

Diagnosis of *Photobacterium sanguinancrri* in Smooth-Hound Shark (*Mustelus mustelus*, Linnaeus 1758)

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Research Article

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Abstract

This study aims to determine the cause of mortalities in smooth-hound sharks (*Mustelus mustelus*, Linnaeus 1758) under stressful conditions from a marine aquarium in Turkey. Moribund smooth-hound sharks externally had haemorrhages on various body parts, especially the pectoral fins and certain areas of their skin. A pale liver and spleen pseudo-tubercles were the main pathological findings internally. For bacteriological examination, samples were taken from visceral organs and blood and streaked onto marine agar (MA) plates, which were incubated at 22°C for 72 hours. Gram-negative motile rods that displayed bipolar staining were isolated from several tissues, including the spleen, kidney, and liver. According to their morphological and biochemical characteristics as well as 16S rRNA gene sequencing, these isolates were identified as *Photobacterium sanguinancrri*. Therefore, it was concluded that photobacteriosis was responsible for this disease outbreak in aquarium-kept smooth-hound sharks. According to the literature survey, this study is the first report describing photobacteriosis caused by *Photobacterium sanguinancrri* in a smooth-hound shark.

Keywords: Smooth-Hound Sharks, Photobacteriosis, *Photobacterium sanguinancrri*, Aquarium

Tazı Köpekbalıklarında (*Mustelus mustelus*, Linnaeus 1758) *Photobacterium sanguinancrri*'nin Tanısı

Özet

Bu çalışma, Türkiye'de bir deniz akvaryumunda stresli koşullar altında bulunan tazı köpekbalıklarında (*Mustelus mustelus*, Linnaeus 1758) meydana gelen ölümlerin nedeninin belirlenmesini amaçlamaktadır. Dış bakıda, ölmek üzere olan tazı köpekbalıklarının çeşitli vücut kısımlarında, özellikle göğüs yüzgeçlerinde ve deride belirli bölgelerde hemorajiler vardı. İç bakıda ise, soluk karaciğer ve dalakta psödotüberküller ana patolojik bulgularıdır. Bakteriolojik inceleme için iç organlardan ve kandan alınan örnekler marine agar (MA) içeren petrilere ekilmiş ve 22°C'de 72 saat boyunca inkübe edilmiştir. Bipolar boyama özelliği gösteren gram negatif hareketli basiller dalak, böbrek ve karaciğer dahil olmak üzere çeşitli dokulardan izole edilmiştir. Morfolojik ve biyokimyasal özelliklerine ve 16S rRNA gen dizilimine göre, bu izolatlar *Photobacterium sanguinancrri* olarak tanımlanmıştır. Bu nedenle, akvaryumda tutulan tazı köpekbalıklarında bu hastalık salgınına photobacteriosis'in neden olduğu sonucuna varılmıştır. Literatür araştırmasına göre, bu köpekbalığı türünde *Photobacterium sanguinancrri*'nin neden olduğu Photobacteriosis'i tanımlayan ilk rapordur.

Anahtar kelimeler: Tazı Köpekbalığı, Photobacteriosis, *Photobacterium sanguinancrri*, Akvaryum

INTRODUCTION

Photobacteriosis caused by *Photobacterium damsela* subsp. *piscicida* (formerly *Pasteurella piscicida*), was first described in white perch (*Morone americana*) and striped bass (*Morone saxatilis*) as a causative agent of a systemic bacterial infection in Chesapeake Bay, USA (Sniezko et al., 1964; Janssen and Surgalla, 1968). Agent of the disease is the halophilic bacterium, Gram-negative, non-motile rod, oxidase, and catalase-positive, fermentative, sensitive to the vibriostatic agent O/129, and has a strict salt requirement. The disease has become a problem in Mediterranean countries since 1990 and described in many marine fish species, such as Atlantic salmon (*Salmo salar*), yellowtail (*Seriola quinqueradiata*), and sole (*Solea solea*) (Magarinos et al., 1996). The first epizootic incidence of photobacteriosis was described by Çağırğan (1993) in sea bream in the Aegean Sea coasts of Turkey. Since then, this fish disease has been reported in sea bass (Candan et al., 1996; Timur et al., 1999; Korun and Timur, 2005) and mullets belonging to genus Mugil (Tanrıkuş and Çağırğan, 2001) and rainbow trout (Savaş and Türe, 2017) in the different regions of Turkey.

