

**PHOTOBACTERIUM DAMSELAE CASE IN FARMED SEA
BREAM AND SEA BASS: MOLECULAR IDENTIFICATION
AND ANTIBIOTIC SUSCEPTIBILITY**

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Abstract

In the current study, gilthead sea bream ($n = 100$) and European sea bass ($n = 100$), with suspected infection by *P. damsela*, which showed skin lesions, were sampled under aquaculture conditions. *P. damsela* was diagnosed by the BD Phoenix rapid diagnosis system, and its subspecies were identified by polymerase chain reaction. Initially, BD Phoenix identified 26 *Photobacterium damsela* isolates from all tissue samples. Also, as to polymerase chain reaction analysis, 8 isolates from 26 identified isolates showed positive results for the 16S rRNA, ureC, and CPS genes and were identified as *P. damsela* subsp. *damsela*. On the other hand, isolates ($n = 18$) with 16S rRNA and CPS gene-positive and ureC gene-negative results were defined as *P. damsela* subsp. *piscicida*. Antibiotic tests showed that *P. damsela* subsp. *damsela* isolates were susceptible to amoxicillin-clavulanic acid, ciprofloxacin, cefotaxime, and ampicillin. Conversely, *P. damsela* subsp. *piscicida* isolates were sensitive to ciprofloxacin and gentamicin.

Key words: *Photobacterium damsela*, European sea bass, gilthead sea bream, molecular typing, rapid diagnostic systems

Introduction. Bacterial fish pathogens are quite a major threat factor to fish health under aquaculture conditions. *Photobacterium damsela* is a significant pathogen for marine fish culture in Türkiye [1–3], as well as in other Mediterranean

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countries [4–6]. *Photobacterium damsela* has two notable subspecies, *Photobacterium damsela* subsp. *damsela* and *Photobacterium damsela* subsp. *piscicida*, which cause dark skin pigmentation, exophthalmia, pale gills, and epidermal ulcers in marine fish [7,8]. Also, these pathogens affect internal organs, mainly the spleen and liver [9,10]. In the identification of *P. damsela*, besides molecular techniques [7,8], rapid diagnostic systems like the BD Phoenix, Vitek, and Vitek MS have been thoroughly and effectively used [11,12]. Additionally, *P. damsela* and its subspecies have been diagnosed rapidly and accurately by molecular techniques [13–15]. The 16S rRNA [6,7,13,15] and CPS (capsular polysaccharide gene) gene [14,15] were frequently utilized in *Photobacterium damsela* isolation; also, during subspecies differentiation in the identification, the ureC gene was used in different studies [6,7,13,15]. The present study is regarding *P. damsela* subsp. *piscicida* and *P. damsela* subsp. *damsela* infections in infected European sea bass and gilthead sea bream cultured in the southwestern part of Türkiye. Isolates were isolated and identified by both molecular and BD Phoenix rapid diagnosis systems; also, their susceptibilities to various antibiotics were reported.

Material and method. Sampling. A total of 200 cultured European sea bass (*Dicentrarchus labrax*) ($n = 100$, 0.2–114 g) and gilthead sea bream (*Sparus aurata*) ($n = 100$, 0.2–110 g) suspected to have died from *Photobacterium damsela* were collected from Muğla, Türkiye between June and September in 2018 and 2019, and also in September in 2021.

Isolation. A loopful of tissue samples (kidney, spleen, and liver) crushed between two slides was taken and inoculated onto tryptic soy agar (TSA) containing 1% NaCl. The incubation temperature was 25 °C for 24–48 hours. Also, TSA medium containing 1% NaCl was used for purification of single colonies. Hemolysis test was carried out with blood agar containing 5% sheep blood. In bacterial isolate typing, Gram stain and catalase and oxidase tests were used.

Identification. *Photobacterium* spp. suspected isolates on TSA were transferred into ID broth and put in the panels (NMIC/ID433) and placed in the device for biochemical identification in the BD-Phoenix M50 rapid diagnostic system device.

