Here's the Dish: Epitheliocystis and Chlamydia in Fish

Angelina Cooper and Wilmore C. Webley

Dept. of Microbiology, University of Massachusetts Amherst, M.A.

Abstract

The demand for aquatic food products has increased significantly over the past decade, with global aquaculture production attained 90.4 million tons in 2012, generating an incomes US$ 144.4 billion, and the production of food fish was 66.6 million tons. This has lead to a boom in aquaculture farms around the world. Epitheliocystis is a serious skin and gill disease in fish, that is often lethal, leading to millions of dollars lost each year. While the disease is believed to be caused by pathogenic intracellular bacteria in the parachlamydiaceae family, empirical data and direct evidence relating to the etiology, treatments and epidemiology remain limited, highlighting the need for more work to better characterize this disease across the different hosts and locales affected.

We hypothesize that *Chlamydia*-like organisms are an important etiologic agent of epitheliocystis. We believe that a better understanding of disease transmission, mechanism of disease process and host range will provide a basis for preventing this emerging disease in aquaculture. However, chlamydia-like organisms are problematic to grow and maintain. The specific objectives of my summer project was to utilize a tissue culture model to grow known, closely related Chlamydiaceae organisms in the lab to be later used in experimental epitheliocystis studies.

I successfully cultivated and propagated HeLa (human epithelial cells) and RTg1 (Rainbow Trout Gill Epithelial cells) as substrates for generating progeny chlamydia-like organisms. I then cultivated S*imkania negevensis* and *Waddlia chondrophila*, chlamydia-like organisms to be used as models for epitheliocystis. Our data shows that we can grow these bacteria in tissue culture and we are waiting to confirm that the gill epithelial cells support their growth effectively.

Background

Bacterial disease costs animal producers as well as consumers billions of dollars annually, when the animals that become infected grow poorly or die. Aquaculture provides about 50% of seafood for global human consumption, and epitheliocystis threatens the industry, affecting over 90 species of fish, including salmon and trout [1]. Systematic studies of epitheliocystis in specific fish species are currently lacking and there are extensive gaps in our basic knowledge of the pathogenesis of this disease and the factors that may influence it [1, 2].

Figure 1: Epitheliocystis with gill tumors and fusion

The pathophysiology is however, clear – as a result of the swelling of the cells of the gills and the increase in secreted mucus around the heavily infected gills, fish can become lethargic and show respiratory distress, leading to death. An aquaculture farm can therefore lose an entire hatchery in a short time. Commercial fish farms require a steady, predictable source of juveniles from hatcheries in order to stay in operation and provide a consistent product.

Preliminary research shows that the causative agent of epitheliocystis replicates intracellularly in the cysts and, since 1969 epitheliocystis has been associated with *Chlamydia*-like bacteria, based on the ultrastructural characteristics of the content of the cysts [3]. Attempts to definitively identify the causative agent using modern molecular and serological tools have not been successful, and results are often inconsistent. Chlamydiaceae are obligate intracellular bacteria that are very hard to grow, further hampering efforts to confirm the etiology. However, the electron microscopic morphology of the inclusion bodies in eptheliocystis fish gills are identical to those of human and animal chlamydial infections (Figure 2). The current study seeks to confirm the sitology through cultivation of the causative agent and confirmation of disease in a zebrafish model.

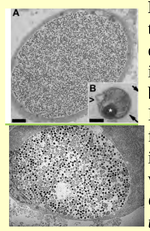

Figure 2: top – epitheliocyst is inclusion; bottom – Inclusion from infection with *Chlamydia trachomatis*

Methods

Cultivation of Cells : HeLa cells were first cultured in 25cm and 75cm culture flasks to expand and store cells for future experiments. Cells were cultured in RPMI/DMEM minimal growth media , supplemented with 10% Fetal Bovine Serum (FBS). These cells were grown at 37^{0}C in 5% CO_2 and humidity.

Splitting and freezing cells: When flasks reached approximately 95% confluence, they were trypsinized to lift the cells, which are adherent to the surface of the flask. Cells were then centrifuged and resuspended in media with FBS, neutralizing the activity of the trypsin. Cells were further propagated to increase their numbers and aliquots resuspended and frozen in a DMSO/ growth media/FBS solution at -80^{0}C.

