Efficacy of tilapia oral vaccine coupled with a nanocomposite biomaterial as carrier for vaccine delivery

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Introduction

Tilapia

- second most farmed fish worldwide
- major freshwater fish species in Southeast Asia
- widely cultured in several countries due to its fast growth rate, high market demand and reasonable prices.
- top 3 aquaculture species in the Philippines, with a total production of 279,385.87 tons corresponding to an estimated value of US$447,000 (PSA, 2020).
- susceptible to bacterial pathogens

Aeromonas spp. in fish

- Several species can cause diseases in both wild and farmed freshwater and marine fish species impacting the aquaculture sector.
- *A. veronii* has been recently identified as causative agent of motile aeromonas septicemia (MAS) in Nile tilapia (*Oreochromis niloticus*) often inducing high mortalities.
- Treatment of *A. veronii* infection in tilapia has been hampered by emergence of multiple antibiotic resistance.
- Other MAS-causing *Aeromonas* spp. such as *A. hydrophila*, have also been reported.

Oral Vaccine

- Vaccination is the most appropriate method for disease control.
- Vaccination by injection introduce stress to the fish, very laborious and time consuming.
- Therefore, oral administration, which can induce higher immune response compared to other routes, was used in this study.
- Oral vaccine was prepared using *Aeromonas* sp. coated with phyllosilicate, a non-specific organophilic carrier (Dong et al., 2005; Argayosa et al., Phil. Patent May 2017) mixed with feeds and used for immunization.

Fig. 1. SEM of the phyllosilicate (orange and yellow green), the encapsulation of bacterial cells. (The sample was sputter-coated with Au and Pd and color enhanced for presentation)

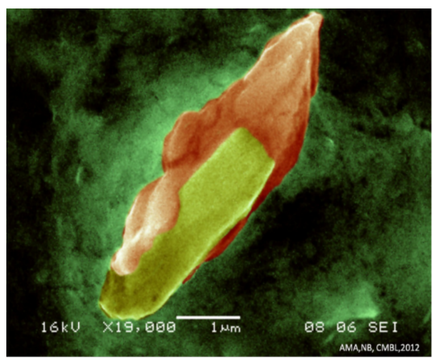
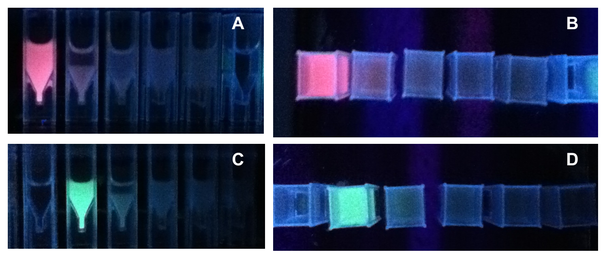
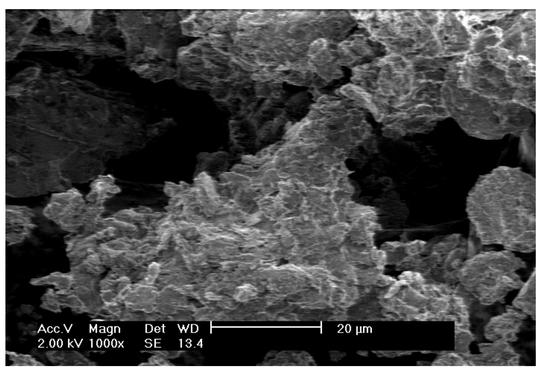


Fig. 2. Fluorophore-labeled (EtBr: A-B; FITC: C-D) inactivated *A. hydrophila* I2DF2 cell suspensions in cuvette viewed under UV transilluminator. (0, 0.5, 1, 2, 3% MMT-KSF, buffer only)



The bacteria-clay mixture of fluorescent-labeled cells showed decreased fluorescence due to the clay encapsulation of bacterial cells. Adsorption of clay material blocked the emission of light from the fluorophore. A higher concentration of clay allowed better encapsulation of cells.

