

NOTE

Catfish hybrid *Ictalurus punctatus* × *I. furcatus* exhibits higher resistance to columnaris disease than the parental species

Covadonga R. Arias^{1,*}, Wenlong Cai¹, Eric Peatman², Stephen A. Bullard³

¹Aquatic Microbiology Laboratory, Department of Fisheries and Allied Aquacultures, 253 Upchurch Hall, Auburn University, Auburn, Alabama 36849, USA

²Fish Genetics Laboratory, and ³Parasitology Laboratory, Department of Fisheries and Allied Aquacultures, 203 Swingle Hall, Auburn University, Auburn, Alabama 36849, USA

ABSTRACT: We present experimental data on susceptibility to columnaris disease, caused by the bacterium *Flavobacterium columnare*, in hybrid catfish (female channel catfish *Ictalurus punctatus* × male blue catfish *I. furcatus*) (C×B). Under our experimental conditions, C×B hybrids were significantly more resistant to columnaris disease caused by the highly virulent strain of *F. columnare* BGFS-27 (genomovar II) than channel catfish and blue catfish. Channel and blue catfish cumulative mortalities after immersion challenge were 74 and 87 %, respectively, whereas mortality in the C×B hybrid was 31 %. Susceptibility to the strain ARS-1 (genomovar I) was lower among all catfishes, although channel catfish was the least resistant species at 32 % cumulative mortality. By contrast, C×B hybrid and blue catfishes were strongly resistant to the ARS-1 strain, with <10 % mortality. Our data suggest enhanced disease resistance of the C×B hybrid to columnaris disease.

KEY WORDS: Channel catfish · Blue catfish · *Flavobacterium columnare*

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Channel catfish *Ictalurus punctatus* is the most economically important cultured fish in the USA, accounting for >60 % of all US aquaculture production (www.ers.usda.gov/). Historically, one of the main factors limiting expansion and profitability of the channel catfish industry has been disease control (Wagner et al. 2002, USDA 2003). Current best management practices for the channel catfish industry include prophylactic measures such as vaccination and use of approved therapeutants after disease onset. In addition, improving disease resistance is a main target for genetic stock enhancement programs including strain selection, hybrids, and transgenics.

Hybrids have been investigated for >40 yr (Giudice 1966), but apparently only 1 cross (female channel catfish × male blue catfish *Ictalurus furcatus*, C×B) has proved to exhibit better aquaculture performance than either of its parents (Dunham et al. 2008). Some of the superior characteristics exhibited by C×B hybrids are faster growth, better feed conversion ratios, tolerance to low oxygen (1.0 mg l⁻¹), better harvestability by seining, and higher tolerance to crowding (Masser & Dunham 1998, Dunham et al. 2008). Because of these positive traits, an increasing number of catfish producers have opted to grow C×B hybrids despite the additional costs associated with their production, which requires artificial spawning, in contrast to the pond spawning methods used for

*Email: ariascr@auburn.edu

channel catfish (Masser & Dunham 1998). Regarding disease susceptibility, the resistance of C×B hybrids to enteric septicemia of catfish (ESC) is intermediate between that of channel catfish and that of blue catfish (Wolters et al. 1996), but these hybrids show no difference in resistance to channel catfish virus (CCV), proliferative gill disease (PGD), or freshwater white spot disease (caused by *Ichthyophthirius multifiliis*) (Bosworth et al. 2003, Silverstein et al. 2008, Xu et al. 2011).

No experimental data on susceptibility to columnaris disease in C×B hybrids and blue catfish has been published, but anecdotal observations by Dunham et al. (2008) suggest that C×B hybrids may be less susceptible to columnaris than blue catfish. Columnaris disease, caused by the Gram negative bacterium *Flavobacterium columnare*, is considered the second most important disease affecting the channel catfish industry after ESC. In the present study, we evaluated the susceptibility of C×B hybrid catfish to *F. columnare* as compared to both parental species.

