Novel Immune Booster for Fish

(OTT ID 1222)

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Fish Husbandry and Disease: Problems

Problems:

- Few compounds are available for disease treatment and prevention in aquatic organisms
- Lack of vaccines and well worked out prophylaxis regimens for pet fish
- Currently only four compounds are approved by the FDA for use in aquatic species and the use is extremely limited in terms of species, indication, and route of administration
- Once fish disease is recognized it is often too late for effective treatment
- Lack of data showing efficacy for commercially available fish immune boosting products

http://www.exoticpetvet.com/breeds/fish6.htm

Fish Husbandry and Disease: Solution

Solution:

- The inventors have demonstrated that the use of small cyclic sugar molecules known as cyclodextrins, stimulate increases in several inflammatory cytokines to aid in fighting infection
- The inventors have shown that cyclodextrins protect fish larvae in an *in vivo* bacterial infection challenge model
- Treatment with these molecules is inexpensive and safe
- Cyclodextrins are already approved by the FDA for use in food and pharmaceuticals
- The supporting data shows that this protection is more effective than competitive products on the market
- This product is stable, highly soluble, and easy to use and can be applied through multiple methods including use directly in the water, in fish food, or in vaccine adjuvants

Market Potential

Applications in Fish Husbandry

- Treatment of infection in ornamental fish and in aquaculture
- Prevention of infection by boosting the immune system in ornamental fish and aquaculture
  - Addition to the water prior to stressful activities such as water changes, sorting, handling or transport
- Can be used directly in water (for recreational aquariums), as a food additive, or in vaccine adjuvants during immunization

Market

- The entire ornamental fish industry has been estimated to be worth around $15 billion and the market shows an annual growth rate of 8%
- The largest importer of ornamental fish is the USA with over $500 million worth of ornamental fish imported each year followed by Europe and Japan
- It is reported that 30-50% of new aquarists leave the hobby each year out of frustration due to fish disease and death
- Nearly 50% of the fish consumed in the U.S. is farmed with the annual market for all aquaculture in the U.S. at approximately $1 billion
- In 2006, world aquaculture had a value of almost $80 billion

*UNEP-World Conservation Monitoring Centre; www.fishchannel.com; www.thefishsite.com; Agricultural Marketing Resource Center, Food and Agricultural Organization of the United Nations

Road to Commercialization: Next Steps

- **U.S. Patent Application US 20140086961 A1**

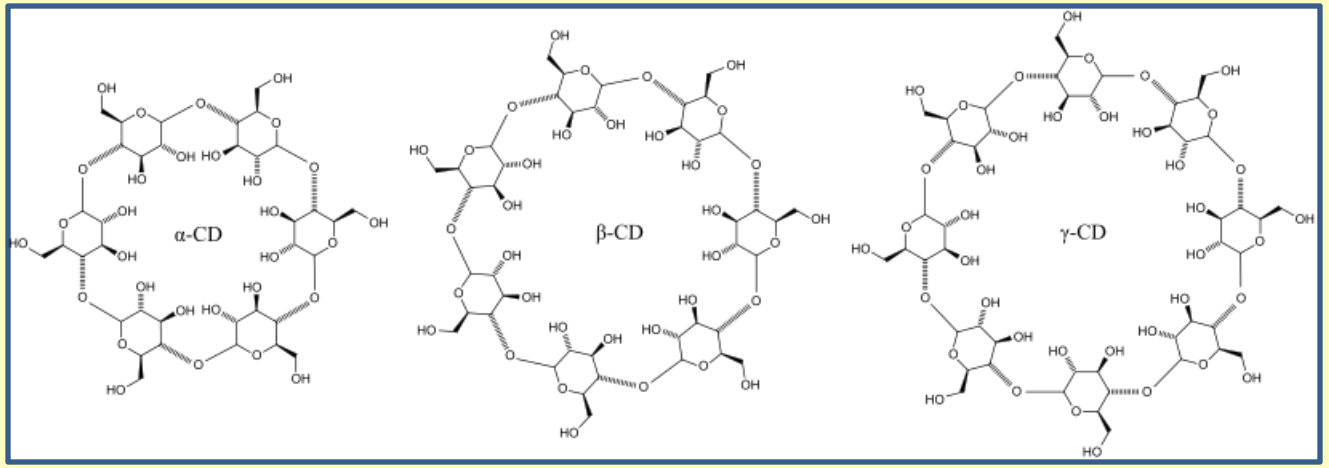
- **Looking for a development partner to:**
  - **Collaborate in fish trials in ornamental species using cycloD in water or food**
  - **Collaborate in fish trials with cycloD as a vaccine adjuvant**
  - **Product development for aquaculture or ornamental fish**
  - **Obtain regulatory approval as needed**

