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First Report of *Candida* sp. from Rainbow Trout (*Oncorhynchus mykiss,* Walbaum) Fry in Turkey

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Research Article

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Abstract

In this study, we isolated *Candida sp.* for the first time from fry of rainbow trout (*Oncorhynchus mykiss*) in Turkey. Candida species caused disease in fry (average 0.2 g) which was observed at 10°C water temperature in February 2016. Due to yeast accumulation on gills, whitish areas was seen and fusion of secondary lamella in gills were observed under microscope. The microbiological samples of kidney, spleen and gills were streaked onto trypticase soy agar (TSA) and tryptone yeast extract salts (*TYES*) agar plates and incubated at 22°C in TSA and 18°C in TYES agar for 48 h. Thirteen isolates were obtained and Gram staining was performed. The all of the isolates were determined as yeast cell based on cellular morphology by using light microscopy. The identification was performed by ITS (Internal transcribed spacer) region sequence analysis and sequence data demonstrated that representative isolate D11 belonged to genus *Candida*. The yeast infection might have seen in early development stages (larvae and fry) due to the intensive antibiotic use against rainbow trout fry syndrome (RTFS) disease.

Keywords: Candida sp., rainbow trout, *Oncorhynchus mykiss*, identification, sequence analysis

Türkiye'deki Gökkuşağı Alabalığı(*Oncorhynchus mykiss,* Walbaum) Fryları'ndan *Candida* sp.'nin İlk Bildirimi

Özet

Bu çalışmada, Türkiye'de gökkuşağı alabalıklarında (*Oncorhynchus mykiss*) ilk defa Candida sp. izole edilmiştir. Hastalık, yavrularda (ortalama 0,2 gr) 2016 Şubat ayında 10°C su sıcaklığında gözlenmiştir. Hasta balıkların solungaçlarında maya hücrelerinin birikimine bağlı olarak beyaz renkli alanlar ve mikroskop altında sekonder lamellalarda yapışma gözlenmiştir. Böbrek, dalak ve solungaçlardan alınan mikrobiyolojik örnekler tryptik soya agar (TSA) ve trypton yeast extract salts (TYES) agar üzerine ekilmiş ve TSA 22° C ve TYES agar da 18° C'de 48 saat boyunca inkübe edilmiştir. Hasta balıklardan on üç izolat elde edilmiş ve izolatların tümünden Gram boyama yapılmıştır. Işık mikroskobu kullanılarak hücresel morfolojiye göre tüm izolatların maya oldukları saptanmıştır. İdentifikasyon, ITS (Internal Transcribed Spacer) bölgesinin sekans analizi ile gerçekleştirilmiş ve sekans verilerine göre D11 izolatının Candida'ya ait olduğu tespit edilmiştir. Maya enfeksiyonunun, alabalıkların erken gelişim evrelerinde (larva ve yavru) görülen gökkuşağı alabalığı fry sendromu (RTFS) hastalığının tedavisinde yoğun olarak antibiyotik kullanımına bağlı olarak ortaya çıktığı düşünülmektedir.

Keywords: Candida sp., gökkuşağı alabalığı, *Oncorhynchus mykiss*, identifikasyon, sekans analizi

INTRODUCTION

Yeasts are ubiquitous microorganisms, which spread through animals, air and water currents. Yeast cells can grow in various environments where the organic substrates are available. Yeasts infections in fish were previously reported on skin, gills, mouth, faeces and gut contents (Kutty and Philip, 2008). Ross and Morris (1965) determined that the highest number of yeast cells on the skin of fish. Sometimes, yeasts have commonly been isolated from the gastrointestinal tract with high cell densities obtained in healthy fish.

Their presence has been noted in fish guts for some time in wild, as well as farmed animals. It appears that yeasts can constitute a significant part of the microbiota in fish gut (Gatesoupe, 2007). Most previous studies on yeast infecting fish in freshwater concerned rainbow trout (*Oncorhynchus mykiss*) or other *Oncorhynchus* spp. *Debaryomyces hansenii* was found dominant, *Candida* sp., *Saccharomyces cerevisiae* and *Leucosporidium* sp. were also dominant in intestinal samples of some rainbow trout (Gatesoupe, 2007).

