



NOTE

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

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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*

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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

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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, $27 \pm 1^\circ\text{C}$, $\text{pH } 7.8 \pm 0.2$ [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 nomenespecies 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 posteriorly 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.

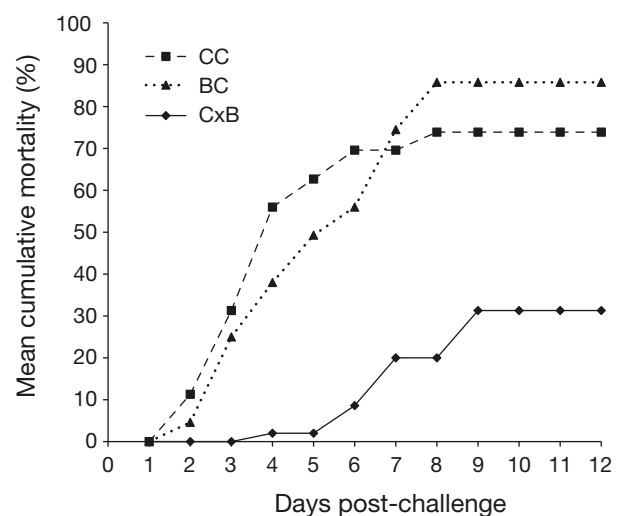


Fig. 1. Mean cumulative mortality of channel catfish *Ictalurus punctatus* (CC), blue catfish *I. furcatus* (BC), and CxB hybrid catfishes following immersion challenge with *Flavobacterium columnare* BGFS-27 strain (genomovar II). Infectious dose was 1.0×10^6 CFU ml⁻¹

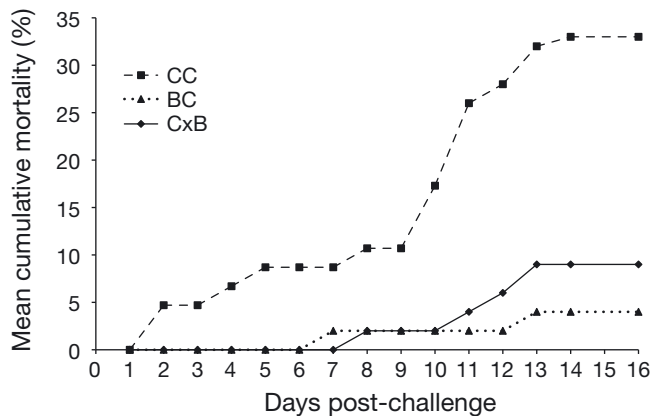


Fig. 2. Mean cumulative mortality of channel catfish *Ictalurus punctatus* (CC), blue catfish *I. furcatus* (BC), and CxB hybrid catfish following immersion challenge with *Flavobacterium columnare* ARS-1 strain (genomovar I). Infectious dose was 9.6×10^6 CFU ml⁻¹

DISCUSSION

Our results show a significant difference in resistance to columnaris disease between 2 species of catfish (*Ictalurus punctatus* and *I. furcatus*) and their hybrid under experimental challenge conditions. This is the first study in which columnaris disease has been experimentally re-created in CxB hybrid and blue catfish. The CxB hybrid was more resistant to infection by *Flavobacterium columnare* BGFS-27 strain than both parental species, supporting the premise of hybrid vigor (Scribner et al. 2000). In a previous study, we showed a marked difference in virulence between strains of *F. columnare* genomovar I and II when we infected channel catfish by immersion (Shoemaker et al. 2008). We selected BGFS-27 as representative of genomovar II for the present study because it has been shown to attach and colonize channel catfish tissues at a higher rate than the ARS-1 strain (Olivares-Fuster et al. 2011). Originally isolated from wild channel catfish (Mobile River, Alabama, USA), BGFS-27 consistently achieves >70% mortality in channel catfish in our challenge model (Olivares-Fuster 2010). The mortalities observed in the present study were therefore expected for channel catfish. Mortalities in blue catfish and in channel catfish were not significantly different, both reaching ~80%. By contrast, onset of the disease in CxB hybrids lagged for 3 d and only reached 31% mortality.

Results obtained with the genomovar I strain ARS-1 were markedly different. Previous studies have shown that ARS-1 can cause up to 46% mortality in channel catfish fry (Shoemaker et al. 2008), but <40% in channel fingerlings (Shoemaker et al. 2011).

However, the high standard errors reported in those studies make comparisons difficult between ARS-1 virulence in channel catfish fry and fingerlings (Shoemaker et al. 2008, 2011). Under our challenge conditions, we typically obtained 30% mortality when challenging channel catfish fingerlings with ARS-1 (data not shown). In the present study, the cumulative mortality obtained with ARS-1 in channel catfish was within the expected values, while CxB hybrid susceptibility to ARS-1 was significantly lower than in channel catfish. Interestingly, blue catfish showed the lowest mortality when challenged with *Flavobacterium columnare* genomovar I strain ARS-1 (although not statistically significant from the cumulative mortality observed in the CxB hybrid). This result contradicts some published literature in which blue catfish is considered more susceptible to columnaris disease than channel catfish (Masser & Dunham 1998, Dunham et al. 2008). However, these are both review papers in which no experimental data or primary references regarding blue catfish resistance to columnaris were provided. According to our data, blue catfish is equally or less susceptible to columnaris diseases than channel catfish, depending on the strain tested.

It is not surprising that *Flavobacterium columnare* genomovar II is highly virulent for both channel and blue catfish since this genomovar has been associated almost exclusively with these fish species in the wild (Olivares-Fuster et al. 2007b). Genomovar I has also been isolated from wild channel and blue catfish, but at a much lower rate (Olivares-Fuster et al. 2007b).

Our results emphasize the importance of testing >1 bacterial strain from the same bacterial species when conducting pathogenicity studies, since blue catfish can be considered highly susceptible or highly resistant to columnaris disease depending on the strain used for challenge. This is of particular importance with columnaris disease, since at least 2 markedly distinctive genetic groups exist (Decostere et al. 1999, Arias et al. 2004, Soto et al. 2008). Similarly, future examination of the susceptibility profiles of different strains of channel catfish, blue catfish, and their resulting hybrids to columnaris is needed to broaden our understanding regarding the contribution of fish strain to disease resistance. Challenges of different channel catfish strains with *Edwardsiella ictaluri*, for example, have revealed significant differences in survival rates among strains (Wolters et al. 1996).

Two main conclusions result from the present study. First, channel catfish were equally or more susceptible to columnaris disease compared to blue

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

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