Why Partner with UWMRF?

- This novel immune boosting technology is in excellent alignment with the needs of the fish pharmaceutical, aquaculture, and aquarist communities
- This technology provides an excellent opportunity to expand further into the fish prophylactic and disease treatment market
- The compounds are safe and relatively inexpensive
- The overuse of antibiotics has the public looking for safer alternatives to disease treatment
- UWMRF flexibility in licensing terms

What are Cyclodextrins (CycloD)?

- Cyclodextrins are cyclic sugar molecules produced from starch
- Cyclodextrins are currently used in a wide range of applications due to their ability to form stable complexes with hydrophobic molecules
- The alpha, beta, and gamma forms are all generally recognized as safe by the FDA
- Since the 1970's many applications have been explored using encapsulation by cyclodextrins and their derivatives in both pharmaceutical and industrial settings

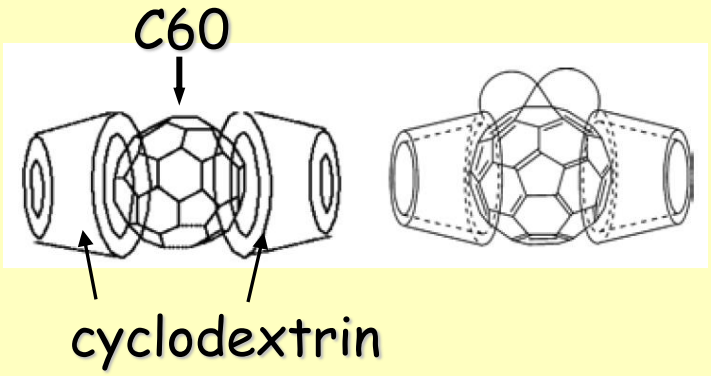


The Fish Immune System

- The two arms of the vertebrate immune system include the innate and the adaptive system
- The innate system is the first to respond to pathogens, and while it is a more general response, it can be a powerful and quick block to infection
- The adaptive immune response involves the production of specific antibodies towards a foreign pathogen, but this system can take days to become effective
- The focus of this technology is to charge the innate immune system in the event of an impending infection or to be able to address an infection more intensely
- Many fish diet products are available which claim an immune boosting function, but there is little data to support the efficacy in protecting against disease