Most yeasts are likely harmless to healthy fish reared in good condition. The natural proliferation of yeasts in fish mucus may be generally considered as commensalism, in spite of a few cases of pathological infections mainly due to opportunistic yeast strains (Gatesoupe, 2007). Some yeast and other fungi may be potentially harmful for fish in an immunocompromised host or under adverse environmental conditions (Chi et al., 2010). Also the use of wide-spectrum antibiotics increases the risk for yeast infections in fish. Because antibiotics alter the bacterial microflora and thus allow for overgrowth of yeast cells (Meurman et al., 2007; Achkar and Fries, 2010).

Yeasts such as *Candida* sp. have caused internal lesions in Chinook salmon *Oncorhynchus tshawytscha* (Mueller and Whisler, 1994), stomach distended with fluid in Japanese Amago *Oncorhynchus rhodurus* (Hatai and Egusa, 1975), swollen swim bladder in *Sparus aurata* (Galuppi et al., 2001) and overinflation of cardiac stomach in sterlet sturgeon *Acipenser ruthenus* fingerlings (Park et al., 2012). *Candida* sp. has also been found in the gut of healthy rainbow trout (Sakata et al., 1993; Gatesoupe et al., 2005). The yeast *Metschnikowia bicuspidata* var. *bicuspidata* infection reported in chinook salmon caused blood-tinged ascites and numerous melanomacrophages in kidney (Moore and Strom, 2003).

The aim of this study was the characterization of *Candida* sp. which isolated in fry of rainbow trout for the first time in the Mediterranean region of Turkey.

MATERIAL and METHODS

Fish

Sick fish (average 0.2 g) were observed in February 2016 and submitted to our laboratory for examination from a fish farm in the Mediterranean region of Turkey. The water temperature was 10°C. The disease outbreak occurred after a florfenicol treatment to fry, due to a bacterial infection caused by *Flavobacterium psychrophilum* pathogen. A total of 14 fish were microbiologically examined

Isolation

The samples from kidney, spleen and gills of diseased fish were streaked on both trypticase soy agar (TSA, Merck) and tryptone yeast extract salts agar (TYES) mediums and incubated at 22°C and 18°C for 48 h, respectively (Holt et al., 1994). Gram staining was used for primary identification of the isolates. Thirteen isolates were further used for identification.

Identification of a Representative Strain by ITS Sequence Analysis

The ITS (Internal transcribed spacer) region was used for genetic identification (Gago et al., 2014). For this purpose, a representative strain D11 was selected based on colony morphology among the isolates. The ITS region sequence analysis of the strain D11 was performed by the Macrogen (Seoul, Korea). Briefly, DNA segment comprising ITS region of the ribosomal DNA (rDNA) was amplified with primers ITS-1(TCCGTAGGTGAACCTGCGG) / ITS-4 (TCCTCCGCTTATTGATATGC), and the amplicon was purified using a commercial PCR purification kit and sequenced in both directions with the same primer pairs. Sequence data was compared with previously published data for identification with the Basic Local Alignment Search Tool (BLAST) via GenBank (http://www.ncbi.nlm.nih.gov/genbank).

RESULTS and DISCUSSION

Affected fish were generally in a good condition and showed normal behavior, feed intake. However, low mortality was obtained by reaching 1-2 % during the outbreak. Whitish areas on gills due to yeast accumulation and fusion of secondary lamella (Figure1) were observed in diseased fish.

In the internal examination of the fish, the clinical deviations or disease signs were not found. In contrast, in the previous studies the most typical symptoms were obtained as distended stomach and swim bladder of fish in Candida infection (Hatai and Egusa, 1975; Mueller and Whisler, 1994; Galuppi et al., 2001; Park et al., 2012). This difference may be due to disease severity and the virulence of the pathogen in these studies.