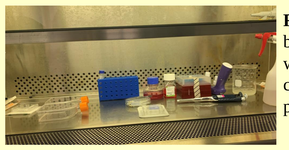

Figure 3: Inside biosafety cabinet where tissue culture work takes place

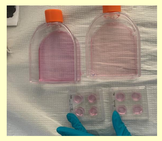

Figure 4: Picture of tissue culture flasks and 4 –well plates containing media and cells

Growth of Chlamydia-like organisms: S*imkania negevensis and Waddlia chondrophila*, Chlamydia-like organisms were obtained from the American Type Culture Collection (ATCC). Since they are obligate intracellular microbes, there were grown on a HeLa cell substrate of cells that were allowed to reach 80% confluence in T-25 flasks. The infection was incubated at 37^{0}C in 5% CO_2 and allowed to grow for 72 – 96h.

Simkania and **Waddlia** progeny were used to infect 12mm coverslips in 4-well plates that had HeLa cells grown to 80% confluence. The infection was allowed to run for 48h, after which cells were fixed with cold methanol for 10 minutes.

Cultivating gill epithelial cells: I also cultivated a model cell line of Rainbow trout gill epithelial cells (RTgill1). These were grown at just below room temperature with no CO_2 or humidity in a specialized medium (L15). The goal is to determine if these fish cells can sustain an infection by our model organisms.

Results

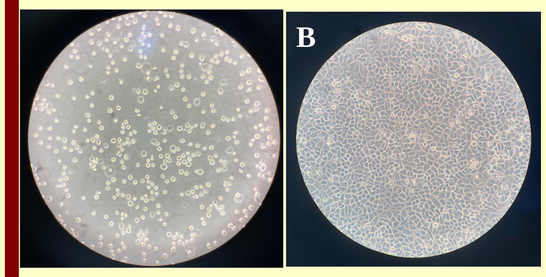

Figure 5: Representative image of non-confluent and confluent HeLa cell monolayers being grown for freeze-down and infection. Cells in A were just starting to attach and grow while those in panel B had become confluent over a 36h period and are ready to be split.

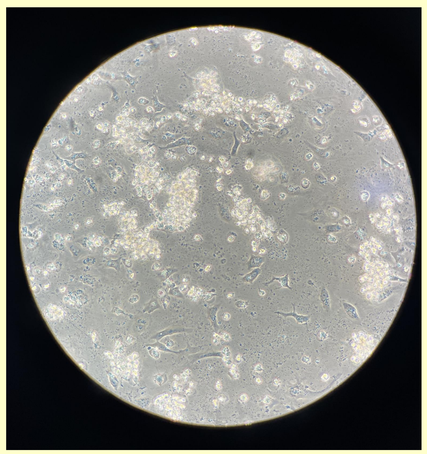

Figure 6: cells infected with Waddlia organisms showing cells death pathology after approximately 60h

Results

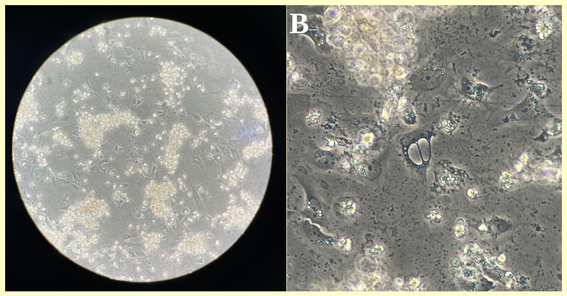

Figure 7: Panel A shows cells that were infected with **Simkania** for 72h. The cells are showing typical infection pathology of death and detachment from the flask surface. Panel B shows a zoomed field of two cells displaying large inclusion vacuoles confirming that the cells indeed harbor these para-Chlamydiaceae organism.

Figure 8: RTgill1 cells at about 70% confluence without infection. Infection would take place after these cells reached a higher confluence.

References

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2. Bradley TM, C. E. Newcomer, and K. O. Maxwell.: **Epitheliocystis associated with massive mortalities of cultured lake trout Salvelinus namaycush**. *Dis Aquat Org* 1988, **4**:9-17.
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Contacts

Angelina Cooper – angelinacoop@umass.edu

Wilmore Webley – wilmore@umass.edu