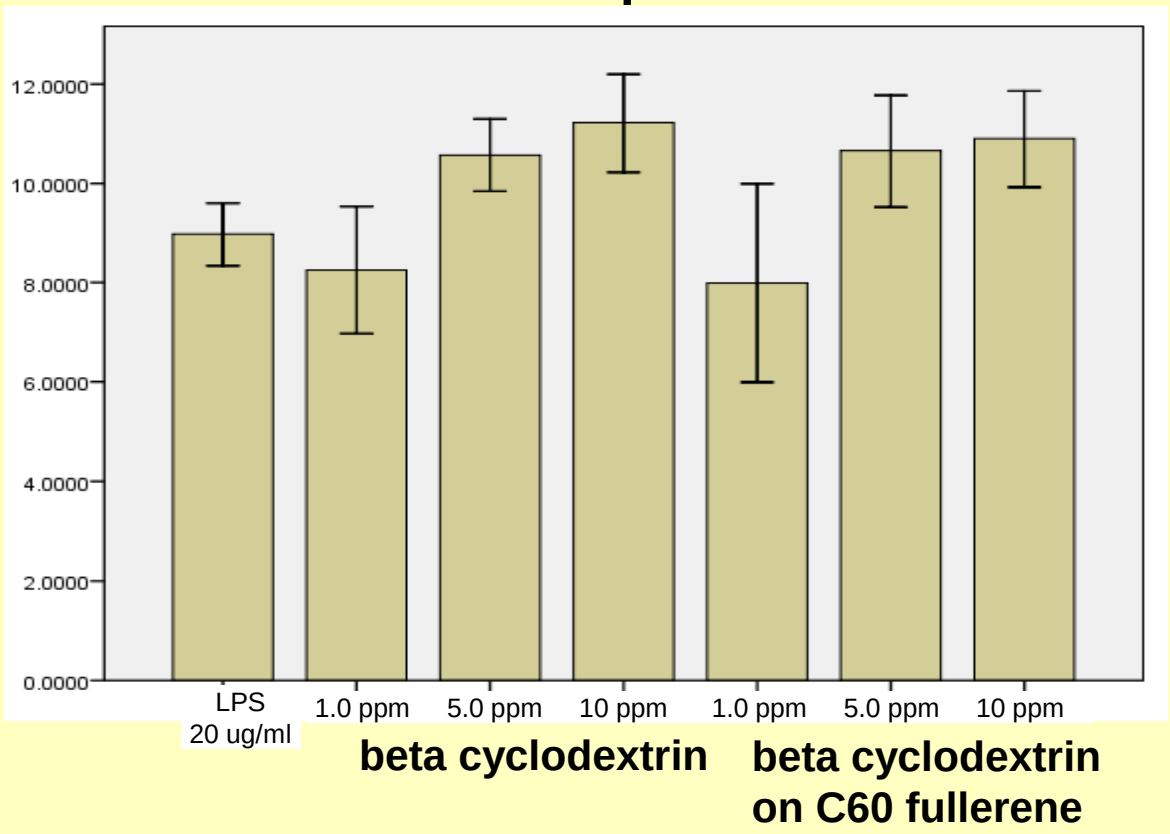
Primary Trout Macrophage Culture System

- In this system, the expression of immunity genes can be monitored upon stimulation of the with various compounds
- Genes such as Interleukin 1B (IL-1B) and tissue necrosis factor (TNF) can be assayed as a measure of the degree of inflammatory stimulation and thus activation of the innate immune system
- In a nanoparticle screen, the inventors discovered that a fullerene (C60) attached to beta cyclodextrin potently induced IL-1B expresssion



C60 fullerene bound to cyclodextrin noncovalently (left) and covalently (right)

