

Skin lesions on different fish species caused by bacteria

Farklı balık türlerinde bakterilerden kaynaklı deri lezyonları

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How to cite this paper:

Kayış, Ş., Er, A., 2014. Skin lesions on different fish species caused by bacteria. *Ege J Fish Aqua Sci*, 31(2): 55-59. doi: 10.12714/egejfas.2014.31.2.01

Özet: Balıklarda sıklıkla rapor edilen hastalıkların semptomları benzerdir, fakat bu semptomlar değişik faktörler sonucu meydana gelmektedir. Bu çalışmada Gram negatif, sitokrom oksidaz pozitif olan bakterilerin farklı balıklarda sebep olduğu deri lezyonları sunulmuştur. *Pseudoalteromonas haloplanktis* ve *Vibrio* spp. istavritten (*Trachurus mediterraneus*), *Chryseobacterium indologenes* melek (*Pterophyllum scalare*) balığından, *Pseudomonas putida* japon (*Carassius auratus*) balığından ve *Aeromonas sobria* kaynak alabalığından (*Salvelinus fontinalis*) izole edilmiştir. Bakteriler hem moleküler hem de biyokimyasal metotlara göre karakterize edilmişlerdir. Doğal ve yaralı istavrit örnekleri, Türkiye'nin Doğu Karadeniz sahillerinden gırgır ve uzatma ağları ile örneklenmiştir ve özellikle bu olay ilk kez bu çalışmada rapor edilmiştir.

Anahtar kelimeler: *Pseudoalteromonas haloplanktis*, *Chryseobacterium indologenes*, istavrit

Abstract: Symptoms of many commonly reported diseases of fishes are similar, but these symptoms may occur by different factors. Different skin lesions on some fishes caused by Gram negative cytochrome oxidase positive bacteria were presented in this study. *Pseudoalteromonas haloplanktis* and *Vibrio* spp. from wild horse mackerel (*Trachurus mediterraneus*), *Chryseobacterium indologenes* from freshwater angelfish (*Pterophyllum scalare*) *Pseudomonas putida* from goldfish (*Carassius auratus*) and *Aeromonas sobria* from brook trout (*Salvelinus fontinalis*) were isolated. Also, bacteria were characterized both phenotypic and molecular methods. Wild and injured *T. mediterraneus* samples were captured with purse seine operation and gill net at the coast of Eastern Black Sea of Turkey and particularly, the phenomena was reported for the first time in the study.

Keywords: *Pseudoalteromonas haloplanktis*, *Chryseobacterium indologenes*, horse mackerel

INTRODUCTION

Fish are sensitive to a wide variety of bacterial pathogens. Different pathogenic and opportunistic bacteria are the cause of high mortalities on fish in aquatic systems. Enteric red mouth disease (*Yersinia ruckeri*), motile *Aeromonas* septicaemia (*Aeromonas hydrophila*, *A. sobria*, *A. veroni*, *A. cavia*), bacterial kidney disease (*Renibacterium salmoninarum*), vibriosis (*Listonella anguillarum*), columnaris disease (*Flavobacterium columnaris*) are important and well-known bacterial fish diseases (Austin and Austin, 2007).

The most commonly reported diseases symptoms of fishes are similar, but these symptoms may occur by different factors. Such as, unilateral or bilateral exophthalmia is very common external symptom in many fish. This symptom may occur by *Philasterides dicentrarchi* (ciliate) (Kayış et al, 2011), *Hoferellus cyprini* (myxozoan) (Grossheider and Körting, 1992), *Renibacterium salmoninarum* and *Aeromonas hydrophila* (bacteria) on fish. Therefore, determination of

diseases agents need to perform detailed studies (Lasee, 1995).

External signs of fish diseases are highly wide and variable. Dark coloration, exophthalmia, haemorrhages on fins or skin, fin erosions some of the most remarkable external fish diseases symptoms (Noga, 2010). *Aeromonas hydrophila*, *Yersinia ruckeri*, *Pseudomonas putida*, *P. luteola*, *Vibrio anguillarum*, *Flavobacterium columnaris*, *Edwardsiella tarda* are the most reported bacterial fish pathogens deal with skin lesions and fin erosions (Lasee, 1995; Altinok et al., 2006; Altinok et al., 2007, Noga, 2010).

