Aquaculture 396–399 (2013) 8–13

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Bacterial diseases of cultured Mediterranean horse mackerel (*Trachurus mediterraneus*) in sea cages

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ARTICLE INFO

Article history:
Received 28 January 2013
Received in revised form 15 February 2013
Accepted 18 February 2013
Available online 26 February 2013

Keywords:
Aeromonas hydrophila
Vibrio vulnificus
Photobacterium damselae damselae
V. alginolyticus
Antibiotic resistance gene

ABSTRACT

We initiated the first cage culture of the Mediterranean horse mackerel (*Trachurus mediterraneus*) in the Black Sea. The wild fish was captured by purse seine and transferred into sea cages. Fish were fed by sea bass feed during grow out period, but fish mortalities were observed. However, the causes of mortalities were unknown. Therefore, cultured Mediterranean horse mackerel have been sampled monthly for two years to determine possible parasitic and bacterial agents. During the study, no parasite was found while several Gram negative bacterial species including *Aeromonas hydrophila*, *Chryseobacterium indologenes*, *Vibrio vulnificus*, *Bulkholderia cepacia*, *Photobacterium damselae damselae* and *Vibrio alginolyticus* were isolated from cultured fish. Antibiotic resistance analyses showed that more than 50% of the bacteria were resistant to streptomycin, sulfamethoxazole, gentamycin, cephalothin, and ampicillin while they were very sensitive to florfenicol and chloramphenicol. The most prevalent resistance genes were found to be beta-lactam ($bla_{TEM\text{-}OT3\text{-}4}$) and Tetracycline (*tetB*). This is the first study reporting isolation and antibiotic sensitivities of bacterial species in horse mackerel, which is expected to be a valuable source in predicting possible disease problems in horse mackerel aquaculture.

1. Introduction

Mariculture is an emerging aquaculture sector requiring innovative scientific and technical developments. In 2010, the world mariculture production was approximately of 37.1 million tons, representing about 48% of capture fisheries used for human consumption (FAO, 2012). Currently, more than 30 marine fish species are being cultured mostly in net cages at shallow areas. In industrialized countries, the main objective is the production of high-value products for human consumption, which are not obtained in sufficient quantity from fishing. The Mediterranean horse mackerel is high-value marine fish, which is distributed throughout the Mediterranean, Marmara and Black Seas, as well as along the eastern Atlantic coast from Morocco to the English Channel (Wheeler, 1987). Recently, we have initiated culture of the Mediterranean horse mackerel (*Trachurus mediterraneus* Steindachner, 1868) in the Black Sea to introduce a new high-value species in mariculture.

Success of aquaculture depends on efficient fish health management programs. In spite of efforts to develop new drugs and improve culture techniques, bacterial diseases are still one of the most serious problems in marine aquaculture in the world (Kusuda and Kawai, 1998). Frequent use of antibiotic drugs is always accompanied with occurrence of drug resistant bacteria, and several mechanisms for acquiring resistance in fish pathogenic bacteria were reviewed by (Aoki, 1992). Resistance of fish pathogens to drugs develops not only year by year but also seasonally in a year (Kusuda and Kawai, 1998).

Although there is no horse mackerel fish farming under controlled conditions in the world yet, we have initiated see cage culture trials using captured horse mackerel in the Southern Black Sea Region of Turkey. During the cultivation of fish, disease outbreaks and high mortalities were observed. Therefore, we aimed to diagnose the diseases in horse mackerel. Antibiotic resistance genes including tetracycline (*tetA*, *B*, *C*, *D*), sulphonamide (*sul1*, *sul2*, *sul3*), beta-lactam (*ampC*, bla_{TEM}, bla_{PSE}), trimetophrim (*dhfr1*), aminoglycoside (*aadA*), and chloramphenicol (*cmlA*) were also analyzed by PCR to better understand the dissemination of resistance genes in the bacteria isolated from horse mackerel.