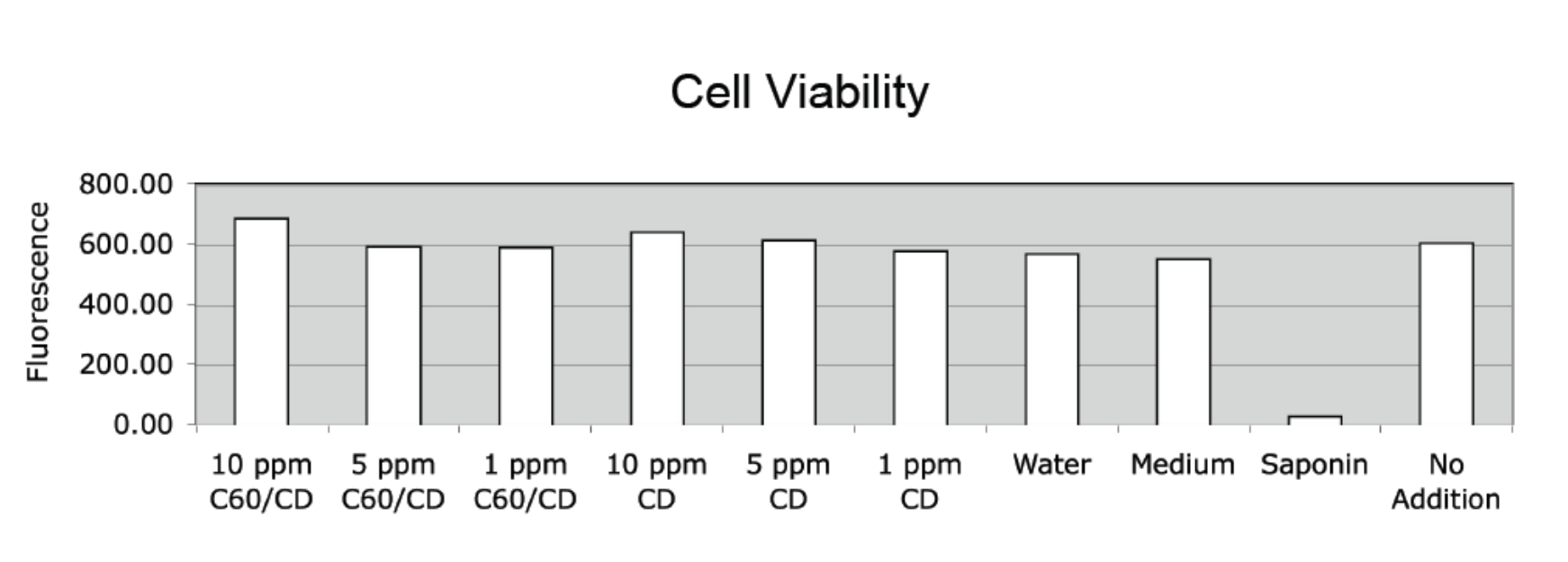
Beta cycloD is a potent inducer of IL-1B expression



*LPS is a positive stimulatory control. Macrophages were isolated from head kidneys of rainbow trout, plated and then stimulated with the preparations for 24 hours. RNA was extracted from cells and analyzed by QPCR for IL-1B expression. Data are means +/- SD for 3 independent trials.

- **Beta cycloD in the absence of C60 fullerene also induced IL-1B expression similar to the bacterial LPS positive control**