PCR conditions. *P. damsela* isolates identified by the BD-Phoenix were selected for molecular identification and boiled. 16S rRNA [13] and CPS gene [5] were used for species identification; also, the urease gene (ureC) was used for subspecies identification [15]. Base pairs are in Table 1. To obtain genomic DNA, a loopful of bacterial colonies was placed in an eppendorf containing 500 µl of distilled water and boiled in a dry block heater for 20–30 min. The reaction conditions for the 16S rRNA gene, the CPS gene, and ureC gene were rearrangement from previous studies [5,13]. A Prima-Trio (Himedia®) DNA thermal cycler was used for PCR amplification. The results of etidium bromide-stained agar gel electrophoresis (2% w/v) were observed under a UV transilluminator in the Vilber Lourmat Gel Documentation System.

Antibiotic testing. Antimicrobial sensitivity was demonstrated by the Kirby–Bauer disc diffusion technique by purified isolates on Mueller Hinton agar, and the CLSI Standard was used to measure and evaluate antimicrobial zones [16]. Antimicrobial agents (Oxoid®) were amoxicillin-clavulanic acid (30 µg), tetracycline (30 µg), ceftizoxime (30 µg), cefotaxime (30 µg), sulfamethoxazole-trimethoprim (25 µg), ampicillin (10 µg), gentamicin (10 µg), and ciprofloxacin (5 µg). Against antimicrobial agents, isolates were reported as resistant, susceptible, or intermediate.

Results. Clinically, diseased juvenile sea bream (0.8–1.3 g) showed skin lesions with detached scales, also, some of them showed an enlarged spleen with white tubercles. Sea bass samples displayed only skin lesions. In the bacterial isolation, strains of both subspecies produced similarly small, white colonies on TSA and blood agar at 25 °C, isolates were Gram-negative, bipolar-stained, and catalase and oxidase-positive. After Gram staining, 26 *Photobacterium* spp. suspected isolates were verified as *Photobacterium damsela* by the BD-Phoenix identification method. These isolates were only from gilthead sea bream, not from European sea bass. All 26 isolates were verified as *Photobacterium damsela* by PCR using 16S rRNA and CPS primers, and at electrophoresis, these primers amplified as 267 bp and 410 bp, respectively. At subspecies differentiation, solely *P. damsela* subsp. *damsela* strains displayed 448 bp PCR product of the ureC (Fig. 1). Totally, 8 *P. damsela* subsp. *damsela* strains were identified. The other 18 strains were *P. damsela* subsp. *piscicida*. At antibiogram test, *P. damsela* subsp. *piscicida* was susceptible to gentamicin, ciprofloxacin, ceftizoxime, ampicillin, amoxicillin-clavulanic acid and cefotaxime. Also, strains were intermediately resistant to sulfamethoxazole-trimethoprim (Table 2). However, *P. damsela* subsp. *damsela* strains were susceptible to ampi-

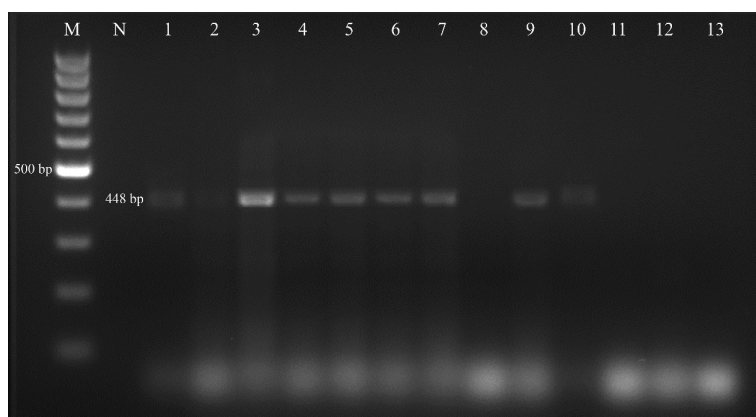


Fig. 1. URE C PCR electrophoresis view of *P. damsela* subsp. *damsela*. M: 100 bp molecular marker; N: Negative control, '1, 3–7, 9, 10': Positive samples of URE C; 8, 11–13: Negative samples of URE C

