BIOASSAY IN THE COMPANY FARALLON AQUACULTURE SA

04/26/2019

Objective:

1. Demonstrate that BioPhoton-X ™ technology is capable of reducing bacterial growth in water and nauplii.
2. Reference the configuration of the best treatment in case of the implementation of the technology.

Methodology.

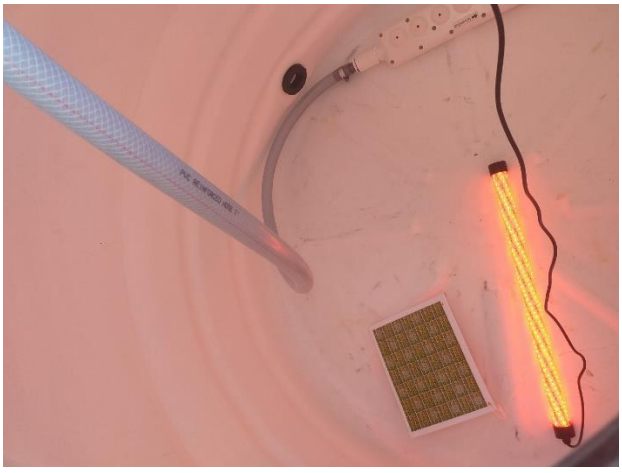
. Everything The test would be carried out in the challenge room where there is an adequate infrastructure for their development.

- From the commercial harvest of the nauplii, a bag would be taken, 500 thousand nauplii, with the aim of forming 2 batteries to be treated each set with different treatments, T1 and T2.
- Each battery will consist of 4 coolers, with 20 liters of water, to be filled to a volume of 18 liters. In addition to the 8 coolers used in the 2 treatments, another 4 will make up the set defined as control 1 or white.
- Each cooler of the different treatments as well as the control were filled with the same volume of water and a quantity of nauplii was placed to allow the samples to be taken expeditiously.
- TREATMENTS
- We will use a Rotoplast, 1000 liters, as the “mother” tank, but it will only be filled with 500 liters to prepare water with BioPhoton-X ™ treatment.
- Treatment 1, is prepared by recirculating water throughands of an air / water exchanger, pulsed red light with PMM modulators and 1 A-7.2 photonic screens with PMM modulators for 45 minutes.
- Treatment 2, still water is brought in a Rotoplast with 500 liters recirculating water throughands of an air / water exchanger, green light (low pulsations) with PMM modulators and 2 photonic screens A-7.2 and H 7.2 with PMM modulators for 45 minutes.

The following image shows the configuration of the tank to carry out the treatments and also allows the photonic plates used to be visualized.

Photo 1. (Conformed system to prepare treatments 1 and 2)

Photo 2. (air / water exchanger, pulsed red light with PMM modulators and 1 A-7.2 photonic screens with PMM modulators for 45 minutes.)



SAMPLE SELECTION.

- From each treatment, 4 coolers, 2 subsamples will be obtained, one of the nauplii to be macerated and one of water to proceed to scratch in TCBS in order to define the cfu colony count. (Colony-forming units.)
- Nauplii and water samples will be taken by the method 4.50 / 9/18/27 minutes.
- Likewise, a sample of the nauplii and the water is taken at the time of harvest in order to quantify the bacterial load.

CONTROL

Control would be made up of the same water placed in the Rotoplast but poured into the 4 coolers before being treated. All the water comes from the larviculture before inoculation of the algae.
Likewise, samples of nauplii and water will be taken at the same frequency as the times indicated above. The control was structured in parallel with treatment 1, labeled as control 1

PROCESS.