MATERIALS AND METHODS

Fish husbandry

Marion strain channel catfish *Ictalurus punctatus* fingerlings (mean weight: 5.2 ± 0.8 g; mean length: 6.2 ± 1.1 cm), Rio Grande strain blue catfish *I. furcatus* fingerlings (mean weight: 5.8 ± 0.9 g; mean length: 6.7 ± 0.9 cm), and Marion × Rio Grande cross C×B hybrid catfish (mean weight: 5.0 ± 0.9 g; mean length: 6.0 ± 0.7 cm) were obtained from the Fish Genetics Research Unit (Auburn University, Alabama, USA). Each strain (Marion and Rio Grande) was represented by individuals from a single family. The hybrid cross was made using standard techniques (strip spawning of the channel female and sacrifice of the blue male to obtain testes). Each family of fish was reared in individual cells (small concrete enclosures) within the partitioned aquaculture system at Clemson University (South Carolina, USA). All cells were placed within a 2 acre pond and shared water by constant exchange through each cell by means of air-lift pumps and a paddle wheel. This rearing system delivers uniform exposure to water quality conditions and the pond microbial environment (Baumgarner et al. 2005). No disease-based mortality was recorded during the course of the fingerling grow-out. Upon arrival at Auburn University, fish were stocked into 37 l aquaria at 15 fish per tank,

acclimatized for 5 d before challenge, and fed daily to satiation with AQUAMAX Grower 400 (Purina Mills). Ten randomly selected individuals of each fish species were examined and proved culture negative for *Flavobacterium columnare* prior to stocking in the tanks. Each aquarium had an individual biofilter and an air stone. Water was checked daily to maintain established parameters (80 ppm alkalinity, 40 ppm hardness, 0.1 ppt salinity, $27 \pm 1^\circ\text{C}$, pH 7.8 ± 0.2 [mean \pm SE], and ammonia and nitrites non-detectable). Tanks were filled with artificial freshwater prepared using 0.97 g of CaCO_3 , 2.26 g of NaHCO_3 , and 110 ml of primary seasalt stock into 55 l of deionized water. Seasalt stock was made using 340 g of Marine Salt (Seachem) in 10 l of deionized water.

Bacterial strains and challenge experiments

Two previously characterized strains of *Flavobacterium columnare*, ARS-1 (genomovar I) and BGFS-27 (genomovar II), were used (Arias et al. 2004, Olivares-Fuster et al. 2007b). A genomovar is defined as a genomic group that shows <70% reassociation by DNA:DNA hybridization with other genomic group(s) within a nomenclature but cannot be differentiated by phenotypic traits (Ursing et al. 1995). Up to 3 different genomovars have been described in the species *F. columnare* (Triyanto & Wakabayashi 1999). Both strains were originally isolated from channel catfish, and experimental challenges have shown ARS-1 as a low virulence strain, while BGFS-27 has proved to be highly virulent in channel catfish (Shoemaker et al. 2008). Strains were routinely cultured in modified Shieh broth (Shoemaker et al. 2005) for 24 h at 26°C with gentle shaking. Stock suspensions of all isolates were made by supplementing 24 h cultures (grown in Shieh broth) with glycerol up to a final concentration of 20%, and kept at -80°C for long-term storage.

Two challenge experiments were conducted, one with each *Flavobacterium columnare* strain. Each experiment consisted of 3 treatments (channel, blue, and C×B hybrid catfish) and 3 unchallenged control treatments (channel, blue and C×B hybrid catfish). Each treatment consisted of 3 randomized replicates (tanks). The immersion challenge was carried out as described in Shoemaker et al. (2008), with approximately 5×10^6 colony-forming units (CFU) ml^{-1} of the pathogen in the immersion bath. Controls were exposed to modified Shieh broth without bacteria. After a 30 min challenge, fish were returned to their individual aquaria and monitored at 12 h intervals for

abnormal behavior, loss of appetite, and mortality. Moribund fish were sampled for *F. columnare* following standard protocols (Thoesen 2004). Putative *F. columnare* colonies were confirmed by specific PCR (Welker et al. 2005) and ascribed to the corresponding genomovar according to Olivares-Fuster et al. (2007a). Experiments were terminated and fish were euthanized with a lethal dose of tricaine methanesulfonate (MS-222; 300 mg l⁻¹) after no sign of disease had been observed for longer than 48 h and all remaining fish in the tank appeared healthy.

