

Missing the target: DNAk is a dominant epitope in the humoral immune response of channel catfish (*Ictalurus punctatus*) to *Flavobacterium columnare*

Miles D. Lange^a, Benjamin H. Beck^a, Jason D. Brown^a, Bradley D. Farmer^a, L. Matthew Barnett^a, Carl D. Webster^a

^a U.S. Department of Agriculture, Agricultural Research Service, Harry K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, AR, USA

Vaccination remains a viable alternative for bacterial disease protection in fish; however additional work is required to understand the mechanisms of adaptive immunity in the channel catfish. To assess the humoral immune response to *Flavobacterium columnare*; a group of channel catfish were first immunized with *F. columnare* LV-359-01 cultured in iron-replete media, before being challenged with wild type *F. columnare* LV-359-01. The immunization protocol did not confer increased protection against *F. columnare*; however both control and immunized responders generated serum and skin IgM antibodies against *F. columnare* proteins. Western blot analyses of individuals from both groups showed that IgM antibodies were generated to the same 70 kDa extracellular protein, which was identified to be the bacterial chaperonin protein DNAk. Antibodies generated were cross reactive to DNAk proteins found in other gram negative bacteria. Our data suggests that DNAk is the dominant epitope in the channel catfish B-cell response to *F. columnare*.