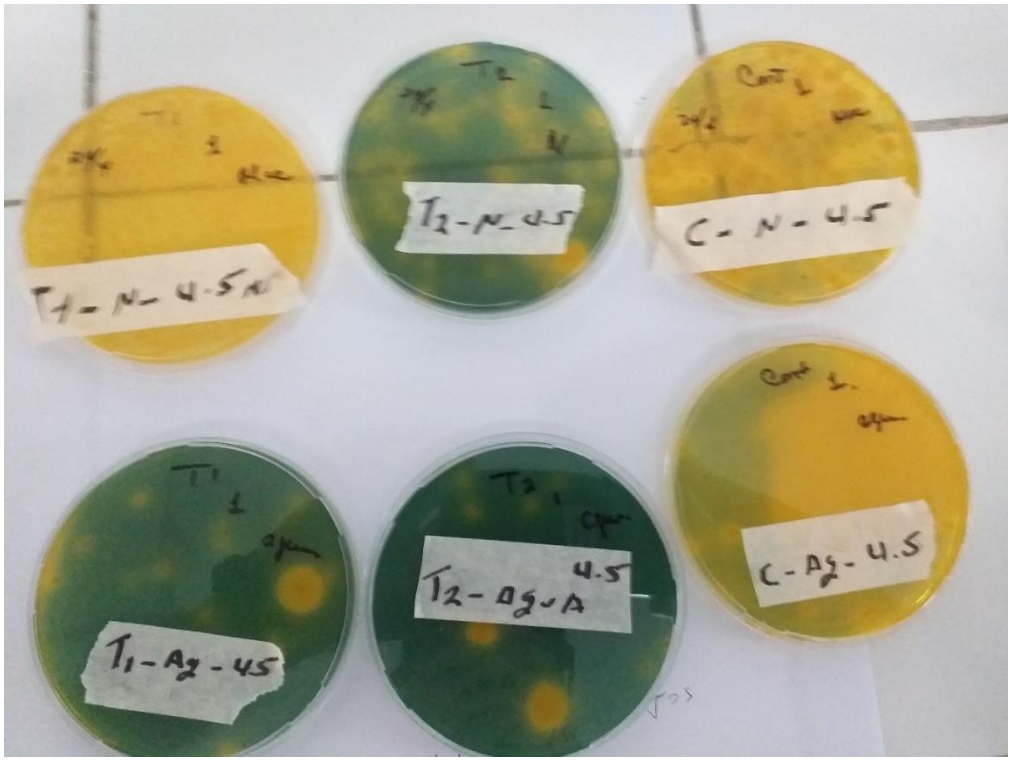
After starting the operation with the Treatment 1, At the time of sampling the nauplii, the technicians drew attention to the difficulty we faced when collecting the nauplii as a low-quantity product in each cooler, contrasting negatively with the times in which we should take the samples.
360 ml of the mother bag had been placed in the same cooler, equivalent to 500,000 nauplii in 20 liters, which yields 9000 nauplii in the cooler, 18 liters, for a density of 500 nauplii per liter.

Due to the above, it was decided to discard Treatment 1 and start with Treatment 2, which was already "running" in its 45 minutes, where we raised the inoculum of nauplii to 400 ml, 10,000 nauplii in the cooler, and it was decided to filter the water at the time of taking the nauplii sample, which allowed us to get enough nauplii to the mash.
The frequency of taking the samples was respected with respect to what was raised. (4.5 minutes, 9 minutes, 18 minutes, 27 minutes,).

Again the Treatment 1 with their respective control, where we placed 750 ml, treatment and control, for an estimated 18,750 nauplii in the 8 boxes used.
The macerate and water samples were scored in TCBS with a volume of 0.1 ml in each one of them by the microbiology team of the Farallón Laboratory.

PLATE RESULTS

The results of the scratching in the plates that are observed in the image are oriented as follows for the 4.5 minutes: (T1-T2 and Control of T1) for nauplii in the first row and in the second row the water sample.



Visually speaking, it is observed that T2 in nauplii and in water inhibited the growth of vibrios. Likewise, treatment 1 has an impact on bacterial growth in water.

The observed in T1 at 4.5 minutes which is very similar to C1, which could be derived from some type of cross contamination.
Sampling time in 4.5 minutes (Formation of Yellow Colonies)

	Treatment 1	Treatment 2	Control1
Macerated	1000	9	300
Water	15	90	200

The second image presents the same sequence at 9 minutes.