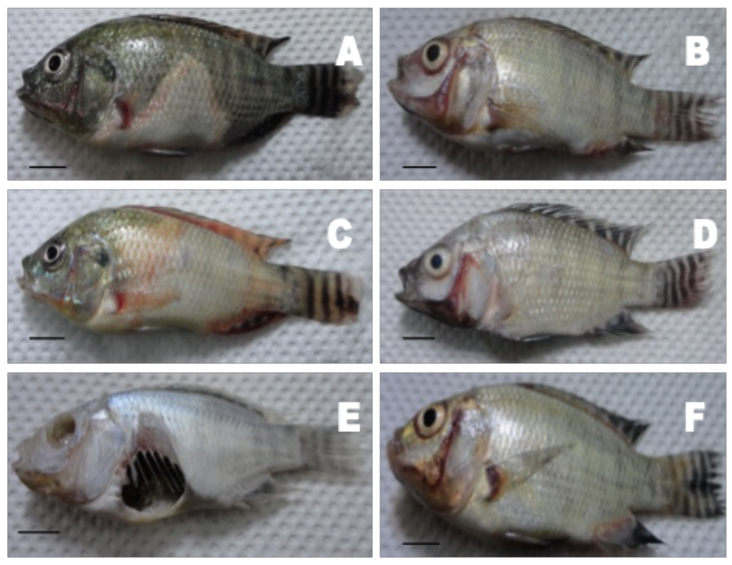
Fig. 3. SEM image of phyllosilicate adsorbed with *A. hydrophila* BIOTECH 10089. No free cells were observed, which indicates complete encapsulation by bulk clay material.



Results and Discussion

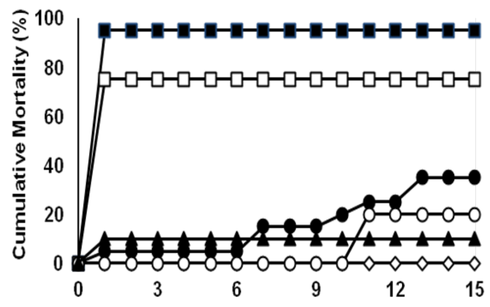
Pathogenicity test of *A. veronii* DFR

Fig. 4. Gross signs in *O. niloticus* experimentally infected with *A. veronii* DFR through intraperitoneal injection



LD50 analysis

Fig. 5. Cumulative mortalities of tilapia juveniles (MBW:6 g; n=20 fish/ inoculum dose) experimentally infected by intraperitoneal injection with *A. veronii* DFR (■). $10^{9.8}$ CFU fish^{-1} ; (□) $10^{8.8}$ CFU fish^{-1}; (●) $10^{7.8}$ CFU fish^{-1}; (○) $10^{6.8}$ CFU fish^{-1}; (▲) $10^{5.8}$ CFU fish^{-1}; (◊) control (NSS buffer only).



Oral vaccination of *O. niloticus* with *A. veronii* DFR (Tank trial)

Fig. 6. Cumulative mortalities of different groups of tilapia juveniles intraperitoneally challenged with *A. veronii* DFR at a dose of 9.2 x 10^6 CFU/fish.

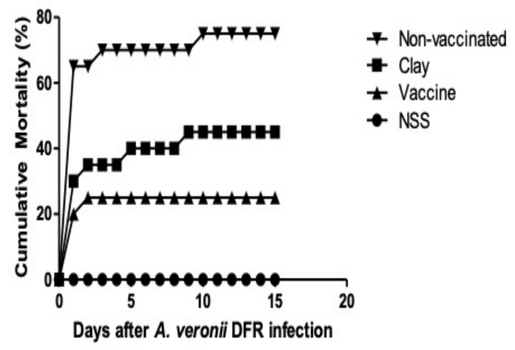


Fig. 7. Relative IgM expression analysis using qRT-PCR A) in different treatments, 22 days post-challenge B) at different timepoints (Tp1-before vaccination, Tp2-after initial vaccination, Tp3-after booster vaccination)

