Susceptibility of Amberjack (*Seriola dumerili*) to Bacterial Fish Pathogens

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Abstract

Amberjack (*Seriola dumerili*) susceptibility to six bacterial fish pathogens (*Lactococcus garvieae, Streptococcus parauberis, Photobacterium damselae* subsp. *damselae, Photobacterium damselae* subsp. *piscicida, Vibrio vulnificus* biotype 2 and *Vibrio parahaemolyticus*) was tested by means of experimental infections, and LD_{50} was calculated. All strains tested were virulent, and symptoms appeared at different times depending on the bacteria injected, and mortalities began 2 to 10 days post-challenge. External signs included haemorrhages, exophthalmia and, occasionally, ulceration.

Introduction

Amberjack (*Seriola dumerili*) is a marine fish which inhabits the Mediterranean and Atlantic European coasts. This fish species has been cultured in Spain since 1980, particularly in Balearic Islands and Murcia (Crespo *et al*., 1994), and it is listed as one of the preferred species for Spanish aquaculture because of its fast growth rate, both in tanks and in sea cages, and its commercial interest (García *et al*., 1995). Bacterial diseases are a serious problem in the species *Seriola quinqueradiata* cultured in Japan, where streptococcosis, caused by *Lactococcus garvieae* (formerly *Streptococcus seriolicida*), is the major bacterial disease (Kusuda *et al*., 1992, Kusuda and Salati, 1993). The species *Photobacterium damselae* subsp. *piscicida* has also been reported as an important pathogen of both *S. quinqueradiata* and *S. dumerili* cultured in Japan (Kubota *et al*., 1970, Kawahara *et al*., 1986). However, there are no references related to bacterial diseases affecting *S. dumerili* cultured outside Japan. The study of bacterial pathogens affecting amberjack is of great interest given the increased culture of this fish species.

The aim of this work is to carry out a preliminar evaluation on the susceptibility of *S. dumerili* to different bacterial pathogens, by means of experimental infections. We included bacterial pathogens of *S. quinqueradiata*, and those associated with bacterial diseases of fish in Spain, such as *Vibrio vulnificus* biotype 2/serovar E (Biosca *et al*., 1991), *V. parahaemolyticus* (Alcaide *et al*., 1999), *Ph. damselae* subsp. *damselae* (Fouz *et al*., 1992) and *Streptococcus parauberis* (Toranzo *et al*., 1994). Moreover, we included two strains belonging to the species *V. parahaemolyticus* and

Strain	Origin	Virulence	
		Intraperitoneal	Immersion
Streptococcus parauberis RA 137-1	Diseased turbot, Spain	3.5×10^5	ND
Lactococcus garvieae ATCC 49156	Diseased yellowtail, Japan	4.6×10^4	ND
Photobacterium damselae subsp. *damselae* RG191	Diseased turbot, Spain	2.0×10^4	3.6×10^7
Photobacterium damselae subsp. *damselae* LP1	Diseased amberjack, Spain	3.6×10^4	6.2×10^7
Vibrio vulnificus biotype 2 E86	Diseased eel, Spain	1.0×10^5	2.4×10^8
Vibrio parahaemolyticus C3	Diseased toothcarp, Spain	5.0×10^3	ND
Vibrio parahaemolyticus GP2	Diseased amberjack, Spain	8.2×10^3	9.3×10^6
Photobacterium damselae subsp. *piscicida* ATCC 17911	Epizootic of white perch *Roccus americanus*, USA	3.0×10^3	8.7×10^5

Table 1. Bacterial strains used in experimental infections. Virulence is expressed as no. of bacteria (cfu/ fish in i.p. and cfu/ml in bath challenge) needed to kill 50% of the inoculated fish in a 15 day period. ND= not determined.

Ph. damselae subsp. *damselae*, isolated from different outbreaks of low mortality which occurred in amberjack cultured in Spain.

Material and Methods

Bacterial strains and growth conditions

Bacterial strains used in this work are listed in Table 1. *Vibrio* and *Photobacterium* species were routinely cultured on tryptone-soy-agar (TSA, Oxoid) or tryptone-soy-broth (TSB, Oxoid) supplemented with 3% NaCl (TSA-3 and TSB-3), and gram positive strains were grown on brain-hearth agar (BHI, Difco) supplemented with 3% NaCl (BHI-3).