In this study, unusual skin lesions on some fishes caused by different bacteria *Aeromonas sobria*, *Chryseobacterium indologenes*, *Pseudomonas putida*, *Pseudoalteromonas haloplanktis* and *Vibrio* spp. were presented.

MATERIALS AND METHOD

Fifty injured *Trachurus mediterraneus* were sampled along the coast of Black Sea in winter 2010. Five moribund *Carassius auratus* and 20 *Pterophyllum scalare* were sampled aquarium units from Recep Tayyip Erdoğan University, Faculty of Fisheries Sciences and 10 *Salvelinus fontinalis* were sampled from fish farms located Eastern Black Sea in Turkey in April 2010. Fish were examined for parasites and bacteria. The lesions of fish skin, kidney, spleen and liver of fishes were sampled bacteriological and bacteria were aseptically streaked on salted (1%)/normal tryptic soy agar (TSA) and thiosulfate citrate bile sucrose agar (TCBS).

The medium incubated at $23.5 \pm 1.5^\circ\text{C}$ temperature and then pure cultured colonies were phenotypic characterized by API 20NE and API 20E (Biomérieux, Marcy l'Etoile, France) according to "instruction for use". API 20E Incubated at $36^\circ\text{C} \pm 2^\circ\text{C}$ for 18-24 hours, API 20NE incubated at $29^\circ\text{C} \pm 2^\circ\text{C}$ 24 hours. Before the biochemical application, cytochrom oxidase, catalase, growth properties at the different temperature (4, 35, 40°C), Gram stain, motility tests (in microscop) and glutamate starch phenol red agar (GSP) were practiced on the bacteria (Table 2). For the molecular characterization of the bacteria, DNA extraction of the isolated bacteria from fish and purification were performed by Ausubel *et al.* (1993) and Altınok *et al.* (2001).

To identify bacteria that isolated on the skin and other tissues of fishes, universal primers (27 F 5' AGA GTT TGA TCC TGG CTC AG-3', 1492 R 5' GTT TAC CTT GTT ACG ACT T-3') specific for 16S rRNA gene of eubacteria were used. The products of the PCR were directly sequenced at

Macrogen Inc. (Seoul, South Korea) and results were compared by BLAST (<http://www.ncbi.nlm.nih.gov>).

Water temperatures were determined as follows; seawater and fish farm water temperature was $9 \pm 0.8^\circ\text{C}$ in December (sea water temperature was measured twice, 8.2 and 9.8°C in December) and $12 \pm 0.5^\circ\text{C}$ (water temperature was measured twice, 11.5 and 12.5°C in April) respectively. Also aquarium water temperature was 25°C .

RESULT

Five different bacteria isolated from the skin of moribund fishes and all bacteria characterized as cytochrom oxidase positive, Gram negative and motile. The bacteria were not isolated from internal organs except for *Salvelinus fontinalis* and *Carassius auratus* tissues. Large yellow colonies were observed in TCBS agar inoculated from the *Trachurus mediterraneus* and white and pale orange colonies (Figure 1) were observed in the TSA. The all bacteria were identified according to the API 20NE, API 20E and molecular method (PCR). All bacteria, identified from fishes, area of lesion on fishes and all characters of bacteria explained in Table 1 and Table 2.

Externally, infected *T. mediterraneus* had advanced skin lesion on the behind of the head (Figure 2). Similarly, large and deep skin lesion was observed on the head of *C. auratus* (Figure 3). Unlike the two described lesion, more widespread but had little diameter skin lesion was observed on *P. scalare* (Figure 4). Also deep lesion and severe tissue erosion were observed on the caudal peduncle of *S. fontinalis* (Figure 5).

Table 1. Bacteria, fish species and isolated area of bacterial pathogens from fish.

