Synergy Effect of Polymyxin B (PMB) on the Bacteriophage 201 Phi2-1 Infection in *Pseudomonas Chlororaphis*

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ABSTRACT

If the antibiotic alone does not kill bacteria, is there another way to treat humans with pathogen infection? Maybe we can try antibiotic with something else. How about phage therapy? If we apply the phage to the bacteria in the presence of antibiotic to the patient, we have two possible conclusions: the antiobiotic will either promote the ability of the phage to enter the bacteria and aid it in killing the bacteria, or it will prevent it from doing so. Therefore, I performed an experiment to find out whether or not the phage therapy along with antibiotic treatment will be more efficient in treating the bacteria. This experiment consisted of many parts such as streaking out *Pseudomonas Chlororaphis*, phage titering, Minimal Inhibitory Concentration (MIC) testing, Growth Curve Determination, Introduction of the drug and phage into cells, and lastly the effect of Polymyxin B (PMB) on the phage infection. After performing these multiple parts of the experiment, I was able to come to one of the two possible conclusions: antiobiotic will be very useful in promoting the phage replication in the bacteria and possibly destroying it. Now, instead of treating the bacteria in patients with antibiotics alone, the doctors could treat the bacteria with antibiotic along with phage therapy.

Streaking out Pseudomonas Chlororaphis

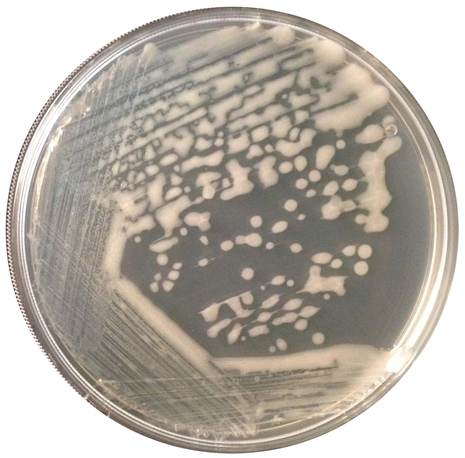

Figure 1: Streaking out PC. Take the hard agar (HA) dish and warm it up in a 30°C incubator. Take a scrip off of the frozen stock and place it on the HA plate. Scrape the frozen stock on area one of the HA plate. Using a new stick, scrape out 1/4th of the first area into the second area and scrape all throughout the second area. Using a new stick, scrape out 1/4th of the second area into the third area and scrape all throughout the third area. Using a new stick, scrape out 1/4th of the third area into the fourth area and scrape all throughout the fourth are. Close the HA dish and place upside down in a 30°C incubator.
Result: The rate of bacteria growth (colonies) decreases as it moves down to area 2,3, and 4. Therefore, a single colony can be picked from the fourth area for later experiments.

Phage titering

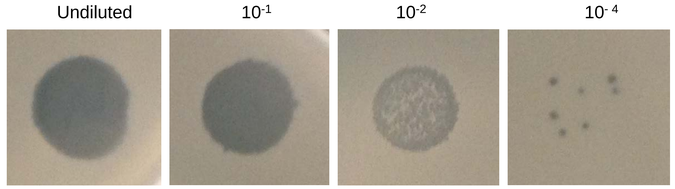

Figure 2: Phage titering. Spot plate titer. For PC's phage, use HA. For PA's phage, use LB. Grow PA at 30°C 300 RPM in 10 ml LB plain/125ml flask. Grow the host (PC) overnight at 30°C in 3 ml HA plain then melt 0.35% top agar(LB or HA depending on organism) in microwave for 3 min. Transfer 4 ml of top agar into small test tube and add 100µl over night culture into the top agar. Mix gently and pour over surface of LB or HA agar plate and swirl until it gets covered. Let agar solidify for five minutes and spot 5 µl of each dilution on phage to the agar corresponding to the indicated square. Let sit until the spot is absorbed for ~ 15 mins. Incubate at 30°C over night (not upside down).
Formula: (PFU/Infection Volume) x (1000µl/1ml) x (dilution factor)
(7pfu/5µl) x (1000µl/1µl) x (1/10^{-4})
Result: 1.4x10^{7}pfu/ml

Minimal Inhibitory Concentration Test (Micro dilution Method

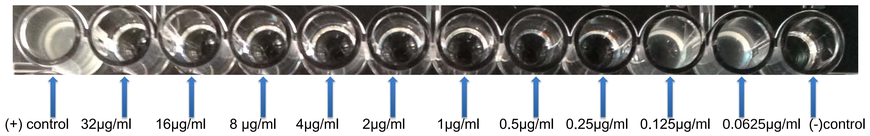

Figure 3: MIC test. Dilute the overnight culture 1/100 into 100µl/10ml and place it into the incubator at 30°C and measure the optical density (OD) constantly every hour until it reaches 0.2. To set up the MIC plate, take out a reagent reservoir and put ~25 ml of the media in it. Using the multi pipetter tips 1-12 except 2, pipette 100µl into each row of the wells. Next, put in 200µl of the highest antibiotic concentration into column 2. Using the 20-200µl draw 100µl out of column 2 and bring it in the 3rd column and mix. Continue diluting until column 11. Take the OD and pour 2ml in the media well and pipette 1µl into every single well except for column 12. Finally, put the cap on the MIC and tape the MIC into the shaker.
Result: According to the data represented above, the MIC is at 0.25 because it is the lowest concentration that kills the cell.

