First Report of *Vibrio harveyi* Infection in Diseased Common Dentex (*Dentex dentex*) Cultured in Turkey*

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Abstract

In the present work, *Vibrio harveyi* was consistently isolated from diseased common dentex (*Dentex dentex*) cultured in Turkey. The outbreak occurred in July (2013), following the transportation of common dentex juveniles from hatchery to offshore floating cages. Five moribund cultured common dentex individuals, about 20-25 g in weight, were selected from the said floating cages and samples were taken from internal organs including liver, spleen, kidney and eye tissue. A total of eight pure cultures were obtained and identified through basic biochemical characterisation as well as 16S rRNA gene sequencing. To our knowledge, this is the first report of *Vibrio harveyi* infection in cultured common dentex in Turkey.

Keywords: Common dentex, *Vibrio harveyi*, 16S

Türkiye'de Kültürü Yapılan Sinarit Balıklarında (*Dentex dentex*) İlk *Vibrio harveyi* Enfeksiyonu Bildirimi

Özet

Bu çalışmada, Türkiye'de yetiştiriciliği yapılan hasta sinarit balıklarından (*Dentex dentex*) *Vibrio harveyi* izole edilmiştir. Hastalık Temmuz ayında (2013), juvenil sinarit balıkları kuluçkahaneden yüzer kafes sistemlerine transfer edildikten sonra ortaya çıkmıştır. Hasta balıklardan 20-25 g ağırlığındaki 5 birey yüzer kafeslerden seçilmiş ve karaciğer, dalak, böbrek ve göz dokusundan örnekler alınmıştır. Bakteriyolojik kültür sonucunda 8 adet izolat saf olarak elde edilerek biyokimyasal karakterizasyonun yanı sıra 16S rRNA geni dizi analizi ile tanımlanmıştır. Bu çalışma, Türkiye'de kültürü yapılan sinarit balıklarında *Vibrio harveyi* enfeksiyonunun rapor edildiği ilk çalışmadır.

Anahtar kelimeler: Sinarit, *Vibrio harveyi*, 16S

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INTRODUCTION

Due to its high growth rate, ease of reproduction in captivity and favourable adaptation to pellet feeds, the common dentex (*Dentex dentex*, Linnaeus, 1758) is held as a strong candidate among new fish species currently under evaluation for cultivation in Turkey. Common dentex has been marketed at high prices and has already a high commercial value in other Mediterranean countries. The most likely problem that could affect common dentex culture in a negative way, is the high mortality rate seen in the early stage of cultivation, which also has been the case for other cultured marine fish species (Abellán, 2000; Rueda and Martínez, 2001).

Among fish pathogens, *Vibrio harveyi* is a well-known bacterial species causing mortalities in marine aquaculture industry and they have been isolated occasionally from moribund fish and identified as the aetiological agents of disease. To date, *V. harveyi*

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infections has been reported in common dentex (*Dentex dentex*) (Company et al., 1999; Pujalte et al., 2003), gilthead sea bream (*Sparus aurata*) (Balebona et al., 1998; Pujalte et al., 2003; Haldar et al., 2010), European sea bass (*Dicentrarchus labrax*) (Pujalte et al., 2003), farmed sole (*Solea senegalensis*) (Zorrilla et al., 2003), cultured wedge sole (*Dicologoglossa cuneat*a) (López et al., 2009), cultured brown spotted grouper (*Epinephelus tauvina*) and silvery black porgy (*Acanthopagrus cuvieri*) (Saeed, 1995) and cage-reared grouper (*Epinephelus awoara*) (Qin et al., 2006).

The aim of this study was to identify the causative agent responsible for a disease outbreak in cultured common dentex that occurred in a commercial fish farm located in Aegean Region of Turkey.

MATERIALS and METHODS

The present study was approved by Istanbul University Local Committee on Animal Research Ethics (Decision No: 2012/113).

Case History

The outbreak occurred in July (2013), following transportation of cultured common dentex juveniles from hatchery to floating cages in a commercial fish farm located in Aegean Region of Turkey. Water temperature was 22 ºC and salinity was 34‰ at the time of sampling. Overall mortality rate was about 30% according to fish health records of the farm.