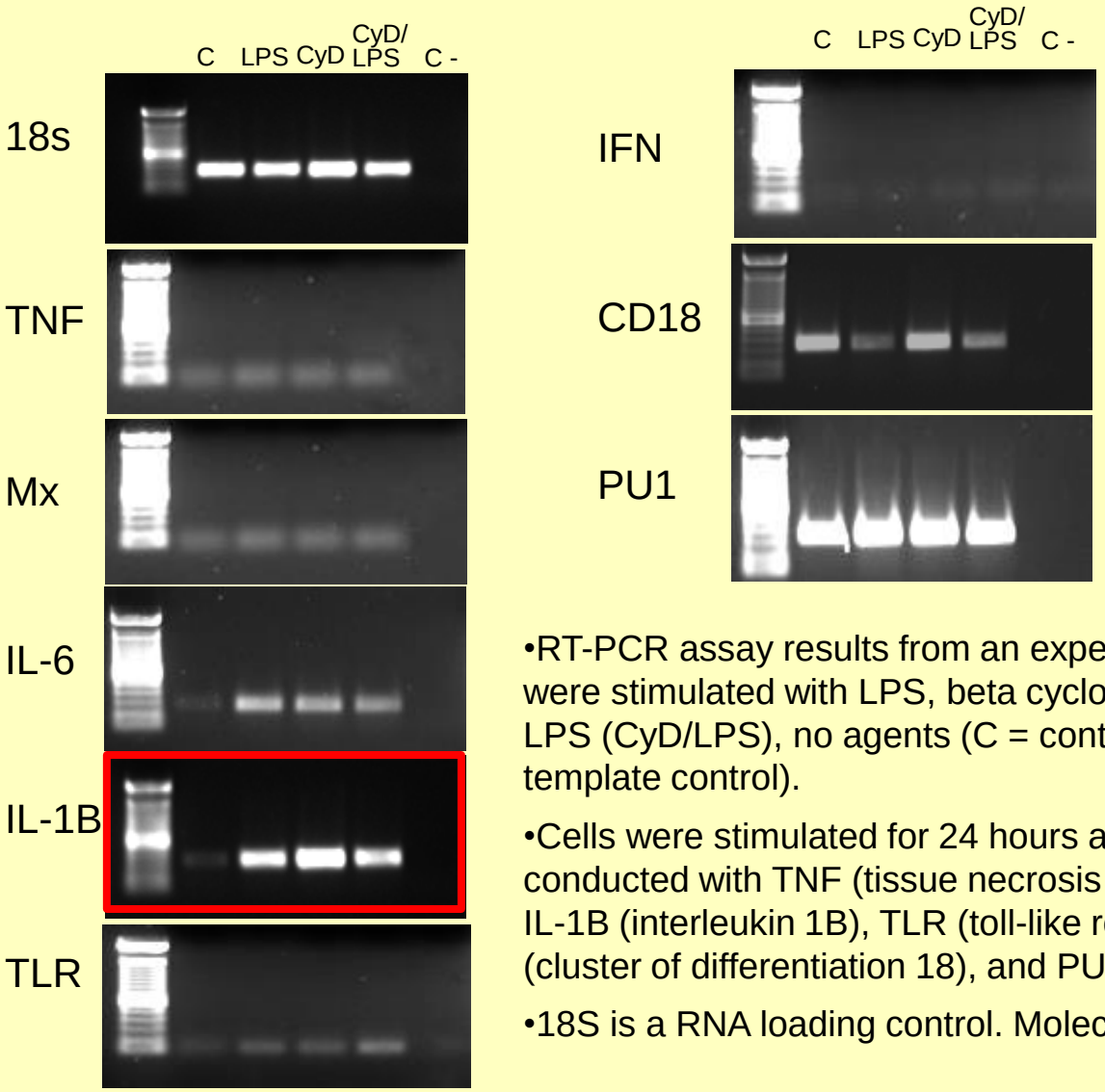
Beta cycloD is not toxic to cells



*Cells were assayed using the QBlue Cell Viability Assay Kit (BioChain); Saponin is a positive control for cell death

