Abstract

Species of *Aeromonas* are pathogenic prokaryotes that invoke a wide range of symptoms in humans as well as other animals. The ability to penetrate these s-layer producing strains would help lead the pathway to treating diseases caused by *Aeromonas* in a sustainable manner.

Predation assays were used to evaluate the predation effects of *Aeromonas* by *Myxococcus xanthus*. Diluted colony plates were analyzed to see the colony forming units on the predation spot compared to the prey spot. The predation assays seen by *M. xanthus* and *Aeromonas* show that the *Aeromonas* strain with the s-layer was more suspectable to predation by the *M.xanthus* strain

There was a reduction in the colonies that formed compared to the control and the predation spots as expected. There was a greater decrease in the variable strain compared to the other strains. The s-layer strain had the least number of colonies from the predation assay. The next experimental steps would be to insert the s-layer gene into a strain of *Aeromonas* that does not produce the s-layer in order to properly evaluate the effects of the s-layer in *Myxococcus xanthus* predation.

Background

Species of *Aeromonas* are Gram-negative, non-spore-forming, rod-shaped, facultatively anaerobic bacteria that occur ubiquitously in aquatic environments. A. salmonicida is a fish pathogen and has not been associated with human infection. By contrast, the mesophilic species like A. Hydrophila, have been associated with a wide range of infections in humans. The mesophilic aeromonads have been commonly isolated from patients with gastroenteritis although their role in disease causation remains unclear. They are also associated with sepsis and wounds, and with eye, respiratory tract, and other systemic infections.

In a few instances, S-layers have been shown to be virulence factors on pathogens. A S-layer is a paracrystalline protein thin layer attached to the outermost portion of the cell wall of some species of Aeromonas. Three species of Aeromonas were chosen to evaluate the protective nature of the S-layer using a predatory strain of Myxococcus xathus (Dk1622). The fruiting bodies produced by the M.xanthus during nutrient starvation would scavenge the environment for prey species to feed upon. The preys were strains of Aeromonas: Ahy1127 (A.hydrophila), ATCC 7966 (A.hydrophila), and Asa1287 (A.salmonicida). The strains of Aeromonas produced the s-layer, did not produce the s-layer, and did not encode for the s-layer but phenotypically was variable for the presence of the s-layer, respectively.

Method

A streaked colony of mycocccus xanthus was inoculated in a 250 ml flask. The strain was grown at 32C in 12.8ml of CTTYE broth with shaking for 24 hours. Simultaneously, the strains of Aeromonas were streaked out and inoculated in a culture tube with 10ml of Brain Heart Infusion broth at 37C for 24 hours. After the 24 hours were completed, 2ml of the myxo culture was put in a new 250ml flask and 48ml of CTTYE broth was added and shaken at 32c for 24 hours. The Aeromonas strain was centrifuged for 10 min at 7000 rcf and the supernatatnt was taken out. 10 mL CFL broth was added to the pellet and vortexes until mixed. The strains of Aeromonas were again incubated in 37c for 24hours. The optical density was measured for each culture to ensure that that it was less than 1. 3 sections were created to spot the prey, predator, and the prey-predator assay. Myxo was the predatory strain, one of the Aeromonas strain was put into each cfl plate, and the chosen Aeromonas strain with the myxo strain

was spotted 1mm apart from each other. Pictures were taken with an imaging microscope to determine the progress of the predation assay during 48 hours and 72 hours. Series dilutions were made of the prey spot and the predation spot of myxo and one of the strains of Aeromonas using mc7 from 10-1 to 10-5. The dilutions were plated on LB agar and incubated for 24 hours. The colonies were counted to calculate the Colony forming units per mL.

Results

The Aeromonas strain with the positive for the S-layer was more suspectible to predation by the myxococcus. The myxo was less aggressive in preying upon on the negative strain. The variable strain was preyed upon more than the negatuve strain but less than the positive strain. All strains showed a decrease in the colony forming units per mL compared to the control with just the prey and the predation assay with the prey and the predation strain. The greatest decrease from the control compared to the predation was the variable strain. The least difference was the strain that was negative for the s-layer.

Conclusion

The initial hypothesis was that the thin peptiglycan layer outside of the Aeromonas strains would protect the strain from predation but that was not supported. Certain receptors embedded in the s-layer would signal the myxococcus to prey upon the species compared to the strain that was negative for the s-layer. The next steps to further test would be to insert the positive s-layer gene into a negative Aeromonas strain in order to further assess the suspectivlibity of the s-layer in myxococcus predation. Another possible experiment would be to test the capsule layer in Klebisella to see if the species is more suspectible to predation by myxo.