Growth Curve Determination Results

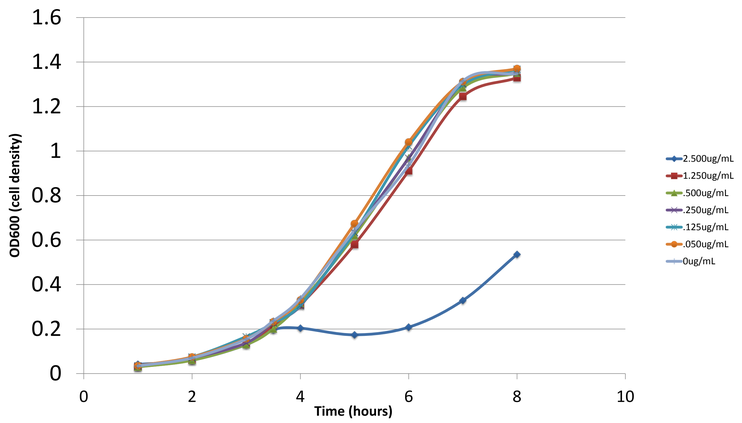

Figure 4: Growth Curve Results. Take 10 ml of HA broth and place it into 125 ml flask. Pick one single colony and put it into the 10ml broth. Incubate the falst at 30°C overnight in water bath shaking. Dilute out 1/100 of the overnight culture stock by taking 250µl of the overnight culture and putting it into 25ml of new HA medium. Incubate shaking in water bath at 30°C and take 1 ml of the culture to measure the OD_{600} every hours.
Result: Concentration of the drug at 2.500µg/ml effects the growth rate of P. Chlororaphis compared to the control while at the lower concentration of the drug does not effect the growth rate of the bacteria.

Introduction of the drug and phage into the cells

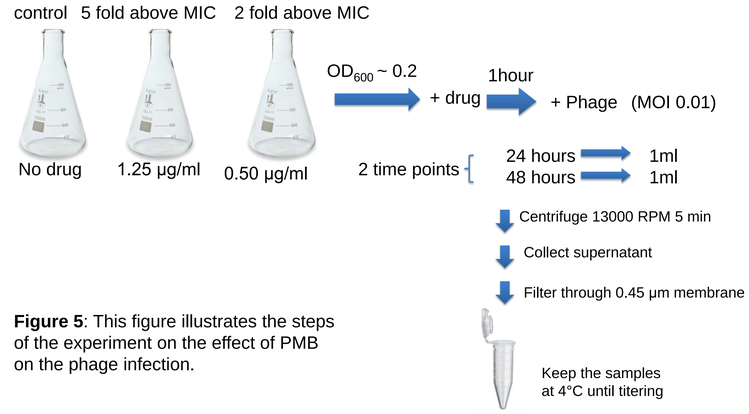

Figure 5: This figure illustrates the steps of the experiment on the effect of PMB on the phage infection.

Effect of PMB on the phage infection

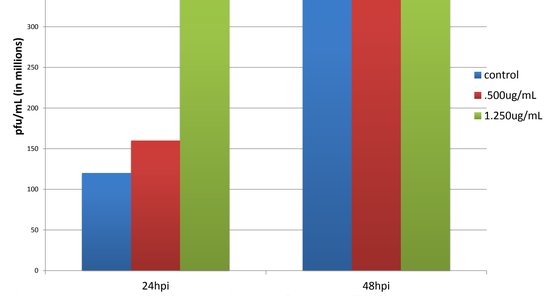

Figure 6: This figure illustrates the results of figure 5 above. To get these results, take 3 flasks and label each one. Label the first one as the "control" the second one as "5 fold above MIC" and the third one as "2 fold above MIC". In other words, the first flask should contain no drug, the second flask should contain 1.25µg/ml, and the third flask should contain 0.50µg/ml. Then, place all three flasks into the shaking water bath at 30°C and measure the OD_{600} of each one every hour until it reaches 0.2. Once the OD is at 0.2, add the drug. After an hour, add the phage at MOI 0.01. Take 1 ml at 24 hours from each flask, centrifuge 13000 RPM 5 min, and collect supernatant. Then, filter through 0.45 µm membrane and keep at 4°C. After 48 hours repeat the same steps that were done for the 24 hour time period.
Result: The concentration of the phage obviously increased around 3.0 fold in the presence of the drug at 1.250µg/ml and around 1.33 fold in the presence of the drug at .500µg/ml at 24 HPI. At 48 HPI, it does not make much difference among the samples.

CONCLUSIONS AND FUTURE DIRECTIONS

- When Pseudomonas Chlororaphis (PC) is treated with Polymyxin B (PMB) the phage can infect the bacteria more efficiently. Therefore, it is possible that the PMB drug can promote the infection. According to this study, phage therapy along with antibiotics could be very useful to treat patients in the near future.

References

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Characterization of Pseudomonas chlororaphis myovirus 201varphi2-1 via genomic sequencing, mass spectrometry, and electron microscopy. Thomas JA, et. al., Virology. 2008