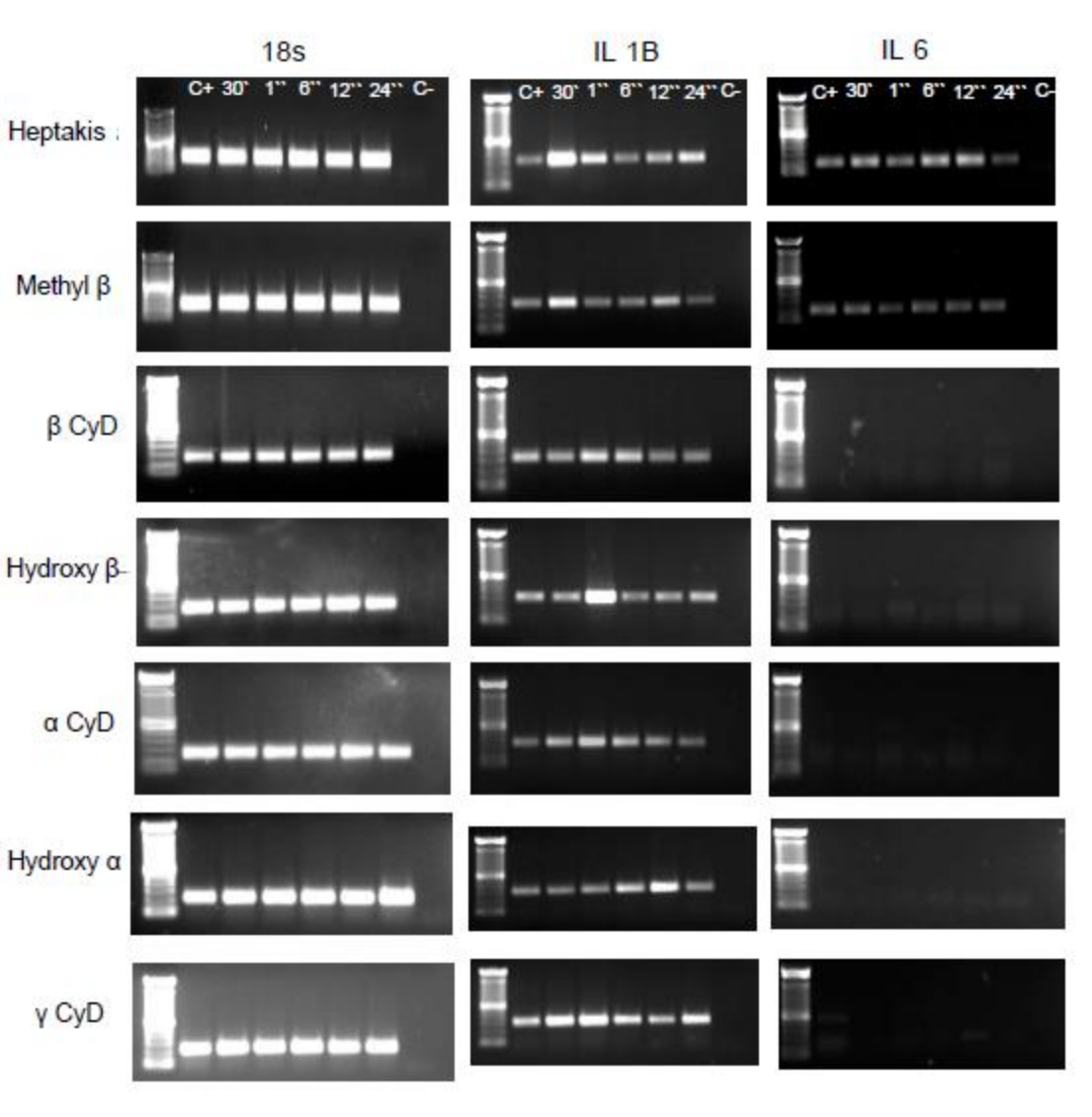
- **Application of either cycloD attached to C60 or cycloD alone did not lead to a loss in cell viability**

Beta cycloD specifically induces IL-1B pro-inflammatory gene expression



- RT-PCR assay results from an experiment in which trout macrophages were stimulated with LPS, beta cyclodextrin (CyD), beta cyclodextrin and LPS (CyD/LPS), no agents (C = control) or no DNA template (C- = no template control).
- Cells were stimulated for 24 hours and RNA extracted and RT-PCR conducted with TNF (tissue necrosis factor), Mx protein, IL-6 (interleukin 6), IL-1B (interleukin 1B), TLR (toll-like receptor), IFN (interferon), CD18 (cluster of differentiation 18), and PU1(transcription factor PU1).
- 18S is a RNA loading control. Molecular weight standard in first lane.

