Development and efficacy of genomovar II vaccines against columnaris disease

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Abstract

Flavobacterium columnare, the causative agent of columnaris disease, is pathogenic to most species of freshwater fish in the USA and worldwide. Columnaris disease is the second highest killer of farmed catfish and is currently the most prevalent illness in farm-raised channel catfish, *Ictalurus punctatus* (Rafinesque), which constitutes the largest aquaculture industry in the USA [1]. The species *F. columnare* has been divided into three distinct genetic groups or genomovars based on DNA: DNA hybridization. Genomovars I and II are the most prevalent types in catfish aquaculture in the USA in where columnaris disease is responsible for major economic losses every year. Different genetic groups of *F. columnare* have different degrees of virulence in channel catfish. Studies on the virulence and pathogenicity of these two types demonstrated that genomovar II strains are significantly more virulent towards channel catfish than genomovar I strains [2]. Besides, genomovar II strains have been found to be almost exclusively associated with catfish species in the wild. Due to the cosmopoliating distribution of this pathogen in aquaculture settings, prevention strategies such as vaccination are being sought after to reduce disease incidence. A modified-live *F. columnare* vaccine derived from a genomovar I strain has been commercialized under the trade name AQUAVAC-COL^{TM} (Merck) and is currently used by the industry to prevent columnaris disease. However, the efficacy of this vaccine under field conditions has been questioned in some forums.

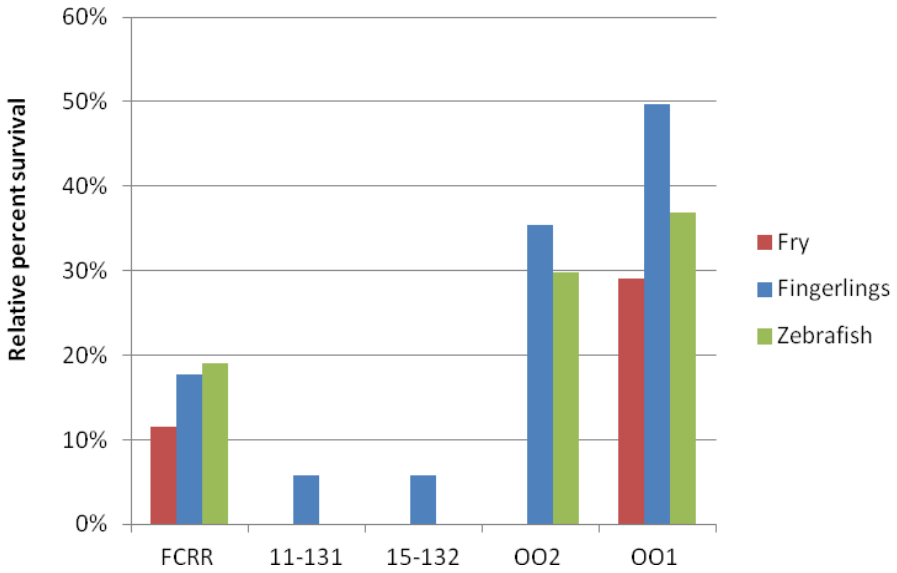
Since genomovar II strains are more virulent towards catfish, a specific vaccine against this genomovar is likely to increase the protective effect of vaccination against highly virulent strains. Our objective in this study was to develop a modified-live vaccine derived from genomovar II strains. We have recently generated stable and safe mutants from genomovar II strains by multiple passages of virulent wild-type strains on increasing concentrations of rifampicin in the culture medium. Out of four selected genetically different genomovar II strains, we were able to obtain 13 rifampicin-resistant avirulent mutants. Extensive characterization of these mutants showed a marked genetic diversity, indicating that not all mutations were introduced in identical loci [3]. Preliminary data showed that the new mutants outperformed the commercial vaccine strain (FCRR) in controlled laboratory challenges. These new mutants were then further evaluated in channel catfish fingerlings, zebrafish, and channel catfish fry. The efficacy of genomovar II and genomovar I avirulent mutants as vaccines against columnaris disease was compared in parallel.