Mortality data were compared by ANOVA using the Duncan's multiple range test (SAS Institute) to determine significant differences ($p < 0.05$) between the means.

RESULTS

Acute columnaris disease was successfully induced in the 2 species of catfish (*Ictalurus punctatus* and *I. furcatus*) and their hybrid after challenge with either *Flavobacterium columnare* strain. Columnaris signs include the development of pale or discolored areas beneath the dorsal fin toward the pectoral fins and posterior to the pelvic fin that, in most cases, evolved into typical saddleback lesions (Grizzle & Rogers 1976, Bullard et al. 2011). Gross clinical signs were similar among CxB hybrids, channel catfish, and blue catfish, and *F. columnare* colonies were isolated and confirmed by PCR from all diseased fish. Table 1 summarizes the cumulative mortalities observed in all 3 catfish species. Challenge doses were 1.0×10^6 and 9.6×10^6 CFU ml⁻¹ for BGFS-27 and ARS-1 strains, respectively. No mortality occurred in any of the unchallenged control tanks. Cumulative mean mortalities obtained with genomovar II strain BGFS-27 were significantly lower in CxB hybrids (31%) than in both parental species. Blue catfish appeared

Table 1. Cumulative percent mortality (means \pm SE) from CxB hybrid catfish and parental species, channel catfish *Ictalurus punctatus* and blue catfish *I. furcatus* after challenge with *Flavobacterium columnare* BGFS-27 (genomovar II) and ARS-1 (genomovar I) strains. Within a column, means followed by different superscript letters are significantly different ($p < 0.05$)

Catfish species	<i>F. columnare</i> BGFS-27	<i>F. columnare</i> ARS-1
CxB catfish hybrid	31 (± 7.7) ^a	9 (± 2.4) ^a
Channel catfish (Marion)	74 (± 5.2) ^b	32 (± 4.5) ^b
Blue catfish (Rio Grande)	87 (± 8.3) ^b	4 (± 2.8) ^a

as the most sensitive species to this strain, exhibiting a cumulative mortality of 87%, although this value was not significantly different from the observed channel catfish mortality (74%). Challenge with the genomovar I strain resulted in fish showing the clinical signs of columnaris disease but with a lower mortality rate in all 3 catfish species. Channel catfish was the most susceptible species to ARS-1, with a 32% cumulative mortality. Blue catfish and CxB hybrids were significantly less susceptible to ARS-1, showing minimal mortalities at 4 and 9%, respectively.

Fig. 1 shows the mortality progression caused by *Flavobacterium columnare* strain BGFS-27 in all 3 species. Channel and blue catfish began dying within 48 h post-challenge, while no mortality was observed in CxB hybrids until Day 4. Mortality was more severe in channel catfish than in blue catfish during the first 6 d post-challenge, but more blue catfish succumbed to columnaris disease by the end of the experiment. CxB hybrids experienced a longer incubation phase, and mortalities plateau at 31%. Channel catfish was the most susceptible species to genomovar I strain ARS-1, with mortalities starting within 48 h post-challenge and reaching 32% by Day 14 (Fig. 2). Blue catfish and CxB hybrids were refractive to colonization by ARS-1, with no mortalities observed until Day 7. Blue catfish exhibited the highest survival rate of all species, but this was not significantly different from CxB hybrids. Both blue catfish and CxB hybrids were significantly more resistant to *F. columnare* genomovar I strain ARS-1 than channel catfish.

