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Identification and expression profile of multiple genes in the anterior kidney of channel catfish induced by modified live *Edwardsiella ictaluri* vaccination

Julia W. Pridgeon *, Craig A. Shoemaker, Phillip H. Klesius

Aquatic Animal Health Research Unit, USDA-ARS, 990 Wire Road, Auburn, AL 36832, USA

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ABSTRACT

Using PCR-select subtractive cDNA hybridization technique, 57 expressed sequence tags (ESTs) were isolated from 240 clones of a modified live *Edwardsiella ictaluri* vaccinated *vs.* sham-vaccinated channel catfish anterior kidney subtractive library. The transcription levels of the 57 ESTs in response to *E. ictaluri* vaccination were then evaluated by quantitative PCR (QPCR). Of the 57 ESTs, 43 were induced at least 2-fold higher in all three vaccinated fish compared to unvaccinated control fish. Of the 43 upregulated genes, five were consistently upregulated greater than 10-fold, including two highly upregulated (>20-fold) glycosyltransferase and Toll-like receptor 5. The transcriptional levels of GTPase 1, coatomer protein complex zeta 1, and type II arginine deiminase were consistently induced greater than 10-fold. MHC class I α chain and transposase were upregulated greater than 10-fold in two of the three vaccinated fish. The 43 upregulated genes also included 19 moderately upregulated (3–10-fold) and 17 slightly upregulated (2–3-fold). Our results suggest that subtractive cDNA hybridization and QPCR are powerful cost-effective techniques to identify differentially expressed genes in response to modified live *E. ictaluri* vaccination.

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1. Introduction

Enteric septicemia of catfish (ESC), the most prevalent disease affecting farm-raised channel catfish, *Ictalurus punctatus*, is caused by *Edwardsiella ictaluri*, a facultative intracellular Gram-negative flagellated bacterium akin to phylogenetically related *Salmonella* (Thune et al., 1997; Zhang and Arias, 2007). ESC is generally an acute septicemia that develops very quickly, especially in the temperature range of 22–28 °C. Signs of the disease have been observed within 2 days after immersion challenge and heavy mortalities have been reported as early as 4 days after infection (Newton et al., 1989; Wolters and Johnson, 1994; Thune et al., 1997).

To control ESC, live attenuated *E. ictaluri* vaccines have been developed to protect catfish (Wise et al., 2000; Shoemaker et al., 1999, 2002, 2007; Karsi et al., 2009). Several studies have demonstrated that protective immunity in channel catfish against *E. ictaluri* is largely mediated by cellular immune responses with humoral antibodies having a secondary function (Ellis, 1999; Shoemaker and Klesius, 1997). Like *Salmonella*, *E. ictaluri* can survive and replicate intracellularly (Steele-Mortimer et al., 2000; Skirpstunas and Baldwin, 2002; Thune et al., 2007; Russo et al., 2009), further suggesting that the cellular immune response plays an important role in combating ESC.

The innate immune system is the first line of host defense against pathogens and plays a vital role in maintaining host–microbe homeostasis (Bingle and Craven, 2004). Bony fish have very quick and powerful defense mechanisms to a wide range of pathogens (Bayne et al., 2001; Ellis, 2001). The host immune system recognizes invading pathogens by their

* Corresponding author. Tel.: +1 334 887 3741; fax: +1 334 887 2983.
E-mail address: Julia.Pridgeon@ars.usda.gov (J.W. Pridgeon).

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highly conserved pathogen-associated molecular patterns (PAMPs), which are unique to these pathogens and are normally not shared by host cells. Recognition mediated by pattern recognition receptors (PRRs) will then initiate the inflammatory processes (Medzhitov and Janeway, 2002; Magor and Magor, 2001; Hoffman et al., 1999). Many components of PRRs, such as Toll-like receptors (TLRs), are evolutionarily conserved from insects to humans (Kimbrell and Beutler, 2001). TLRs function in innate immunity through recognizing the conserved pathogen-associated molecular patterns (PAMPs) of an invading pathogen and eliciting inflammatory and immune responses (Medzhitov and Janeway, 2000). To date, 13 TLRs (TLR1–TLR13) have been identified in mammals and five TLRs (TLR2, TLR3, TLR5, TLR20, and TLR21) have been reported in channel catfish (Bilodeau and Waldbieser, 2005; Baoprasertkul et al., 2007a,b). The best characterized ligands that TLRs recognize include: (1) lipoproteins by TLR2; (2) dsRNA by TLR3; (3) lipopolysaccharide (LPS) by TLR4; and (4) flagellin by TLR5.