Currently, this is one of the most significant fish diseases in marine aquaculture, causing important economic losses (Magarinos et al., 1996). Although photobacteriosis may occur in both acute and chronic form, the disease eventually develops into a septicemia. In the acute form, the disease usually does not show any clinical signs, but in some cases, several disease signs have been reported, including anorexia, loss of mobility, pale gills, dark pigmentation, and petechiae at the base of fins and on the operculum. In the chronic form, affected fish have white nodules in the internal organs, so the disease is also known as pseudotuberculosis; but during acute form disease, affected fish usually have no obvious gross pathological signs (Magarinos et al., 1996; Timur et al., 1999).

Photobacterium sanguinancrri is one of the motile newest members of genus *Photobacterium* and was originally isolated from the hemolymph of spider crabs (*Maja brachydactyla*) captured in Spain and from a diseased adult blue mussel (*Mytilus edulis*) caught in the Netherlands (Gomez-Gill et al., 2016). However, *P. sanguinancrri* has never been isolated from any fish species so far.

Mustelus mustelus (Linnaeus, 1758), belongs to Triakidae, is a widespread coastal species from Northern Europe to South Africa, including the Mediterranean Sea and usually found in shallow waters at 5-50 m depth, although it occurs to at least 350 m depth (Compagno, 1984; Serena et al., 2009). In Turkey, only two cases have been reported about parasites of this species of shark. *Pandarus bicolor* (Leach, 1986) from Copepoda collected from the ventral surface and fins of smooth-hound sharks by Ökter and Trilles (2009) and *Lernaepoda galei* (Kroyer, 1837) corresponding order Siphonostomatoida were reported by Karaytuğ et al. (2004) in cloacal region of this species from Edremit Bay in the Aegean Sea. In our previous study, we reported and identified that *Photobacterium damsela* subsp. *piscicida* along with *Lernaepoda* sp. as a main causative agent of photobacteriosis in same shark species (Yardımcı et al., 2017).

The present study which is the first report describing photobacteriosis caused by *Photobacterium sanguinancrri* in shark was carried out to determine the cause of mortalities in stressed smooth-hound sharks kept in a public marine aquarium in Turkey.

MATERIALS and METHODS

Smooth-hound sharks (*Mustelus mustelus*) (20 specimens, approx. 1.5 m long and 3 kg weight) were caught by trawling the Edremit Bay of the Aegean Sea, then transported to Istanbul and placed into the quarantine tanks of a public aquarium in 2018. A few days later, mortalities were observed in the shark population. The water parameters of the aquarium were measured as follows: the temperature was 24°C, salinity was 28 ‰, pH was 8, the dissolved oxygen concentration was 7,6 mg L⁻¹ and oxidation-reduction potential was 175 mV. Two moribund sharks were sampled according to standard methods (Hall, 1999; Noga, 2000).

For bacteriological examination, samples were taken from visceral organs and blood. They were streaked onto marine agar (MA) plates and the plates were incubated at 22°C for 72 hours. The morphological and physiological characteristics of isolates were determined using conventional biochemical and physiological tests as well as rapid identification kits (API VITEK GN ID Card), according to Buller (2004), Austin and Austin (2007), Gomez-Gill et al. (2016) and Bergey's Manual (Holt et al., 1984).

Molecular methods were used for the identification of bacterial strains. The isolates on marine agar plates were inoculated into marine broth 2216 (Difco) and incubated overnight at 22°C. Then total DNA was extracted from liquid cultures and samples using the PureLink™ Genomic DNA Mini Kit (Invitrogen) according to the manufacturer's instructions. For bacterial identification, a 540-bp-long fragment of the 16S rRNA gene was amplified and sequenced using the universal bacteria primer set: primer S-D-Bact-0008-a-S-20 and primer S-*-Univ-0536-a-A-18 (Suau et al., 1999).

RESULTS

In this study, affected sharks had haemorrhages on the skin, ventral side of the body, and in the claspers (Figure 1). They also had ulcerative lesions and haemorrhage on the pectoral fin, the skin, upper jaw, head, and also the torn fin (Figure 2a-d). Internally, there were no symptoms other than a pale liver or haemorrhages in the liver and pseudo-tubercles in the examined fish spleen (Figure 2e-f). 100 % mortality range was determined inside two weeks.

Shiny, raised, and translucent bacterial colonies were formed on MA. These six bacterial isolates appeared as Gram-negative, motile rods with a bipolar staining and were oxidase and catalase positive.

