AQUACULTURE

VALIDATION OF FERMENTATION AND PROCESSING PROCEDURES FOR THE COMMERCIAL SCALE PRODUCTION OF A LIVE ATTENUATED EDWARDSIELLA ICTALURI VACCINE FOR USE IN CATFISH AQUACULTURE

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"ENTERIC SEPTICEMIA OF CATFISH IS ONE OF THE MOST PROBLEMATIC DISEASES AFFECTING THE COMMERCIAL CULTURE OF CATFISH. FURTHER DEVELOPMENT OF THIS EXPERIMENTAL LIVE, ATTENUATED, ORAL VACCINE HOLDS PROMISE AS AN EFFECTIVE CONTROL MEASURE AGAINST THIS DISEASE."

David Wise

Edwardsiella ictaluri, a gram negative enteric bacterium, is the causative agent of enteric septicemia of catfish (ESC) and a major hindrance to channel catfish (*Ictalurus punctatus*) aquaculture in the southeastern United States. Recently, a live, patent pending, attenuated *E. ictaluri* orally delivered vaccine has been developed by Mississippi Agricultural and Forestry Experiment Station scientists in the Thad Cochran National Warmwater Aquaculture Center. The vaccine has been shown effective in protecting fish against ESC. A single oral immunization dose was shown to greatly improve survival and increase production efficiency in small scale experimental ponds trials. Under controlled laboratory settings, oral vaccination resulted in heightened protection of vaccine recipients, with relative percent survival upwards of 90%.

This work investigates fermentation protocols for industrial production of the experimental *E. ictaluri* vaccine (S97-773-340X2) for use in catfish aquaculture and evaluates the viability and potency of multiple industrial scale vaccine serials after prolonged cryogenic storage (<24 months; -74° C). Further vaccine development is contingent on the successful completion of large scale field trials that reflect industry conditions.

Vaccine Serial

Vaccine serials were produced in 2013 and 2014 (four serials per year produced within a three month period). Serials were subjected to a series of validation tests to determine cell viability of processed vaccine, vaccine potency and stability (shelf-life). The 50-L fermentation cultures were concentrated by centrifugation prior to cryogenic storage and the concentration of viable cells in the 50-L fermented culture (pre-processing), as well as the concentrated vaccine before and after freezing, was evaluated. There was an approximate 10-fold increase in vaccine cell density after concentrating the fermented 50-L culture. Serials produced in 2013

demonstrated an average 9.6-fold increase in viable cells, while 2014 serials exhibited an average 9.8-fold increase in viable cells. Statistically, there was no difference in viable cells counts between pre- and post-frozen harvested cells within a given year. However, viable cells after processing were significantly higher in 2014 due to an increased fermentation yield.

Each vaccine serial was evaluated for potency, safety and cold storage (-74° C) shelf-life stability (Table 1, page 66). Safety, defined as a lack of mortality related to vaccine administration, was evaluated by immunizing fish at 10X the target dose (>1x10^8 CFU/g feed). In the initial testing, (between 4-52 days after freezing), all frozen serials conferred a high level of protection against *E. ictaluri* infection with no adverse effects, as no post-vaccination, pre-challenge mortality was observed. Although some variation in yield was observed between serials produced in 2013 and 2014, there were no differences in mean relative percent survival (RPS) between the respective years (2013 = 90.7%; 2014 = 90.0%;).

Vaccination resulted in RPS in excess of 90% in 12 of 17 trials, with one trial showing complete protection (100% RPS) against *E. ictaluri* infection. Three of the four, 2014 serials were tested for potency more than 300 days post production with RPS values between 85.6% and 95.6%. Serials BCL-042513 and BCL-050913 both demonstrated RPS values in excess of 90% after more than 600 days in cold storage.

This data demonstrates the live, attenuated, orally delivered vaccine can be stored at -74° C for at least two years with no reduction in cell viability or vaccine potency. The vaccine is currently being used to conduct field tests in support of obtaining a USDA/APHIS license for the commercial use in catfish aquaculture and refining commercial scale delivery protocols.

Sartarios Floor Model 50 L Fermentation Reactor

Table 1. *Storage stability of frozen harvested vaccine cells. Vaccine serials produced in 2013 and 2014 were periodically tested for cell viability and potency in laboratory tests. For all serials, mortality of vaccinated fish was significantly less than non-vaccinated controls (One-ANOVA, P value for all challenges was < 0.0001). Challenge trials were not conducted for each test date. (-) = no challenges performed; ND= Not determined; RPS = Relative percent survival.*

	Date Serial tested	Days in Storage	Vial CFU/ml (Log10)	Feed CFU/g (log10)	% mortality (naive)	% mortality (vaccinated)	RPS
BCL-042513	5/13/2013	18	10.3	7.3	76.5	6.7	91.3
	8/12/2013	109	10.2	-	-	-	-
	3/10/2015	730	10.2	7.2	56.5	2.5	95.6
BCL-050913	5/13/2013	4	10.5	7.5	76.5	10	91.2
	9/10/2013	154	10.4	7.5	93.3	9.1	90.2
	3/10/2015	670	10.5	7.7	56.6	3.8	93.4
BCL-051613	5/21/2013	4	10.6	7.6	76.1	5.1	93.3
	7/29/2013	74	10.5	7.6	97.9	11.8	88
	11/21/2013	196	10.4	ND	78.8	1.7	97.8
BCL-061613	6/20/2013	7	10.4	7.4	95.1	22.8	76
	2/3/2014	235	10.3	7.3	55.8	5.3	90.6
BCL-022714	3/26/2014	27	10.6	7.6	46.9	0	100
	7/2/2014	124	10.7	-	-	-	-
	7/28/2014	150	10.6	-	-	-	-
BCL-030614	3/26/2014	20	10.3	7.2	47.5	3.69	92.2
	7/22/2014	141	10.4	-	-	-	-
	3/2/2015	378	10.5	7.7	34	2.1	93.8
BCL0-041714	5/8/2014	52	10.7	7.7	98.3	15.1	84.7
	6/11/2014	86	10.7	-	-	-	-
	3/27/2015	375	10.7	7.6	60.8	8.8	85.6
BCL-052214	6/4/2014	13	10.8	7.6	93.5	20.8	77.7
	3/27/2015	309	10.7	7.6	60.8	2.5	95.9
	4/14/2015	327	10.7	-	-	-	-