Fish Species	Bacteria	Isolated area
Goldfish (<i>Carassius auratus</i>)	- <i>Pseudomonas putida</i>	Head, liver
Angelfish (<i>Pterophyllum scalare</i>)	- <i>Chryseobacterium indologenes</i>	All body of fish
Brook trout (<i>Salvelinus fontinalis</i>)	- <i>Aeromonas sobria</i>	Caudal peduncle, Kidney, liver, spleen
Horse mackerel (<i>Trachurus mediterraneus</i>)	- <i>Pseudoalteromonas haloplanktis</i> - <i>Vibrio</i> spp.	Between the behind of head and dorsal fin

Table 2. All characters of bacteria isolated from fish. Molec. Ch.; molecular characterization, NA; not application, Color in Medium; GSP; glutamate starch phenol red TSA; tryptic soy agar, TCBS; thiosulfate citrate bile sucrose.

Tests	<i>Pseudomonas putida</i>	<i>Chryseobacterium indologenes</i>	<i>Aeromonas sobria</i>	<i>Pseudoalteromonas haloplanktis</i>	<i>Vibrio</i> spp.
Molec. Ch.	99.7%	96%	97%	98%	96%
API 20E Profile	NA	NA	NA	NA	7 3 1 6 1 0 5
API 20NE Profile	0 1 4 2 4 5 7 (99.8%)	2 6 5 0 2 0 5 (99.9%)	7 1 3 6 7 5 5 (98.4%)	NA	3 4 4 4 1 4 6
Cyt. Oxidase	+	+	+	+	+
Catalase	+	NA	+	+	+
Temp. (°C)					
4	+	+	+	-	-
35	+	+	+	+	+
40	+	-	NA	+	+
Gram stain	-	-	-	-	-
Motility	+	-	+	+	+
Col. in Med.					
GSP	Violet	NA	Yellow	NA	NA
TSA	Cream	Orange	Cream	White	Cream/White
TCBS	NA	NA	NA	NA	Yellow

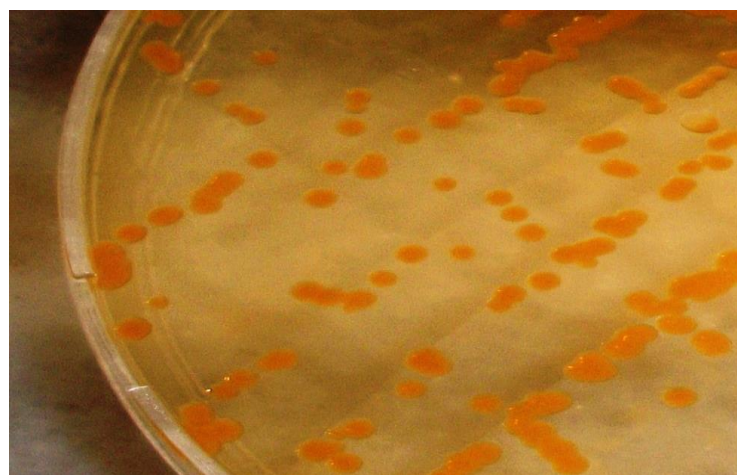


Figure 1. Colony of *Chryseobacterium indologenes* on TSA isolated from *Pterophyllum scalare*



Figure 2. Deep lesions on the skin of *Trachurus mediterraneus* caused by *Vibrio* spp. and *Pseudoalteromonas haloplanktis*.

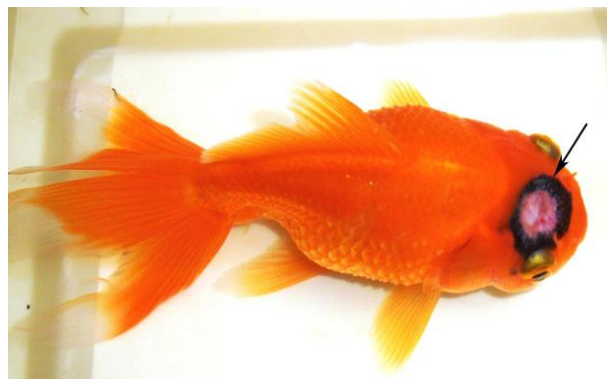


Figure 3. Deep and black ring-shape lesion on the skin of *Carassius auratus* caused by *Pseudomonas putida*.