Their morphological, biochemical characteristics and API VITEK GN ID Card results were shown in Table 1. These isolates were found biochemically homogeneous. According to their morphological and biochemical characteristics as well as 16S rRNA gene sequencing, these isolates were identified as *Photobacterium sanguinicancri*. One of the sequence was deposited in the GenBank database with MG696892 accession numbers.

Table 1. Morphological and phenotypical characteristics and API VITEK GN ID Card results of the isolates

Morphology	R	Indole	-
Motility	+	Voges Proskauer Reaction	+
Gram Staining	-	Methyl Red	+
Catalase	+	Nitrate Reduction	+
Cytochrome Oxidase	+	Degradation of Arginine	+
Growth on TCBS	-	Degradation of Starch	+
Ala-Phe-Pro- Arylamidase	-	L-Arabitol	-
H ₂ S Production	-	D- Glucose	-
Beta-Glucosidase	-	D-Mannose	-
L-Proline Arylamidase	+	Tyrosine Arylamidase	-
Saccharose	-	Citrate (Sodium)	-
L-Lactate Alkalinisation	-	Beta-N-Acetyl-Galactosaminidase	-
Glycine Arylamidase	-	L-Histidine Assimilation	-
0129 Resistance	S	L-Malatat Assimilation	-
Adonitol	-	D-Cellobiose	-
Beta-N- Acetyl-Glucosaminidase	-	Gamma-Glutamyl-Transferase	-
D-Maltose	-	Beta-Xylosidase	-
Lipase	+	Urease	-
D-Tagatose	-	Malonate	-
Alpha-Glucosidase	-	Alpha-Galactosidase	-
Ornithine Decarboxylase	-	Coumarate	+
Glu-Gry-Arg Arylamidase	-	L-Lactate Assimilation	-
L-Pyrroldonyl-Arylamidase	-	Beta-Galactosidase	-
Glutamyl Arylamidase pNA	-	Fermentation/Glucose	-
D-Mannitol	-	Beta-Alanine Arylamidase pNA	-
Palatinose	-	D-Sorbitol	-
D-Trehalose	-	5-Keto-D-Gluconate	-
Succinate Alkalinisation	-	Phosphatase	-
Lysine Decarboxylase	-	Beta-Glucoronidase	-

R: rods; S:sensitive; +: positive; -: negative



Figure 1. Moribund sharks showed haemorrhages on the skin.



Figure 2. Moribund sharks showed haemorrhages and ulcerative lesions on the pectoral fin (a), on the torn fin (b), on the upper jaw and head (c), pale liver (d), haemorrhages on the liver (e) and pseudo-tubercles in the spleen (f).

DISCUSSION

Sharks are predators within the marine environment and recent studies have shown that abiotic factors may be a significant role for caused died. Both acute and chronic changes have also been linked to their conditions. The marine ecosystems have a relatively more stable environment than freshwater in terms of water temperature, oxygen, and other water-quality parameters; therefore, much wild fish usually cannot adapt to artificial conditions, and when they are placed in aquaria, many disease problems may arise (Schlaff et al., 2014). Few diseases have been reported in species of shark. Especially, *Vibrio* species as agents of elasmobranch diseases was founded. *V. charchariae* is the most recently recognized species within the genus that can cause lethal infections in shark such as chronic skin ulceration (Grimes et al., 1984).

In this study, affected sharks have some gross pathological signs in chronic form and these similar situations decelerated other previous studies (Çağırğan, 1993; Magarinos et al., 1996; Candan et al., 1996; Timur et al., 1999; Korun and Timur, 2005). In this study, six bacteria isolated, and these isolates were found biochemically homogeneous. Their morphology such as molecularly, the ability of lipase production and degradation of arginine and starch had made them distinct from other *Photobacterium* spp. (Holat et al., 1984; Çağırğan, 1993; Magarinos et al., 1996; Candan et al., 1996; Timur et al., 1999; Buller, 2004; Korun and Timur, 2005; Austin and Austin, 2007). And also, these isolates characteristic is so similar to *Photobacterium sanguinancrri* which isolated adult blue mussel by Gomez-Gill et al. (2016). As a result, all isolates were identified as *Photobacterium sanguinancrri* by using universal 16S rRNA primer pairs sequence similarities in the GenBank database with the highest similarity index (each isolate was 99%).

In conclusion, this study represents the first report of *Photobacterium sanguinancrri* isolation and identification in aquarium-kept smooth-hound sharks. Environmental conditions and stress probably play a major role in determining the severity of this disease. It was concluded that the high losses observed might in part be due to stress during catching and/or transporting of the sharks.

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