For each vaccination/challenge experiment, fish were appropriately acclimated to lab conditions to reduce stress during the actual experiment. Fish were transferred from their holding aquaria into a bucket with 2 L of water and 20 mL of an overnight culture of the tested vaccine strain in

modified Shieh (MS) broth. 20 mL of sterile MS broth was added to the control group bucket. Aeration was provided to each individual bucket by means of air pump and air stone. Fish were kept in the vaccination suspension for 30 minutes. After that period, fish were returned to their original aquaria and monitored throughout the vaccination period. All challenges were performed at 28 days post-vaccination following the same procedure using *F. columnare* strain BGFS-27, which has been identified as a highly virulent genomovar II strain. Mortalities were recorded twice per day for 15 days post challenge.

In the first vaccination/challenge trial using catfish fingerlings, we tested four of the new genomovar II mutants (11-131, 15-132, OO1 and OO2). The active ingredient used in AQUAVAC-COL^{TM} (*F. columnare* strain FCRR) was included as one of the treatments and a sham vaccinated group as control. A total of 1,440 fingerlings, average weight/fish = 2.4 g, were divided (40 fish/aquarium, 6 treatments/6 replicates each) and immersed in ~10^6 CFU/ml of the corresponding vaccine. Survival in the vaccinated and control fish was 100% in all tanks. At 28 days post-vaccination, fish were challenged with a highly virulent strain of *F. columnare* (BGFS-27) by immersion following standard protocol procedures. The relative percent survival (RPS) values were 5.8%, 5.8%, 49.7%, 35.4% and 17.7% for 11-131, 15-132, OO1, OO2, and FCRR, respectively.

In the second vaccination/challenge experiment, we immersed a total of 336 adult zebrafish, average weight/fish = 0.5 g (28 fish/aquarium, 4 treatments/3 replicas each) in ~10^6 CFU/ml of the experimental vaccines OO1 and OO2 or in the FCRR, and a sham vaccinated control group. Survival in the vaccinated and control fish was 100% in all tanks. At 28 days post-vaccination, fish were challenged with a highly virulent strain of *F. columnare* (BGFS-27) by immersion following standard protocol procedures. The relative percent survival (RPS) values were 36.9%, 29.8% and 19% for OO1, OO2, and FCRR, respectively. Both genomovar II vaccines resulted in higher RPS than the genomovar I vaccine, however, RPS of only OO1 was statistically significantly higher than the AQUAVAC-COL^{TM}. Our third experiment was performed similarly, but we used only OO1 and AQUAVAC-COL^{TM} as vaccines in channel catfish fry. We immersed a total of 480 channel catfish fry, average weight/fish = 0.05 g (40 fish/aquarium, 3 treatments/4 replicates each) in ~10^6 CFU/ml of the experimental vaccines and a sham vaccinated control group. Survival in the vaccinated and control fish was 100% in all tanks. At 28 days post-vaccination, fish were challenged with a highly virulent strain of *F. columnare* (BGFS-27) by immersion following standard protocol procedures. Our results were similar to our previous results obtained in channel catfish fingerlings and to those obtained in zebrafish, in that OO1 outperformed the AQUAVAC-COL^{TM} vaccine after fish had been challenged. The RPS values were 29.8% and 11.6% for OO1 and FCRR, respectively.



Overall, the genomovar II mutant OO1 outperformed the genomovar I mutant FCRR. Although both genomovar II vaccines resulted in a higher RPS than the genomovar I vaccine, only OO1 showed a statistically significant higher RPS than FCRR. Relative percent survival was significantly higher when OO1 was used as vaccine in all trials conducted to date. The results suggest that administration of genomovar II avirulent mutants as potential live-modified vaccines is safe and elicits greater protection against columnaris disease than the use of genomovar I mutants.

References

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