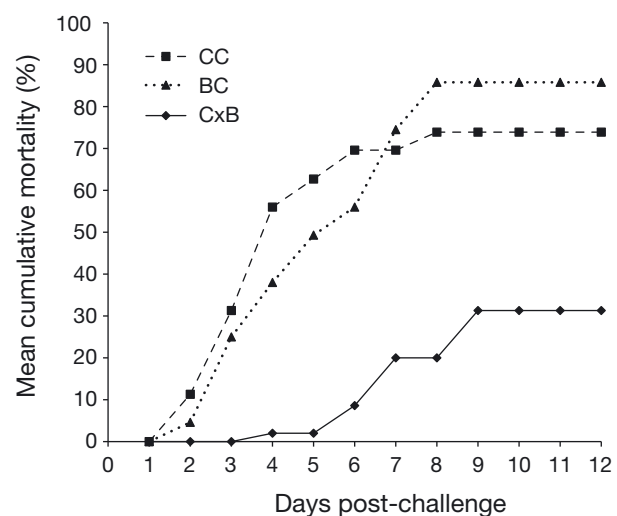


Fig. 1. Mean cumulative mortality of channel catfish *Ictalurus punctatus* (CC), blue catfish *I. furcatus* (BC), and CxB hybrid catfishes following immersion challenge with *Flavobacterium columnare* BGFS-27 strain (genomovar II). Infectious dose was 1.0×10^6 CFU ml⁻¹

catfish. Second, the studied C×B hybrid outperformed both parental species when challenged with a highly virulent *Flavobacterium columnare* strain.

Acknowledgements. We thank Stacey LaFrenz for facilitating the immersion challenge and helping with fish husbandry. This work was supported in part by Alabama Agricultural Experiment Station HATCH funds awarded to Stephen A. Bullard, Covadonga R. Arias, and Eric Peatman (Auburn University) and by the USDA-ARS/Auburn University Specific Cooperative Agreement 'Prevention of Diseases of Farmed Raised Fish' and USDA-ARS CRIS Project No. 6420-32000-022-00D awarded to Covadonga R. Arias (Auburn University). Wenlong Cai thanks Shanghai Ocean University (Shanghai, China) for partially funding his graduate research assistantship.