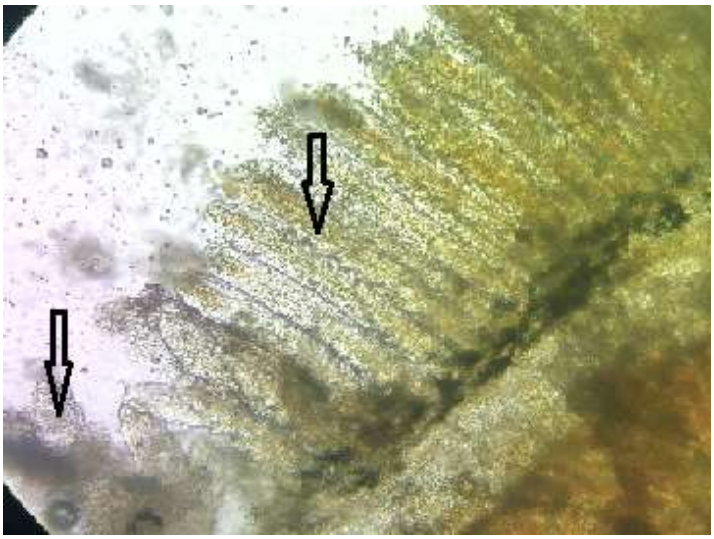

Figure 1. a) Whitish areas due to yeast accumulation on gills (left arrow)
b) fusion of secondary lamella (right arrow)

After incubation of agar plates, thirteen isolates were obtained from the kidney (n=3), spleen (n=7) and gills (n=3) of thirteen diseased fish. Only one type of colony morphology was determined after 48 hours incubation. The isolates produced shiny, round, white colonies on TSA and TYES agar (Figure 2) and were preliminary identified based on cellular appearance in Gram staining. Bacterial pathogens were not isolated from the fish. After Gram staining, Gram-positive, typical large oval cells were defined as yeast (Figure 3). The ITS region sequence analysis of the representative isolate was contributed to microscopic identification and based on the sequence data this strain was identified as a *Candida* spp. The ITS sequence of strain D11 has been deposited in GenBank library under accession numbers MF411159. Similarly, Hatai and Egusa (1975) noted that *Candida sakei* cells isolated from distended stomach of *Oncorhynchus rhodurus* which were oval or elongate cell types. In another study, *Metschnikowia bicuspidata* var. *bicuspidata* was showed entirely large club-shaped cells that stained strongly Gram positive (Moore and Strom, 2003).

Figure 2. Growth of *Candida* sp. on TSA

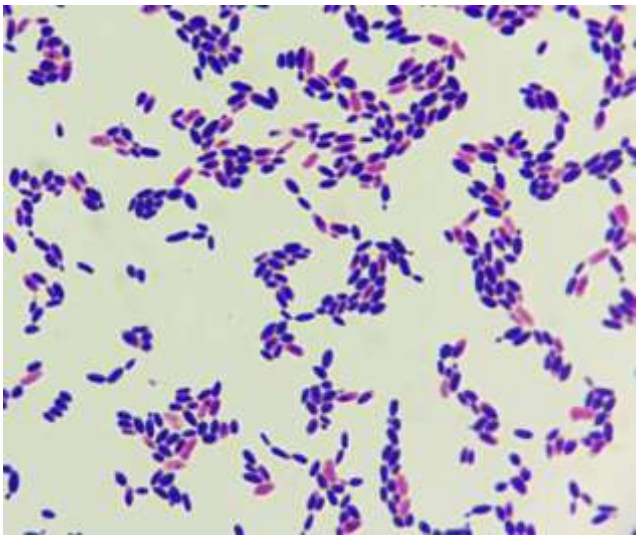

Figure 3. Gram morphology of *Candida* sp. cells (X1000)

CONCLUSIONS

In this study, *Candida* sp. was isolated from fry of rainbow trout, *Oncorhynchus mykiss* following the RTFS outbreak, for the first time in Turkey. *Flavobacterium psychrophilum* is one of the main pathogen caused disease among farmed rainbow trout fry and fingerlings. For the treatment of RTFS, antibiotics have been intensively used in hatcheries of rainbow trout. This study showed that inappropriate use of antibiotics, increased risk of yeast infections in fish due to disruption of microflora. Therefore, it will be useful to take protective measures against bacterial diseases and need to support the flora with probiotic bacteria in fish.

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