Isolation of the Causative Agents

Five moribund cultured common dentex (20-25 g in weight) were selected from offshore floating cages. Samples were taken from internal organs (liver, spleen, kidney and eye tissue) and streaked onto Marine Agar 2216 (Difco) using aseptic techniques. Agar plates were incubated at 22 ºC for 48-72 h. After incubation period, single colonies on the primary plates were selected and re-streaked onto the same media to obtain pure cultures.

Morphological and Biochemical Characteristics

Isolated pure cultures were examined using standard protocols for some basic characteristics including colony and cell morphology, Gram staining, oxidase activity, motility, oxidation/fermentation (O/F) reaction, growth and appearance on TCBS agar, susceptibility to vibriostatic agent O/129 (10 µg and 150 µg) (Oxoid) (Whitman, 2004).

Genomic DNA Extraction

The isolates were inoculated into Marine Broth 2216 (Difco) and incubated overnight at 22 °C in a shaking incubator. Genomic DNA was then extracted by the PureLink Genomic DNA Mini Kit (Invitrogen) according to the manufacturer's instructions and used as template for PCR.

PCR and 16S rRNA Gene Sequencing

An approximately 540 bp long fragment of the 16S rRNA gene was amplified using the universal bacteria primer set; primer S-20 (5’ AGA GTT TGA TCC TGG CTC AG 3’) and primer A-18 (5’ GWA TTA CCG CGG CKG CTG 3’) (Suau et al., 1999).

The PCR mixture (50 µl) included approximately 50 ng template DNA (2 µl), 0.4 µM of each primer (2x 2 µl), PCR Master Mix (2X) (Thermo Scientific) (25 µl) and nuclease-free water (Thermo Scientific) (19 µl). Amplification was performed using a thermal cycler (Biometra, TPersonal) with the following parameters: initial denaturation at 95 °C for 3 min, followed by 30 cycles of amplification (denaturation at 95 °C for 30 s, annealing at 56 °C for 1 min, extension at 72 °C for 1 min) and a final extension step of 72 °C for 4 min.

After amplification, 10 µl of PCR sample was loaded on a 1.5% (wt/vol) agarose gel in TAE containing ethidium bromide (0.5 ug/ml) and electrophoresis was performed (for 40 min at 90 V). PCR products were visualized on a UV transilluminator and size estimated against GeneRuler 100 bp DNA Ladder (Thermo Scientific).

PCR products were purified and sequenced bidirectionally by BM Labosis (Ankara, Turkey). Sequence editing and analysis was performed in Bioedit v7.0.0 (Hall, 1999) using the ClustalX 2.1 (Larkin et al., 2007) and BLASTN 2.2.20 algorithm (Zhang et al., 2000). A ≥99% similarity criterion in 16S rRNA gene sequence was used for identification of the isolates at the species level (Clarridge, 2004). The 16S rRNA gene sequences were deposited in GenBank database.

RESULTS

Clinical Signs

Hemorrhaging, especially on the ventral side of the body, scale rots on the skin surface along with skin depigmentation, pale gills and corneal opacity were common findings of all sampled fish. In some individuals hemorrhaging were also observed in the opercular region, at the base of the fins and around the vent. Some individuals had also erosions on the tail and the cranial region. Internal examination revealed that the intestine was swollen containing yellowish fluid and the abdominal cavity was distended due to ascitic fluid. The liver was hyperaemic and the spleen was swollen in all affected fish (Figure 1, 2, 3, 4).

Figure 1. Hemorrhaging in the opercular region and at the base of the fins

Figure 2. Hemorrhaging on the ventral side of the body

Figure 3. Erosion on the cranial region

Figure 4. Swollen spleen and the intestine containing yellowish fluid

Morphological and Basic Characteristics of the Isolated Bacteria

From the five fish samples, a total of eight pure cultures were obtained and processed for 16S rRNA gene sequencing. Some morphological and basic characteristics of the isolates are given in Table 1.

