MOLECULAR AND BIOTECHNOLOGICAL APPROACHES TO THE CONTROL OF VIRAL DISEASES IN FISH

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Demand for fisheries products is projected to grow by 25-30% by the Year 2000 and since traditional ocean fisheries production has reached its maximum harvest of 80-90 million metric tons, aquaculture must provide for the increased demand. In fact, it is projected that one out of every four fish consumed in the United States by the Year 2000 will come from aquaculture. In order for the aquaculture industry to meet this increased demand, it must become more efficient in its production and one of the critical factors affecting production has been disease. Aquaculture must bring disease outbreaks in the hatcheries and rearing ponds under control.

The infectious diseases that plague the aquaculture industry are no different from those found in human or veterinary medicine. They include bacteria, fungi, protozoan parasites, and viruses. For many of these agents, antibiotic and chemotherapeutic treatments are available. However, for the viral diseases, the only control methods in use are the strict quarantine of animals and the destruction of all diseased animals followed by restocking with certified disease free animals. For many fish farmers, these control methods are unsatisfactory and not cost-effective. Thus, we have developed vaccines for the control of viral diseases in fish.

In our search for a vaccine, we considered killed vaccines but these only work by injection and that was impractical in hatcheries where millions of fish are reared each year. Also, live modified vaccines were not considered since the licensing of these vaccines was too expensive. Instead, we looked to developing a subunit vaccine by recombinant DNA technology. We have found that the subunit vaccines we have developed are effective, inexpensive, safe and provide immune protection of long duration. All of these are important factors for the acceptance of a viral vaccine by the aquaculture industry.

The work has led to the subunit vaccine development for several viruses. They include infectious hematopoietic necrosis virus (IHNV), viral hemorrhagic septicemia virus (VHSV), infectious pancreatic necrosis virus (IPNV), channel catfish virus (CCV), and spring viremia of carp virus (SVCV). Researchers from around the globe are pursuing recombinant DNA vaccines for these viruses.

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OBJECTIVE 3. Clone and Express Viral Gene

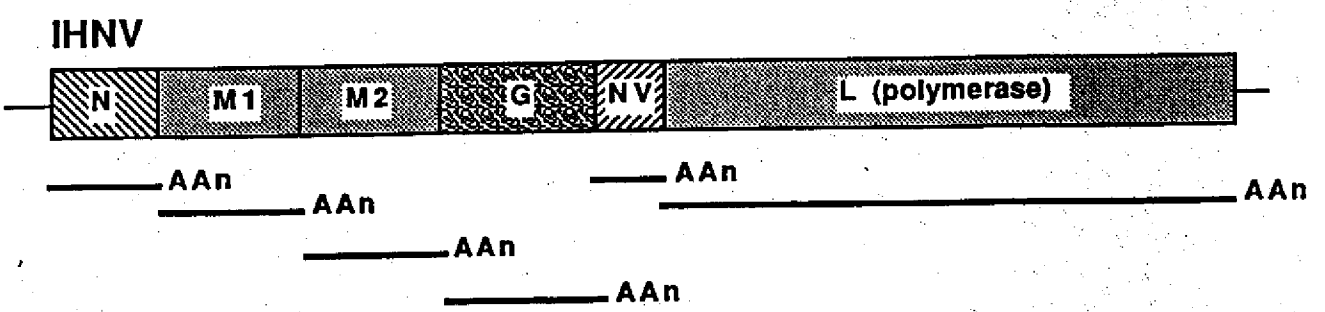

Figure 1

Table 1. Serotypes of IPNV: Group 1

SEROTYPE	GEOGRAPHIC LOCATION	ISOLATES
West Buxton	USA	WB, VR299
Ab	Europe, Asia	Ab, EVE
Sp	Europe	Sp, N-1
Hecht	Europe	Hecht
Tellina	Europe	Tellina
Canada 1	Canada	AS
Canada 2	Canada	
Canada 3	Canada	
Jasper	Canada	Jasper