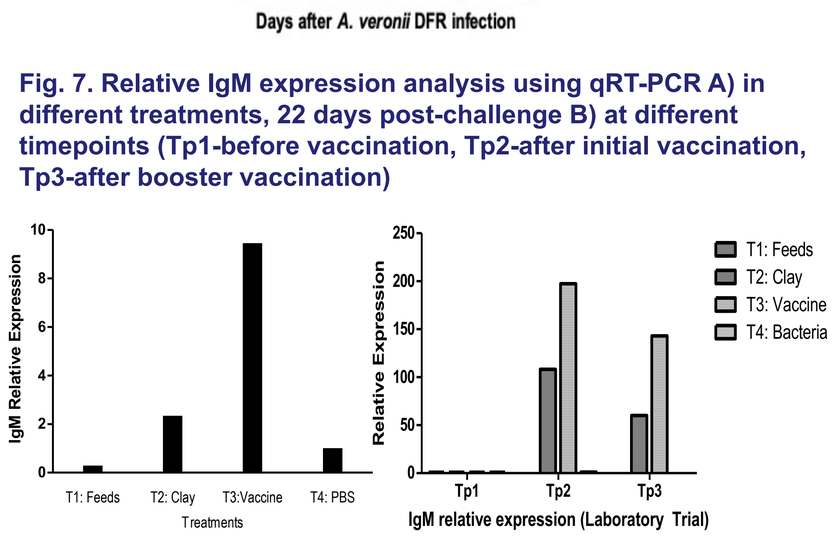
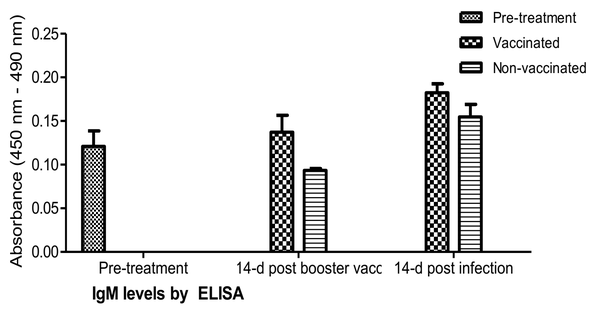


Fig. 8. IgM levels in the sera of unvaccinated and vaccinated *O. niloticus* examined by ELISA at Day 14 post-*A. veronii* challenge



Oral vaccination of *O. niloticus* with *A. veronii* DFR (Field trial)

Primary oral vaccination with *A. veronii* DFR given at days 1-5 in Calauan, Laguna hatchery and booster vaccination given on Day 45-50 in Taal Lake.

Fig. 9. Cumulative mortalities of unvaccinated and vaccinated *O. niloticus* (BFAR GET-EXCEL) after 160 days of culture in floating net-cages

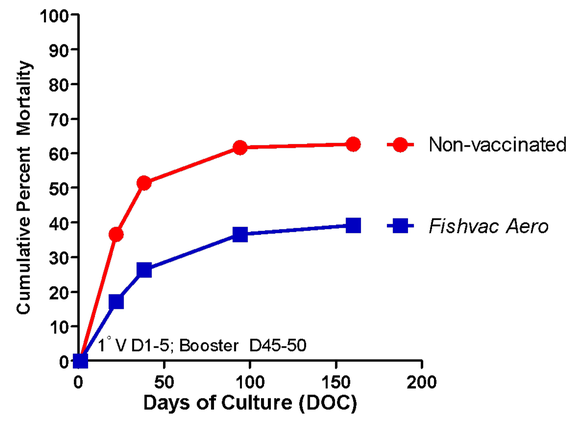
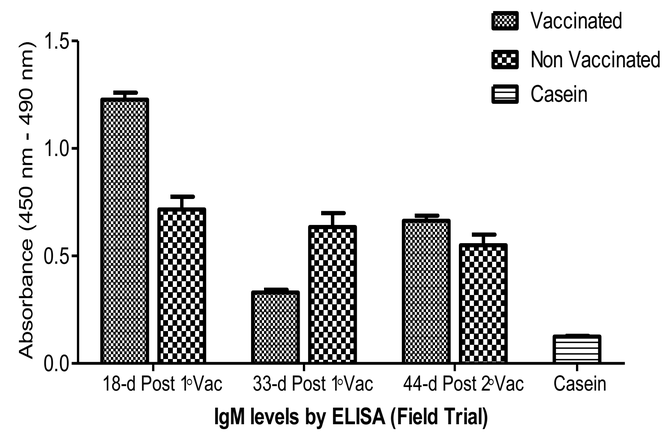


Fig. 10. IgM levels in the sera of *O. niloticus* examined at Day 18 and Day 33 post-primary- and at Day 44 post-booster- oral vaccination with *A. veronii* DFR vaccine.



Conclusion

- *A. veronii* DFR vaccine effectively induced high IgM levels in the sera of tilapia via oral administration
- Tank and field (cages) trials showed higher survival rates in tilapia orally vaccinated with *A. veronii* DFR vaccine compared with unvaccinated fish.
- Oral vaccination of tilapia with U.V. treated-*A. veronii* using phyllosilicate as vaccine carrier potently induced humoral adaptive immune response and conferred significant protection in fish against *A. veronii* infection.
- The potential of oral vaccine using phyllosilicate carrier against other MAS-causing *Aeromonas* spp. warrants further investigation.

References

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