Figure 4. Multiple and widespread lesions on *Pterophyllum scalare* caused by *Chryseobacterium indologenes*.



Figure 5. Deep lesion and severe tissue erosion on *Salvelinus fontinalis* caused by *Aeromonas sobria*.

DISCUSSION

Bacteria isolated from fish have different biochemical and pathogenic characteristics. Many bacteria and their internal and external effects on reared and wild fish have been reported by many researchers. Bacterial pathogens and their antibiotic resistant genes on reared horse mackerel (*Trachurus mediterraneus*) were studied in the Black Sea in Turkey (Boran *et al.*, 2013). The study demonstrated that several Gram negative bacterial species including *Aeromonas hydrophila*, *Vibrio vulnificus*, *Bulkholderia cepacia*, *Photobacterium damsela damsela* and *Vibrio alginolyticus* were isolated from cultured fish. And also externally, affected fish had dark pigmentation, ascites and, petechial hemorrhage on the abdomen in the study. Unlike the previous study, present study show that mix infections of *Pseudoalteromonas haloplanktis* and *Vibrio* spp. was isolated from wild fish population with severe skin lesions.

Many of the bacteria isolated from ornamental fish are opportunists (Love *et al.* 1981). But some of the bacteria including *Aeromonas hydrophila*, *A. sobria*, *Citrobacter freundii*, *Photobacterium damsela damsela*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Vibrio anguillarum*, *V. harveyi* and

V. parahaemolyticus are pathogenic in unsuitable conditions. Continuously a new species of bacteria have been reported from different fish species. In this context, *Chryseobacterium indologenes* isolated from diseased yellow perch (*Perca flavescens*) with skin lesions (Pridgeon *et al.*, 2013). Similarly, we observed that *C. indologenes* cause multiple lesions on angelfish but mortality on fish was not observed. This circumstance may cause due to slow growth feature of the bacteria.

Goldfish (*Carassius auratus*) is the most common reared ornamental fish species. Therefore many disease cases of the species were reported by different paper in all over the world (Crouse-Eisnor *et al.*, 1985; Ostland *et al.*, 1989; Yildiz and Kumantas, 2002; Chanda *et al.*, 2011; Kayis *et al.*, 2013). Particularly, *Aeromonas hydrophila* was reported by Crouse-Eisnor *et al.* (1985), from skin surface of goldfish. *Pseudomonas putida* is an opportunistic human pathogen (Martino *et al.*, 1996) and it is not a common pathogen in aquaculture and was only isolated from ayu, *Plecoglossus altivelis altivelis* and yellowtail, *Seriola quinqueradiata*, in Japan (Kusuda and Toyoshima, 1976; Muroga, 1990;

Wakabayashi *et al.*, 1996). Outside of Japan, *P. putida* infection in fish (rainbow trout, *Oncorhynchus mykiss*) was reported in Turkey (Altinok *et al.*, 2008). Similarly, this study presented that *Pseudomonas putida* can cause advanced damage on skin surface of goldfish.

Aeromonas salmonicida, *Edwardsiella tarda*, *Flavobacterium branchiophilum*, *Nocardia spp.* and *Yersinia ruckeri* (Bullock *et al.*, 1978; Ostland *et al.*, 1999, Uhland *et al.*, 2002) were reported on brook trout (*Salvelinus fontinalis*). Because of to can cause skin ulcers and septicaemia, *A.*

salmonicida differ from the other bacteria (Bullock *et al.*, 1978). *Aeromonas sobria* and *Aeromonas caviae* were reported with skin lesions on *Oncorhynchus mykiss* (Rehulka, 2002). *Aeromonas sobria* was reported from cultured rainbow trout fingerlings (skin hemorrhages), gizzard shad (*Dorosoma cepedianum*) (liver, kidney and spleen) (Toranzo *et al.*, 1989). Clinical signs of motile aeromonad infection are generally superficial deep skin lesions (Noga, 2010). However, *A. hydrophila* commonly reported motile *Aeromonas* species from fish with the regard to skin lesions. We observed that *A. sobria* can cause deep skin ulcer and severe tissue loses.

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