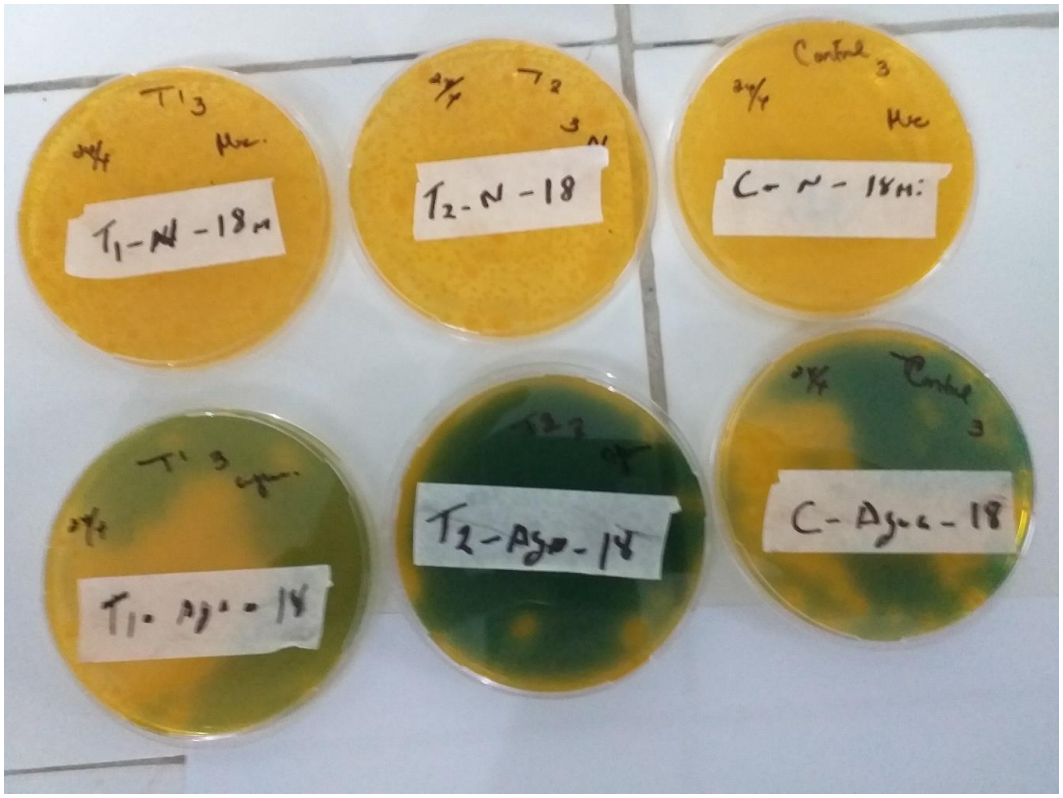
Treatment 1 indicates an inhibitory power of the bacteria at the nauplii level, but not what is observed in T2. Control 1 continues to express a high bacterial content as expected at the nauplii level.

For water, T1 presents an acceptable result, 56 cfu, while T2 in water defines an indicator of better performance. It should be noted that the control allows a low colony count to be counted.

Sampling time in 9.0 minutes (Formation of Yellow Colonies)

