/No Growth	Intended ug/ml of Tetracycline	Actual ug/ml of tetracycline
1	No Growth	5000.0	25.6
2	No Growth	500.0	2.56
3	Growth	50.0	0.256
4	Growth	5.0	0.0256
5	Growth	0.5	0.00256
6	Growth	0.05	0.000256
7	Growth	0.005	0.0000256

Table 2. Table of Triclosan of different concentrations, Growth/No Growth and ug/ml of tetracycline in the plate of *P. aeruginosa*

Plates	Growth/No Growth	Intended ug/ml of triclosan	Actual ug/ml of triclosan
1	Growth	1000.0	12.8
2	Growth	100.0	1.28
3	Growth	10.0	0.128
4	Growth	1.0	0.0128
5	Growth	0.10	0.00128
6	Growth	0.010	0.000128

DISCUSSION

P. aeruginosa was more resistant to triclosan than to tetracycline. The bacterium shows high resistance to triclosan because *P. aeruginosa* has both the FabI and FabK gene. The lowest concentration of tetracycline that could destroy the bacteria was 500.0ug/ml. The experimental findings may have been affected by errors in dissolving the solutions of triclosan and tetracycline

in water. The limits solubility of triclosan in water is 0.01g in 1000ml. The actual concentration for triclosan was 1000.0ug/ml, this make sense because much of triclosan was dissolved into a small amount of distilled water. In tetracycline the actual concentration was 5000.0ug/ml and limits solubility of tetracycline in water is 1g in 10ml of distilled water. In the tetracycline experiment, bacteria in the 50.0ug/ml solution seemed to have developed resistance, because when they were moved to new sterile plates that contain 50.0ug/ml concentration of tetracycline, from the 5.0ug/ml plate, they grew, although bacteria had not grown there originally. In other studies compare with this research, the MIC of triclosan was much higher and MIC of tetracycline, which 11 strains had lower concentration and 7 other strains has higher concentration than what was found in the result. In between 2.56ug/ml and 0.256ug/ml there maybe MIC could be found but this experiment was not taken farther to find the exact number of MIC in between these two concentrations. In other studies cross-resistance between triclosan and tetracycline in *P. aeruginosa* and found amino acid changes due to exposure to triclosan makes it cross-resistant to other antimicrobial agents (Karkhoff-haeizer, 2000).

This study could be refined in future studies by using limited concentration of triclosan and tetracycline in order to dissolve in water. Furthermore, mutation could be encouraged in the bacteria by exposing them to ultraviolet light. More genetic mutations in the bacterium might lead to a greater possibility of its developing resistance.

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