Table 1. Morphological and basic characteristics and originated tissues of the isolates

Isolate	Gram	Motility	Oxidase	O/F	TCBS agar	O/129 (10 µg-150 µg)	Organ
IUET-AK01	-	+	+	F	Yellow	R-S	Eye
IUET-AK05	-	+	+	F	Yellow	R-S	Spleen
IUET-AK07	-	+	+	F	Yellow	R-S	Gill
IUET-AK08	-	+	+	F	Yellow	R-S	Spleen
IUET-AK09	-	+	+	F	Yellow	R-S	Spleen
IUET-AK14	-	+	+	F	Yellow	R-S	Kidney
IUET-AK19	-	+	+	F	Yellow	R-S	Kidney
IUET-AK20	-	+	+	F	Yellow	R-S	Liver

Key: -: Negative, +: Positive, F: Fermentative, R: Resistant, S: Sensitive

All isolates showed the same characteristics including being cytochrome oxidase positive, fermentative, resistant to vibriostatic agent O/129 at 10 µg and sensitive at 150 µg and observed as Gram negative, curved-rod shaped, motile bacterial cells under light microscopy, while visually appearing as white shiny colonies on Marine Agar and yellow colored colonies on TCBS agar.

16S rRNA Gene Sequencing Results

According to 16S rRNA gene sequence analysis, all isolates (IUET-AK01, IUET-AK05, IUET-AK07, IUET-AK08, IUET-AK09, IUET-AK14, IUET-AK19, IUET-AK20) were identified as *V. harveyi* (Table 2).

Table 2. Bacterial species identified by 16S rRNA gene sequence analysis

Isolate	Species name	Similarity (%)	Accession no.
IUET-AK01	*Vibrio harveyi*	99.8	KJ158253
IUET-AK05	*Vibrio harveyi*	99.4	KJ158254
IUET-AK07	*Vibrio harveyi*	99.6	KJ158255
IUET-AK08	*Vibrio harveyi*	99.6	KJ158256
IUET-AK09	*Vibrio harveyi*	99.0	KJ158257
IUET-AK14	*Vibrio harveyi*	99.8	KJ158258
IUET-AK19	*Vibrio harveyi*	99.8	KJ158259
IUET-AK20	*Vibrio harveyi*	99.8	KJ158260

DISCUSSION

V. harveyi is a well-known pathogen of both invertebrates and vertebrates and infections in fish by this bacterium have been reported from both wild and cultured

species (Austin and Zhang, 2006). Eye lesions have been reported as a common clinical finding in several disease outbreaks, affecting common dentex (*D. dentex*) (Company et al., 1999), gilthead sea bream (*Sparus aurata*) (Haldar et al., 2010), milkfish (*Chanos chanos*) (Ishimaru and Muroga, 1997), common snook (*Centropomus undecimalis*) (Kraxberger-Beatty et al., 1990) and short sunfish (*Mola mola*) (Hispano et al., 1997). In accordance with these reports, all individuals had corneal opacities in our examination. In addition to eye lesions, our findings such as pale gills, abdominal swelling due to ascitic fluid, hemorrhaging in the liver and at the base of the fins as well as skin lesions are similar to a previous report of *V. harveyi* dominated infections in cultured common dentex (Company et al., 1999). We also observed that the spleen was enlarged while the intestine was swollen containing yellowish fluid in all affected fish and that some individuals had erosions in the cranial region.

The reported disease outbreak occurred during the first summer following transportation juveniles from hatchery to floating cages. Likewise, Company et al., (1999) indicated that in a two-year survey, the highest mortality rates in common dentex culture occurred at the end of the first summer, after their transport from the hatchery. The same authors also mentioned that the peak of mortalities coincided with high water temperatures.

High mortality rates occurring in the early stages of the rearing caused by bacterial diseases is one of the most important reasons for economic losses during the production period. In order to reduce such losses, it is needed to identify the pathogenic bacterial agents responsible for the mortality. Common dentex is highly sensitive to handling and other stress-inducing factors so stress-related high mortalities may be expected in common dentex culture (Tibaldi et al., 1996; Sodimou and Alexis, 1998).

In this work, *Vibrio harveyi* was isolated and identified from diseased cultured common dentex. To our knowledge this is the first report of *V. harveyi* infection in cultured common dentex in Turkey.

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