2. Materials and methods

2.1. Experimental fish

Because the Turkish fisheries legislation does not allow capture of the Mediterranean horse mackerel less than 13 cm (total length), captured fish that are 13 cm or larger were stocked into sea cages (14 m diameter and 7 m depth) for feeding until they reach 100 g or more. After transferring fish to cages, fish were fed by sea bass feed twice a day (Gumusdoğa, Mugla, Turkey) at 1% body weight. Stocking rate was between 4 and 5 kg/m^3. During the growth period, fish mortalities were observed. Therefore, to diagnose or prevent the diseases horse mackerel that weighed 78.61 ± 13.83 g (mean ± SD) and were 21.25 ± 1.11 cm in total length were sampled monthly for

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http://dx.doi.org/10.1016/j.aquaculture.2013.02.025

Table 1
Water quality characteristics (mean ± SD) during the sampling period.

	Season			
	Spring	Summer	Fall	Winter
Dissolved oxygen (mg/l)	10.83 ± 0.76	8.47 ± 0.72	8.27 ± 0.21	9.83 ± 0.76
Temperature (°C)	12.8 ± 3.3	25.8 ± 0.6	20.2 ± 3.4	9.3 ± 0.8
pH	8.23 ± 0.10	8.35 ± 0.20	8.28 ± 0.23	8.28 ± 0.18
Salinity (‰)	17.32 ± 0.03	17.30 ± 0.10	17.90 ± 0.26	17.73 ± 0.15

parasites and bacteria for two years (2011–2013). Wild Water characteristics (Table 1) were measured every month during the experiments. Dissolved oxygen and water temperature were measured with a WTW 330i polarographic oxygen meter and thermistor (WTW Wissenschaftlich-Technische Werkstatten GmbH, Weilheim, Germany), pH with a WTW 330 glass electrode (WTW Wissenschaftlich-Technische Werkstatten), and salinity with Hach Lange HQ40D model salinometer (Düsseldorf, Germany).

2.2. Bacterial examination of fish

During the two years, 240 fish were sampled during 24 samplings; the samples usually consisted of 10 fish per month. In addition, 30 fish were sampled as a result of disease outbreaks. Fish were randomly sampled when diseased fish were not present in the cages. The use of fish and the experimental protocol were approved by the Animal Experimentation Ethics Committee of the Karadeniz Technical University (KTUAEEC). Fish were randomly sampled when diseased fish were not present in the cages. All sampled fish were examined externally and internally. The gills and body surface were examined microscopically for the presence of parasites and bacteria. After that to prevent contamination of the culture by normal external bacterial flora, body surface of the fish was disinfected by swabbing the lesion with 70% ethyl alcohol. In the lesion, incision was made with sterile scalpel and flamed loop was inserted in the incision and aseptically streaked on Tryptic Soy Agar (TSA) and Marine Agar (MA) and incubated at 20–25 °C for 2–3 days. Liver, trunk kidney, and spleen, were also aseptically streaked on TSA and MA. After incubation at 22 °C for 2 days, bacteria isolated from fish were subcultured on the same medium to check the purity of the isolate when bacterial colonies were overloaded on the first culture medium. Pure cultured colonies were biochemically characterized with API 20NE (Biomerieux, Marcy l'Etoile, France) and the following biochemical tests: Gram staining, cytochrome–oxidase, catalase, ß-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophane deaminase, indole production, voges proskauer, gelatinase, fermentation of glucose, mannitol, inositol, sorbitol, rhamnose, saccharose, amygdalin, methyl red, arabinose, lactose, esculin, xylose, motility, and oxidative/fermentative tests. Isolates were identified to the genus or species level by standard bacterial taxonomy procedures (Austin and Austin, 2007; Holt et al., 1994; Krieg and Holt, 1984). Isolates were stored in a broth culture supplemented with 15% glycerol at −70 °C.

