NOVA SOUTHEASTERN UNIVERSITY
Farquhar College of Arts and Sciences
Division of Math, Science, and Technology

Research updates – NSU/Acme collaboration

October 2014

Bioven project

The major goal of this project is to characterize the molecular mechanism(s) by which Bioven reduces the severity and length of viral infections within human subjects. As a start to better elucidating this mechanism, an *in vitro* cell culture system was established that allowed for infection with different lytic viruses. The first virus that was tested in this new system was herpes simplex virus type 1 (HSV-1) due to the fact that Bioven has been shown to be effective against HSV-1 in human subjects.

The first question that was asked was whether or not Bioven worked in a manner similar to that of interferon. Interferons are small proteins that bind to receptors on host cells and help them establish an anti-viral state in preparation for exposure to virus. When one pre-treats cells with interferon, they become almost completely resistant to subsequent infection with virus. To test whether Bioven exhibits similar ability, Vero cells were pre-treated with different amounts of Bioven overnight prior to being infected with both low and high levels of HSV-1. In all cases, the Bioven failed to protect the cells and all were readily infected by HSV-1.

The next question that was asked was whether or not Bioven stops HSV-1 from being released and spreading to adjacent cells. To test this, HSV-1 was allowed to infect untreated Vero cells for 1 hour and then different amounts of Bioven were placed on the cells after the initial infection. If Bioven restricts viral spread, we would have expected to see smaller viral plaques to form (HSV-1 infects a single cell and then spreads to its immediate neighbors. That clump of infected cells die off, leaving a gap in the cell layer. This is called a plaque and is observable after staining). The results conclusively showed that Bioven failed to inhibit plaque formation to any measurable degree, which indicates it does not significantly stop HSV-1 from spreading cell-to-cell.

This led us to ask the question as to whether Bioven has the ability to directly destroy the HSV-1 viral particles themselves. We mixed various concentrations of Bioven with HSV-1 and let them sit overnight in a warm incubator. The viral particles were then used to infect Vero cells. Amazingly, we found that using just 1 µL of Bioven was enough to lower the infectivity of the HSV-1 by 1000-fold. In other words, untreated HSV-1 has a 1,000-fold better ability to infect Vero cells than viral particles given just 1 µL of Bioven (it was final of 1:200 dilution of the Bioven → 1 µL + 199 µL HSV). When greater amounts of Bioven were added (e.g. 5 µL), nearly 100% of the viral particles were destroyed.

We are in the process of better characterizing how Bioven destroys HSV-1 and to see if it is capable of destroying other types of viruses in the same way.

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Chtinase project

The overall goal of this project is to isolate and identify aquatic bacteria capable of secreting chitinase enzymes and then to purify/concentrate those chitinases for potential use as a novel and all natural insecticide. In addition, chitinase enzymes have potential use as tool to safely breakdown chitin-containing industrial waste (e.g. crab shells, shrimp tails).

The project began around a small pond in Ann Kolb Nature Center in Hollywood, FL. Students took samples of the water and attempted to isolate chitinase-secreting bacteria by spreading the pond water on agar dishes that contained only chitin as an energy source. They were fortunate in that they quickly isolated a single type of bacterium that seemingly had the ability to make chitinases. We isolated DNA from this organism and had it partially sequenced. After plugging in the sequence into a computer search tool, we were able to identify our bacterium as *Shewanella baltica* (a known aquatic bacterium). Interestingly, no one has ever demonstrated that this organism secretes chitinases.

After some time of unsuccessfully quantitating the levels of chitinase secreted by our organism or characterizing the types of chitinases being released, we have recently made some strong headway. Students have recently developed a working assay to quantitate our chitinases and they determined that S. baltica primarily secretes chitobiosidase-type chitinases.

We are now in the process of mass producing, concentrating, and purifying our chitinases. We expect to be assessing its potential as a pesticide within the next several months.

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