Abstract

Introduction

Mycobacteria are members of the order *Actinomycetales* and the only genus in the family *Mycobacteriaceae*. The distinguishing characteristics that are found in mycobacteria include acid-fastness and the presence of mycolic acids. Mycobacteria can be commonly classified as non-spore-forming, aerobic, slender rod-shaped, and are slow-growers. Natural reservoirs that mycobacteria can be found in are aquatic and terrestrial environments. Recent studies have shown that mycobacteria that can cause skin lesions, immune and/or pulmonary dysfunctions and chronic diseases, i.e., *Mycobacterium avium subsp. Paratuberculosis, M. kansasii,* and *M. xenopi,* can be isolated from common household tap water. Tap water can originate from surface water sources (i.e., lakes and rivers) or underground water sources (i.e., wells). Surface water sources are mainly a mix of rainwater run-off and artesian spring water. When rainwater falls onto an area of land occupied by mycobacteria-infected animals, it has the potential to harbor and carry mycobacteria and can survive within the rainwater runoff for long time (i.e., over a year). Studies have also shown that mycobacteria are able to thrive within water distribution systems due to factors biofilm formation, amoeba-associated lifestyle, and high-resistance towards chlorine. Chlorine is the most used decontaminate in domestic conventionally treated drinking water. Bacteriophage therapy is the practice of using bacteriophages, genetically diverse viruses that specifically infect and destroy bacteria hosts, to treat bacteria-based infections as an alternative to antibiotic-based treatment methods. The aim of this study is to determine whether mycobacteria can survive standard water treatment processes for long periods of time with the addition of both chlorine and mycobacteriophages.

Materials and Methods

Different strains of bacteria were used to carry out this study. Bacterial strains included, One strain of *M. smegmatis* (*M. smegmatis mc^{2}155*) and One Strain of *B.subtilis* (MTU Strain Collection). The survivability rate of bacteria was tested by exposing bacteria to sterile double-distilled water with different chlorine concentrations of 0mg/L, 0.5mg/L, 1mg/L and 2mg/L with/without the addition of bacteriophage “MKC-IRE” for periods of 12, 24, 48, and 72 hours at

temperatures of 20°C and 37°C. After the desired contact time had elapsed, 100µL of solution was taken and a 1-in-10 dilution series was carried out in 900 µL of ringers solution. 10 µL of dilution series log was then placed onto BHI agar and incubated at 37°C. *M. smegmatis* was incubated for a period of 48 hours at 37°C. *B.subtilis* was incubated for a period of 24 hours at 37°C. Colony counts were performed after time duration. *B.subtilis* was used as a biological control in order to ensure that chlorine concentrations were effective.

Results

The data showed that when initial inoculum levels were high 10 $\log^6$ CFU/ml^{-1}, *M. smegmatis* strain had a high survivability rate in all chlorine concentration samples, temperatures and contact times applied. *B. subtilis* was shown to been able to survive at both temperatures and all time intervals in the 0mg/L chlorine concentration. However, *B. subtilis* was only able to survive up to 24 hours within the 0.5mg/L, with a CFU concentration of 4.5 x 10^2 CFU/ml^{-1} at 20°C and 6.6 x 10^1 CFU/ml^{-1} at 37°C. *B. subtilis* was shown then to be unable to survive in further chlorine concentrations at both temperatures and contact times. *M. smegmatis* with the addition of bacteriophage "MKC-IRE" was shown to have a log reduction rate of 10^2 CFU/ml^{-1} in 0mg/L water at 37°C after 72 hours and a reduction rate of 10^1 CFU/ml^{-1} at 20°C after 72 hours. The greatest log (10) reduction in CFU in the chlorinated water samples were seen in the 2mg/L water solution, with a log (10) reduction of 10^4 CFU/ml^{-1} after 48 hours at 37°C.

Conclusion

Based off the results gathered, M. smegmatis had the lowest survivability rate when exposed to 2mg/L of chlorine with the addition of bacteriophage "MKC-IRE" after 48 hours at a temperature of 37°C. Results also showed that bacteriophage and chlorine had a higher efficacy at a higher temperature, in this case 37°C. Further research could be done to see the total bactericidal effects of other bacteriophages on *M. smegmatis* at different chlorine concentrations and the survivability rates of other types of mycobacteria.