LITERATURE CITED

- Arias CR, Welker TL, Shoemaker CA, Abernathy JW, Klesius PH (2004) Genetic fingerprinting of *Flavobacterium columnare* isolates from cultured fish. *J Appl Microbiol* 97:421–428
- Baumgarner BL, Schwedler TE, Eversole AG, Brune DE, Collier JA (2005) Production characteristics of channel catfish, *Ictalurus punctatus* stocked at two densities in the partitioned aquaculture system. *J Appl Aquacult* 17:75–83
- Bosworth BG, Wise DJ, Terhune JS, Wolters WR (2003) Family and genetic group effects for resistance to proliferative gill disease in channel catfish, blue catfish, and channel catfish × blue channel backcross hybrids. *Aquacult Res* 34:569–573
- Bullard SA, McElwain A, Arias CR (2011) Scanning electron microscopy of 'saddleback' lesions associated with experimental infections of *Flavobacterium columnare* in channel catfish, *Ictalurus punctatus* (Siluriformes: Ictaluridae) and zebrafish, *Danio rerio* (Cypriniformes: Cyprinidae). *J World Aquacult Soc* 42:906–913
- Decostere A, Haesebrouck F, Charlier G, Ducatelle R (1999) The association of *Flavobacterium columnare* strains of high and low virulence with gill tissue of black mollies (*Poecilia sphenops*). *Vet Microbiol* 67:287–298
- Dunham RA, Umali GM, Beam R, Kristanto AH, Trask M (2008) Comparison of production traits of the NWAC-103 channel catfish, NWAC103 channel × blue hybrid catfish, Kansas select 21 channel catfish and blue catfish grown at commercial densities and exposed to natural bacterial epizootics. *N Am J Aquac* 70:98–106
- Giudice JJ (1966) Growth of a blue × channel catfish hybrid as compared to its parent species. *Prog Fish Cult* 28:142–145
- Grizzle JM, Rogers WA (1976) Anatomy and histology of the channel catfish. Agricultural Experiment Station, Auburn University Press, Auburn, AL
- Masser M, Dunham R (1998) Production of hybrid catfish. Southern Regional Aquaculture Center 190, Stoneville, MS
- Olivares-Fuster O (2010) Development, characterization and early evaluation of new modified live vaccines against columnaris disease. Auburn University Press, Auburn, AL
- Olivares-Fuster O, Shoemaker CA, Klesius PH, Arias CR (2007a) Molecular typing of *Flavobacterium columnare* isolates by single stranded conformation polymorphism analysis. *FEMS Microbiol Lett* 269:63–69
- Olivares-Fuster O, Baker JL, Terhune JS, Shoemaker CA, Klesius PH, Arias CR (2007b) Host-specific association between *Flavobacterium columnare* genomovars and fish species. *Syst Appl Microbiol* 30:624–633
- Olivares-Fuster O, Bullard SA, McElwain A, Llosa MJ, Arias CR (2011) Adhesion dynamics of *Flavobacterium columnare* to channel catfish (*Ictalurus punctatus*) and zebrafish (*Danio rerio*) after immersion challenge. *Dis Aquat Org* 96:221–227
- Scribner KT, Page KS, Bartron ML (2000) Hybridization in freshwater fishes: a review of case studies and cytonuclear methods of biological inference. *Rev Fish Biol Fish* 10:293–323
- Shoemaker CA, Arias CR, Klesius PH, Welker TL (2005) Technique for identifying *Flavobacterium columnare* using whole-cell fatty acid profiles. *J Aquat Anim Health* 17:267–274
- Shoemaker CA, Olivares-Fuster O, Arias CR, Klesius PH (2008) *Flavobacterium columnare* genomovar influences mortality in channel catfish (*Ictalurus punctatus*). *Vet Microbiol* 127:353–359
- Shoemaker CA, Klesius PH, Drennan JD, Evans JJ (2011) Efficacy of a modified live *Flavobacterium columnare* vaccine in fish. *Fish Shellfish Immunol* 30:304–308
- Silverstein PS, Bosworth BG, Gaunt PS (2008) Differential susceptibility of blue catfish, *Ictalurus furcatus* (Valenciennes), channel catfish *I. punctatus* (Rafinesque), and blue × channel hybrids to channel catfish virus. *J Fish Dis* 31:77–79
- Soto E, Mauel MJ, Karsi A, Lawrence ML (2008) Genetic and virulence characterization of *Flavobacterium columnare* from channel catfish (*Ictalurus punctatus*). *J Appl Microbiol* 104:1302–1310
- Thoesen JC (2004) Suggested procedures for the detection and identification of certain finfish and shellfish pathogens. American Fisheries Society — Fish Health Section, Bethesda, MD
- Triyanto K, Wakabayashi H (1999) Genotypic diversity of strains of *Flavobacterium columnare* from diseased fishes. *Fish Pathol* 34:65–71
- Ursing JB, Rosello-Mora R, Garcia-Valdes E, Lalucat J (1995) Taxonomic note: a pragmatic approach to the nomenclature of phenotypic similar genomic groups. *Int J Syst Bacteriol* 45:604
- USDA (United States Department of Agriculture) (2003) Part I: reference of fingerling catfish health and production practices in the United States. USDA, National Animal Health Monitoring System, Fort Collins, CO
- Wagner BA, Wise DJ, Khoo LH, Terhune JS (2002) The epidemiology of bacterial diseases in food-size channel catfish. *J Aquat Anim Health* 14:263–272
- Welker TL, Shoemaker CA, Arias CR, Klesius PH (2005) Transmission and detection of *Flavobacterium columnare* in channel catfish *Ictalurus punctatus*. *Dis Aquat Org* 63:129–138
- Wolters WR, Wise DJ, Klesius PH (1996) Survival and antibody response of channel catfish, blue catfish, and channel catfish female × blue catfish male hybrids after exposure to *Edwardsiella ictaluri*. *J Aquat Anim Health* 8:249–254
- Xu DH, Klesius PH, Peatman E, Liu Z (2011) Susceptibility of channel catfish, blue catfish, and channel × blue catfish hybrid to *Ichthyophthirius multifiliis*. *Aquaculture* 311:25–30