	Treatment 1	Treatment 2	Control1
Macerated	50	500	500
Water	56	16	25

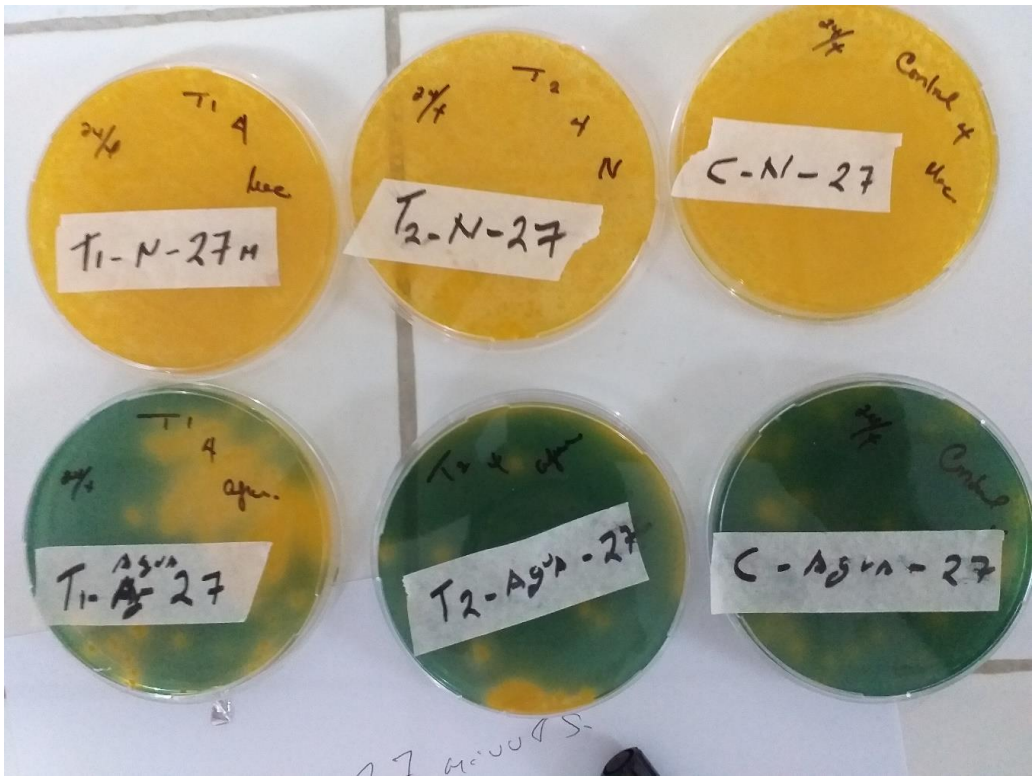
You can see below the results of the 18 minutes



At the nauplii level, no inhibition is observed by T1 and T2. The T1 and T2 in the water show acceptable results. Water control 1 indicates greater growth than both treatments.
Sampling time in 18.0 minutes (Formation of Yellow Colonies)

	Treatment 1	Treatment 2	Control1
Macerated	1000	800	1000
Water	45	29	80

Results at 27 minutes.



At 27 minutes, T1 and T2 did not inhibit growth and T2 on water indicates a favorable result. Control 1 reflects a low value of 40 cfu.

Sampling time in 27.0 minutes (Formation of Yellow Colonies)

	Treatment 1	Treatment 2	Control1
Macerated	1000	600	1000
Water	60	25	40

UFC COUNT SUMMARY (YELLOWS)

	4.5	9	18	27
Macerated (T1)	1000	50	1000	1000
Water (T1)	15	56	45	60
Macerated (T2)	90	500	800	600
Water (T2)	9	16	29	25
Macerate Control	300	500	1000	1000
Water Control	200	25	80	40

The sample taken at the time of washing the nauplii registered 51 yellow colonies, which guides us towards the need to intervene in the water.

CONCLUTIONS.

Treatment 2 presents a greater efficiency in the water with low counts and also did not allow values of 1000 in the nauplii, as observed in the mashes of T1 and the control.

A possible strategy for treating nauplii would be for spawning water and nauplii washing to be done with Treatment 2 to decrease the bacterial load that affects nauplii development.

THANKS.
We must thank the opportunity provided by the Farallón Aquaculture company for allowing the testing of BioPhoton-X ™ treatments and also recognize the great effort made by the laboratory staff, Juan Vlieg, Marquela Herrera, Marvin Pineda, Edgar Carpintero, Alexander Enrique, in the execution of the test despite being carried out in a context of a large number of activities that were carried out in parallel in their commercial production scheme.