Several forms of cyclodextrins induce cytokine gene expression



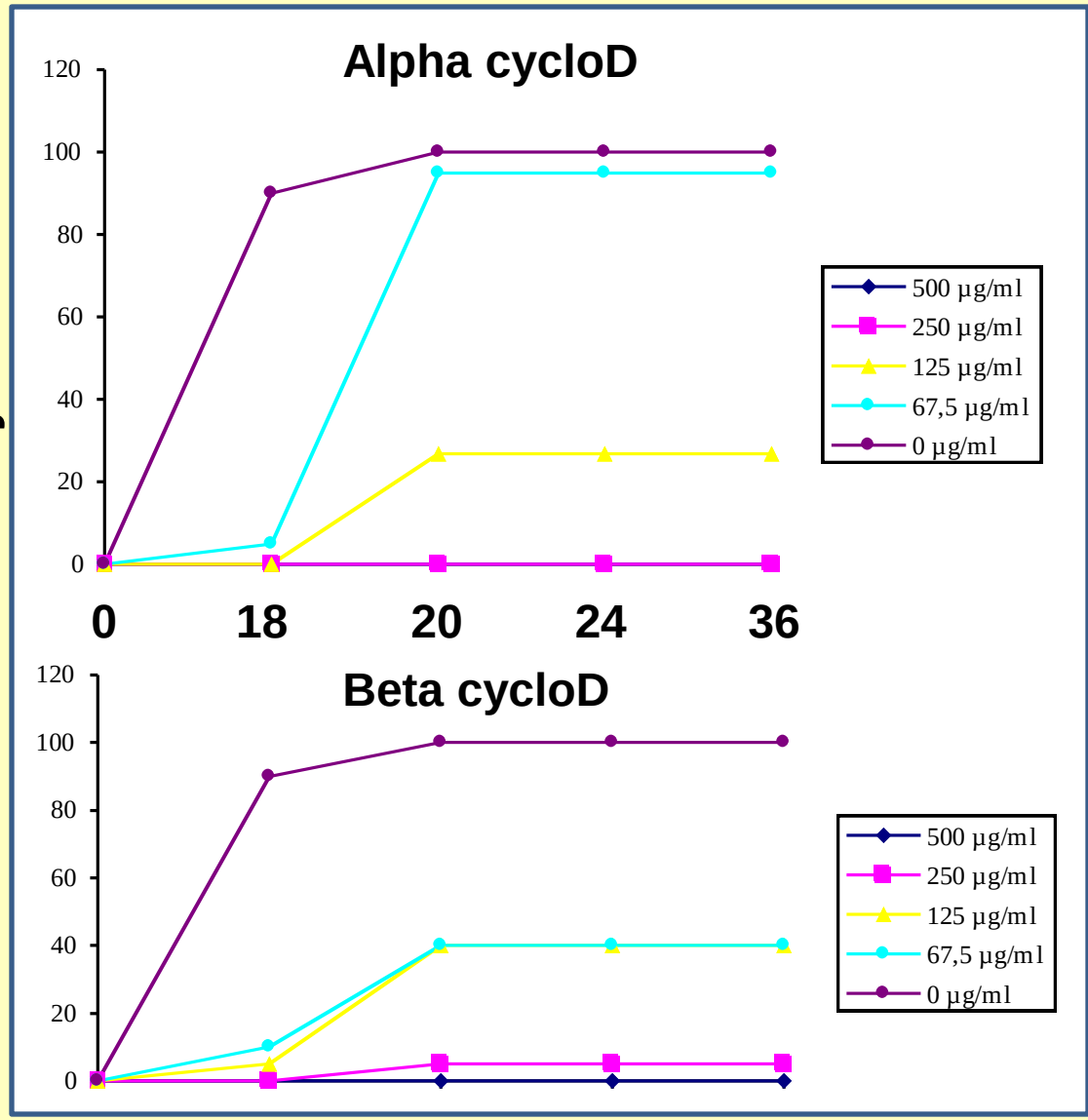
- **Gamma cycloD was the strongest inducer of IL-1B**
- **The heptakis form induced both IL-1B and IL-6**

- Trout macrophages were stimulated for 30 minutes to 24 hours with heptakis (2,6-di-O-methyl)-β-cyclodextrin (Heptakis); methyl-β-cyclodextrin (Methyl β); beta cyclodextrin (β CyD); 2-hydroxypropyl-β-cyclodextrin (Hydroxy β; 2-hydroxypropyl-α-cyclodextrin (Hydroxy α); gamma cyclo-dextrin (γ CyD).
- RNA was extracted and RT-PCR conducted with IL-1B and IL-6.
- 18s was a RNA loading control. Molecular weight standard in first lane.