2.3. Antibiotic susceptibility

Antibiotic susceptibility tests were performed by the disk diffusion method using 9 mm diameter commercial disks (Bioanalyse, Ankara, Turkey) on Mueller–Hinton Agar (MHA) plates (Oxoid Ltd., Basingstoke, U.K.) according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2009). This was done by streaking the surface of Mueller–Hinton agar plates uniformly with 0.5 Mc Farland standardized inoculum and thereafter exposing them to disks impregnated with known concentrations of antimicrobial substances. Commercial antibiotic disks used in the study included sulphamethoxazole/trimethoprim (25 μg), streptomycin (10 μg), tetracycline (30 μg), oxytetracycline (30 μg), chloramphenicol (30 μg), sulphamethoxazole (100 μg), gentamycin (10 μg), cephalothin (30 μg), ampicillin (25 μg) and florfenicol (30 μg). The antibiotic disks were placed on the agar using a disc dispenser (Bioanalyse). Plates were inverted and incubated at 25 °C for 24 h. Then the inhibition zone diameters were measured to the nearest millimeter, and the strains were characterized as susceptible, intermediate or resistant to the antibiotics based on the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2009).

Table 2
Primers used in the PCR reactions.

Primer name	Sequences (5′–3′)	Target Gene or Region	PCR product size (bp)	Annealing temperature (°C)	References
Tet A FW	GCTACATCCTGCTTGCCTTC	*tetA*	210	55	Ng et al., 2001
Tet A RV	CATAGATCGCCGTGAAGAGG				
Tet B FW	TTGGTTAGGGGCAAGTTTTG	*tetB*	659	54	Ng et al., 2001
Tet B RV	GTAATGGGCCAATAACACCG				
Tet C FW	CTTGAGAGCCTTCAACCCAG	*tetC*	418	55	Ng et al., 2001
Tet C RV	ATGGTCGTCATCTACCTGCC				
Tet D FW	AAACCATTACGGCATTCTGC	*tetD*	787	54	Ng et al., 2001
Tet D RV	GACCGGATACACCATCCATC				
Sul1 FW	CGGCGTGGGCTACCTGAACG	*sul1*	433	59	Kerrn et al., 2002
Sul1 RV	GCCGATCGCGTGAAGTTCCG				
Sul2 FW	GCGCTCAAGGCAGATGGCATT	*sul2*	293	59	Kerrn et al., 2002
Sul2 RV	GCGTTTGATACCGGCACCCGT				
Sul3 FW	TCAAAGCAAAATGATATGAGC	*sul3*	787	48	Heuer and Smalla, 2007
Sul3 RV	TTTCAAGGCATCTGATAAAGAC				
AmpC FW	TTCTATCAAMACTGGCARCC	*ampC*	550	48	Schwartz et al., 2003
AmpC RV	CCYTTTTATGTACCCAYGA				
TEM OT-1 FW	TTGGGTGCACGAGTGGGTTA	bla_{TEM}	465	54	Arlet and Philippon, 1991
TEM OT-2 RV	TAATTGTTGCCGGGAAGCTA				
TEM OT-3 FW	ATGAGTATTCAACATTTCCG	bla_{TEM}	859	45	Olesen et al., 2004
TEM OT-4 RV	CAATGCTTAATCAGTGAGG				
PSE1 FW	CGCTTCCCGTTAACAAGTAC	bla_{PSE}	465	50	Zühlsdorf and Wiedemann, 1992
PSE1 RV	CTGGTTCATTTCAGATAGCG				
CmlA FW	TGTCATTTACGGCATACTCG	*cmlA*	455	52	Saenz et al., 2004
CmlA RV	ATCAGGCATCCCATTCCCAT				
dhfr1-FW	CTGATATTCCATGGAGTGCCA	*dhfr1*	433	54	Schmidt et al., 2001
dhfr1 RV	CGTTGCTGCCACTTGTTAACC				
aadA FW	TGATTTGCTGGTTACGGTGAC	*aadA*	284	52	Van et al., 2008
aadA RV	CGCTATGTTCTCTTGCTTTTG				