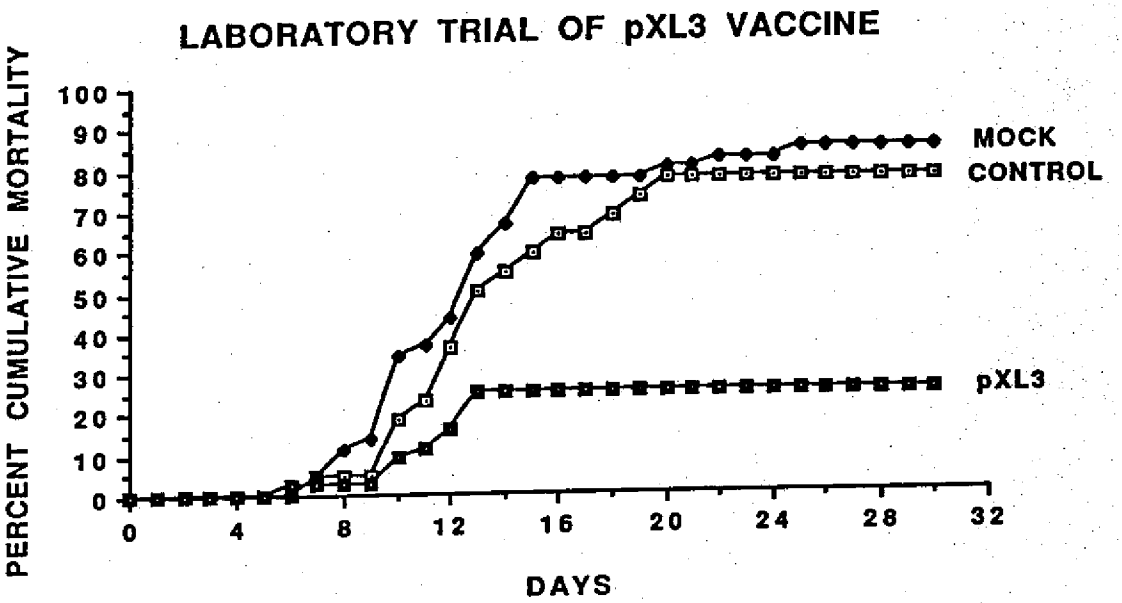
LABORATORY TRIAL OF pXL3 VACCINE
PERCENT CUMULATIVE MORTALITY
100
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
28
32
DAYS
MOCK
CONTROL
pXL3

Figure 3

vaccinated fish were killed by the greater virus challenge dose as if virus infection overwhelmed the immune response in the fish. These results prompted us to redesign the IHNV vaccine clone to include a larger proportion of the viral glycoprotein gene. The original clone contained the region of the glycoprotein gene encoding amino acids 336 to 444 (Gilmore et al., 1988). More recently, the clone pXL3 was generated which encoded amino acids 269 to 453. This particular clone produced a fusion protein which was very protective against the more virulent strains of IHNV (Xu et al., 1991).

Further studies on the IPNV vaccine have not been carried out to determine whether the IPNV-Sp vaccine induces protection against all nine serotypes of IPNV. Nevertheless, the vaccine does induce protection against several serotypes of IPNV, including West Buxton and IPNV-Quebec. In addition, an antigenic region of the IPNV major capsid protein, VP2, has been identified and found to contain a conserved neutralizing antibody domain. The monoclonal antibody, AS-1, was found to recognize a conserved region of VP2 and this reagent neutralizes all known IPNV isolates. These findings indicate that the IPNV vaccine may be effective against all isolates of IPNV.

Accomplishing Objective 5 has been more difficult. The scale-up fermentation of the *E. coli* containing the pXL3 plasmid has enabled us to characterize fermentation conditions which provide 60 OD560 units/ml of bacteria in less than 24 hours with a fusion protein concentration of 35% of the total protein. However, the vaccine produced under these conditions are not as efficacious as the vaccine produced in 2-liter shaker flask fermentations. Several factors including the solubility of the protein, the folding of the fusion protein, and the extraction procedures are under investigation.

Oregon State University has licensed the vaccine to a company, MariGenetics, Inc. of Corvallis, Oregon and this company is conducting trials of the vaccine for U.S. Dept. of Agriculture licensing. Thus, Objective 6, the licensing of the vaccine for commercialization has been accomplished.

The work presented here illustrates the power of recombinant DNA techniques to produce vaccines for viral diseases of aquaculture animals. These vaccines may make it possible to culture these animals at higher densities and maintain the health of these animals against a greater number of pathogens.