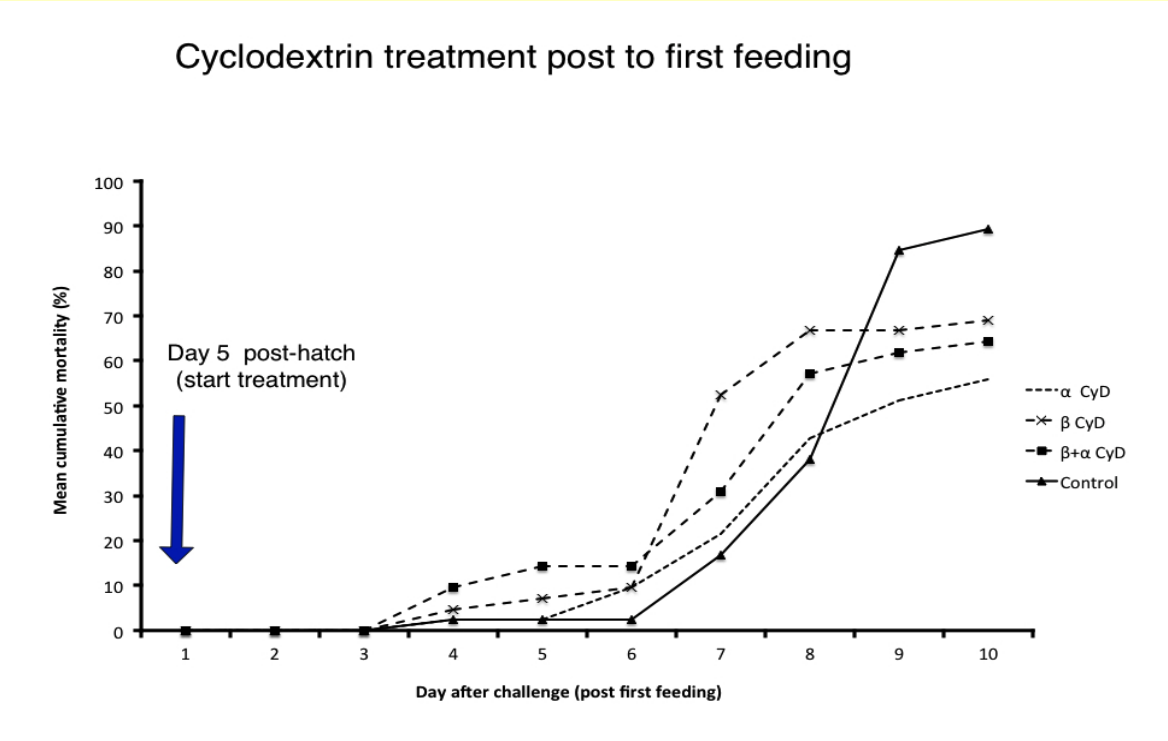
Alpha and beta cycloD protect zebrafish larvae from a lethal dose of LPS

- **Both alpha and beta cycloD were able to completely protect zebrafish larvae against LPS at a concentration of 250-500 µg/ml**

*Zebrafish larvae, 2 days post-fertilization, were bathed with 0, 67.5, 125, 250 or 500 ug/ml of alpha-cyclodextrin and then bathed with a lethal concentration (100% mortality) of LPS (150 ug/ml – *Pseudomonas aeruginosa)* and assessed for mortality over 36 hours.



CycloD is not toxic



Objective: Exploring the larval viability during cyclodextrin treatment and compare the survival during treatment with normal larval growth condition after first feeding (day 5 post hatch)

Results: Survival was slightly better at 10 days with cyclodextrins; larvae motility and morphology were also unaffected

Summary/Next Steps

- **Fish infection challenges are underway using cycloD in fish food with adult aquaculture species**
- **UWMRF is looking for a partner for the further development and testing for vaccine use and ornamental fish**
- Several forms of cyclodextrins induce the pro-inflammatory gene IL-1B while the heptakis form also induces IL-6
- Alpha and beta cyclodextrins protect fish larvae in a bacterial challenge *in vivo*
- Cyclodextrins may provide a novel, inexpensive, safe, easy to use and environmentally friendly solution for disease treatment and prevention in aquaculture and in the ornamental fish industry
- The technology is available for licensing

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