

## Evaluation of the inhibitory effect of APSE <sup>™</sup> technology on probiotic bacteria strains from CAMACO SA and pathogenic vibrios from shrimp Litopenaeus vannamei.

This report presents the comparative results of the tests to measure the inhibitory effect of APSE on probiotic bacteria and shrimp pathogenic vibrios by quantification of the number of cells / ml by spectrophotometry at 540 nm wavelength, carried out at UNICAM between 20 February May to June 7, 2013.

According to the results of advances sent on June 1 and with the use of a unified method for quantifying the number of bacteria cells by spectrophotometry and determining the concentrations of the initial common inoculum for both groups of bacteria, where it was used as model, 4 strains of bacteria for each group.

A final test was carried out from June 3 to 7, 2013, with the objective of evaluating the inhibitory effect of the APSE 7.2 and 8.2 plaques on all the probiotic strains (8) and 7 strains from the group of pathogenic vibrios of the shrimp.

For this test, the concentration of the initial inoculum was unified at 100 thousand cell / ml, this was done on the basis that in larval culture it is common to find in sowings in microbiological TCBS or Marine Agars, values of one thousand CFU up to one hundred thousand CFU in samples of water or any stage of larvae. In vitro concentrations of 10<sup>6</sup> were not included in this test, since according to a previous report, no inhibitory effect of plaques was seen on vibrios, for this reason they were omitted.

The global results show that the APSE 7.2 and 8.2 plates exert an inhibitory effect on bacteria and that this effect is not specific to a bacterial group, that is, it can be considered to have a broad spectrum, although there are both probiotic and probiotic bacteria. pathogenic vibrios, which have the ability to resist the emission of waves from these plates, this may be due to the genetic resistance characteristics of each strain in a particular way. This type of reaction is very frequent when evaluating types and concentrations of antibiotics against sp bacteria. In tests such as Antibiograms or MIC (minimum inhibitory concentration).

The results of this study conclude for the case of the probiotic, APSES 7.2 and 8.2 exert an inhibitory effect on 50% (F, I, 32, SHE) and 63% (F, I, J, 32, SHE) of the strains respectively. The APSE resistant strains were B, 8 and 52, that is, 40%.

It is concluded for the case of pathogenic vibrios, APSES 7.2 and 8.2 exert an inhibitory effect on 14% (V.Azureus3) and 57% (V.Azureus3, V. Campbelli5, V.sp7 and 8) of the strains respectively. The strains resistant to APSE in were V. campbelli1, V. campbelli4 and V. Azureus2, that is, 43%.



## APSE vs Probiotic:



## APSE vs Vibrio pathogens.





## COMMENTS.

- 1. It can be concluded that there is an inhibitory effect of the APSE 7.2 and 8.2 plates, the latter being with a greater inhibitory potential.
- 2. That APSE does not discriminate bacterial genus and has a wide range according to the results of these tests.
- 3. That APSE, by also exerting an effect on probiotic strains, we must always try to maintain a concentration of cells above one million per ml, which is part of the protocol.
- 4. The probiotic strains that showed resistance to APSE were B, 8 and 52. It is very likely that these 3 strains are the ones that exert the effect of controlling the luminescence caused by pathogenic vibrios in CPL San Carlos.
- 5. That according to the integrated infrastructure plan for this technology, to increase efficiency, a freshwater reserve should be installed to complement the APSE effect.
- 6. Considering that these tests have allowed us to clearly observe the APSES effect, the continuous tests of staining and competition that had been proposed are omitted.

The results found here are very interesting in terms of the use of this innovative technology. And in fact, valuable, since it has allowed us to incorporate new information to the evaluation and identification of the strengths in terms of resistance and dominance of part of the strains that we have in the probiotic CAMACO like cocktail.

Sincerely

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UNICAM - CAMACO SA