Virulence tests

The infectivity tests were conducted by both intraperitoneal (i.p.) injection and bath challenge as previously described (Amaro *et al*., 1992, Esteve *et al*., 1993). In i.p. infections, six fish (mean weight 50g/ fish) per dose, were injected with 0.2 ml of a bacterial suspension in phosphate buffer saline pH 7 (PBS, pH 7), containing 10^8 to 10^3 cfu/ ml as determined by plate counts. Sterile PBS was injected i.p. into 6 fish as a control. Fish were held in tanks of 100 L in artificial sea water at 20°C with aeration. In the bath challenge, groups of six fish were exposed for 60 min to serial 10-fold dilution of bacteria, ranging 10^9-10^4 cfu/ml, in a final volume of 50 L of artificial sea water. After challenge, each group of fish was placed in a new aquarium, and the number of bacteria in the challenge suspension was determined by plate-counts in TSA-3. Infectivity experiments were carried out in duplicate. Mortalities were recorded daily for 15 d, and were only considered if inoculated bacteria were recovered from challenged fish. The LD_{50} was calculated by the Reed and Muench method (1938).

Results and Discussion

The results of experimental infections are recorded in Table 1. All strains tested were virulent showing lethal doses between $3.5x10^5$ and $3x10^3$ cfu / fish in i.p. infections, and between $8,7x10^5$ and $2,4x10^8$ in bath challenge. Pure cultures of the strain used in each experiment were reisolated from internal organs (liver and kidney) as well as ulcers of moribund fish. No mortality was detected in the controls.

As could be expected, fish were susceptible to *Ph. damselae* subsp. *piscicida* and *L. gravieae,* described as the main bacterial pathogens for yellowtail cultured in Japan (Kubota *et al*., 1970, Kawahara *et al*., 1986, Kusuda *et al*., 1992, Kusuda and Salati, 1993). *Ph. damselae* subsp. *piscicida* gave the lowest LD_{50} values (Table 1) which agrees with the virulence of this bacterium to various fish species such as filefish, yellowtail and striped jack (Kawakami and Sakai, 1999). Moreover pasteurellosis has been reported in cultured amberjack in Japan (Kawahara *et al*., 1986). The signs presented by fish inoculated with *Ph. damselae* subsp. *piscicida* and *L. garvieae* were similar to previously described for these pathogens (Kubota *et al*., 1970, Kusuda *et al*., 1976).

Amberjack was also susceptible to the other pathogens tested: *V. vulnificus* biotype 2, *S. parauberis, Ph. damselae* subsp. *damselae* and *V. parahaemolyticus*. These strains were isolated from different diseased fish in Spain, including diseased amberjack. The degree of virulence demonstrated by the different species was similar independent of their origin. Fish inoculated with *Ph. damselae* subsp. *damselae* showed a characteristic external reddening of the mouth as previously described (Fouz *et al*., 1992), and pale liver and ascitic fluid were observed as internal signs. The effects of *V. vulnificus* biotype 2 and *V. parahaemolyticus* were similar: haemorrhages, ulcers and pale liver. The most pronounced sign of fish infected with *S. parauberis* was a marked exophthalmia and internally fish showed ascitic fluid and pale liver.

The strains *V. parahaemolyticus* GP2 and *Ph. damselae* subsp. *damselae* LP1 (Table 1) were isolated from kidney and ascitic fluid respectively in two different episodes of mortality that occurred in the hatchery where sample fish were taken. The virulence of these strains demonstrates their role as causative agents of the mentioned outbreaks.

External signs and mortalities began at different times after infection depending on the strain used. Fish inoculated with *Ph. damselae* subsp. *piscicida* were the first to show signs and died 1-2 days after injection. When *L. garvieae, S. parauberis* and *Ph. damselae* subsp. *damselae* were injected, external signs appeared 2 days after infection and mortalities began 1-2 days later. Fish infected with vibrios were the latest to show external signs (5-6 days) and mortality (7-10 days), especially when *V. parahaemolyticus* was used.

Finally, the results obtained in this study indicate that amberjack can be affected by the pathogens tested, which constitute a health hazard as some of these species have been isolated from the water where fish are cultured.

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