To understand the molecular mechanism involved in host immune response to *E. ictaluri* infection in channel catfish, expression profiles of different genes have been selected and studied. For example, the expression of channel catfish TLR3 and TLR5 in response to *E. ictaluri* challenge through immersion has been studied at 2, 5, 8, and 21 days post-challenge and it has been demonstrated that the expression of TLR3 is significantly upregulated in the head kidney of catfish 2 days after immersion challenge (Bilodeau and Waldbieser, 2005). The expression of channel catfish TLR2 in response to *E. ictaluri* challenge through immersion has been studied at 4, 24, and 72 h post-challenge and it has been demonstrated that TLR2 is downregulated at all time points in the head kidney after immersion challenge (Baoprasertkul et al., 2007a), suggesting that anterior kidney play an important role in the hose immune defense system. A recent study has demonstrated that modified live vaccinated catfish are able to control the dispersion of *E. ictaluri* in the anterior kidney (Russo et al., 2009), further suggesting that anterior kidney plays an important role in the immune defense system.

In addition to TLRs, many other genes such as chemokines, antimicrobial peptides, and pro-inflammatory cytokines have been reported to be upregulated in channel catfish challenged by *E. ictaluri* (Peatman et al., 2005, 2006; Chen et al., 2005; Baoprasertkul et al., 2004; Bao et al., 2006; Yeh and Klesius, 2007, 2008). To understand the transcriptional regulation of genes in response to modified live *E. ictaluri* vaccination in the anterior kidney without any preconception of their identities, we used PCR-select suppression subtractive hybridization in this study. Since its first introduction to researchers (Diatchenko et al., 1996), suppression subtractive hybridization has been widely used by researchers in fish innate immunology (Dios et al., 2007; Zhang et al., 2007a,b) and other fields of science (Singh et al., 2008; Zhao et al., 2008; Zhou et al., 2008; Pridgeon et al., 2009) because this technique does not require any previously known genome information for the organism (Sternberg and Gepstein, 2007; Hillmann et al., 2009). We used quantitative PCR (QPCR) to compare the transcriptional levels of genes in vaccinated fish and unvaccinated fish because QPCR has tremendous sensitivity and requires little or no post-amplification processing (Wong and Medrano, 2005). Furthermore, QPCR is a highly reproducible technique for the gel-free detection and quantification of mRNA (Ashton and Headrick, 2007). Using PCR-select subtractive cDNA hybridization technique, we identified 57 different genes from 240 clones of modified live *E. ictaluri* vaccinated *vs.* non-vaccinated channel catfish anterior kidney subtractive library. The transcriptional profiles of the 57 genes in response to *E. ictaluri* vaccination and their putative functions in immune defense were discussed in this study.

2. Materials and methods

2.1. Experimental fish, vaccine strain, and vaccination protocol

Channel catfish fry (NWAC-103 strain) were obtained from the USDA-ARS Catfish Genetic Research Unit, Stoneville, MS and maintained at the USDA-ARS-Aquatic Animal Health Unit at Auburn, AL. Fish were maintained in dechlorinated city water in 340 L tanks to ensure that the catfish fingerlings remained naïve to *E. ictaluri* during grow-out. Catfish fingerlings were grown for 8 months before vaccination and were 186 ± 9 g at time of vaccination. Prior to vaccination fish were moved to 208-L flow-through aquaria and acclimated for 14 days. Fish were vaccinated with AQUAVAC-ESC^{TM} following the established protocol from the manufacturer (Intervet/Schering Plough, Millsboro, DE). The vaccine was based on the modified RE-33 *E. ictaluri* developed by Klesius and Shoemaker (1999). Briefly, the vaccine was thawed at 26 °C in a water bath prior to dilution in 18.9 L of water at 26 ± 2 °C. Three channel catfish (*I. punctatus*) were transferred into one half (9.45 L) of the prepared vaccine bath provided with aeration through air stones via an air blower and held for 2 min. After the 2 min exposure time, an equal volume of water was added and the fish were allowed to remain in the vaccine bath for 15 min prior to release into 208-L flow-through tanks with constant aeration at water temperature of 26 ± 2° C and a 12:12 h light:dark photoperiod. The bacterial concentration was 6.5×10^6 CFU/mL in the vaccine bath. Three additional catfish were sham-vaccinated in immersion water only following the same procedure as above but without the addition of vaccine. All fish were returned to aquarium after vaccination or sham vaccination. At 48 h post-vaccination, fish were euthanized with MS-222 (300 mg/L). The anterior kidney tissue from each fish was collected and flash frozen in liquid nitrogen during collection. All samples were stored at −80 °C until RNA extraction.

2.2. Total RNA extraction and cDNA synthesis

Total RNA was isolated from anterior kidney tissues using TRIzol Reagent (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. All total RNAs were quantified on a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Rockland, DE). Total RNAs were resuspended in distilled water and stored at −80 °C. The first strand cDNAs used for quantitative PCR were synthesized using AMV reverse transcriptase (Invitrogen,