T a b l e 1

The primer sequences utilized in molecular identification

Primers		Sequence
16S rRNA	Forward primer	5'-GCT TGA AGA GAT TCG AGT-3'
	Reverse primer	5'-CAC CTC GCG GTC TTG CTG- 3' [13]
CPS	Forward primer	5'-AGG GGA TCC GAT TAT TAC TG-3'
	Reverse primer	5'-TCC CAT TGA GAA GAT TTG AT-3' [5]
ureC	Forward primer	5'-TCC GGA ATA GGT AAA GCG GG-3'
	Reverse primer	5'-CTT GAA TAT CCA TCT CAT CTG C-3' [5]

T a b l e 2

Antibiotic resistance of *P. damsela* subsp. *piscicida* (Pdp) and *P. damsela* subsp. *damsela* (Pdd) isolates, S: susceptible, I: intermediate, R: resistant

Antimicrobial agents	Concentration	Pdp	Pdd
AMC: amoxicillin-clavulanic acid	30 µg	S	S
AMP: ampicillin	10 µg	S	S
CN: gentamicin	10 µg	S	S
CIP: ciprofloxacin	5 µg	S	S
SXT: sulfamethoxazole-trimethoprim	25 µg	R	I
TET: tetracycline	30 µg	I	I
CTX: cefotaxime	30 µg	S	S
ZOX: ceftizoxime	30 µg	S	S

cillin, amoxicillin-clavulanic acid, cefotaxime, ciprofloxacin, gentamicin, and ceftizoxime. Additionally, both subspecies exhibited intermediate or low-rate resistance to sulfamethoxazole-trimethoprim and tetracycline (Table 2).

Discussion. In the present study, *Photobacterium damsela* was identified by the BD-Phoenix rapid diagnosis system and polymerase chain reaction. *P. damsela* infects both wild fish and cultured juvenile and adult fish in Mediterranean waters [4, 15, 17, 18]. Despite outbreaks in nature, there are few disease problems related to *P. damsela* under culture conditions in Türkiye nowadays by combination vaccine of *Vibrio* spp. and *P. damsela*. Until now, a few outbreaks have been reported, especially in juvenile gilthead sea bream in tanks and rarely in cage systems in sea bass. Thus, scientific reports have been remarkably rare related to *P. damsela* outbreaks (Personal communication with an aquaculture engineer on a farm). In Egypt, there were many infection reports of mass deaths of sea bream, sea bass, and grey mullet infected by *P. damsela* [15, 17, 18]. According to these studies, *P. damsela* subsp. *damsela* infected 38.32% of sea bass and 44.47% of sea bream, and *P. damsela* subsp. *piscicida* infected 16.2% of sea bass

and 26.46% of sea bream in a natural outbreak in culture conditions, sampling throughout the year [15]. As to the report of a natural outbreak in a natural area, 35.38% of sea bass, 25% of sea bream, and 15.38% of grey mullet (*Mugil capitus*) were infected by *P. damsela* subsp. *damsela*, and 55.38% of sea bass, 44.44% of sea bream, and 35.38% of grey mullet were infected by *P. damsela* subsp. *piscicida*, fish sampled all through a year [5]. In another study, thin-lipped grey mullet (*Liza ramada*) was infected at 8.33%, sea bream at 4%, and sea bass at 7.2% for both subspecies of *P. damsela* under farm conditions [17]. Additionally, in a case that collected infected fish from winter to fall in a natural resource, *P. damsela* subsp. *damsela* infected 7.54% of young sea bream and 5.93% of young sea bass and infected 44% of adult sea bream and 28% of adult sea bass [18]. In the summer season, both subspecies of *P. damsela* are highly virulent [5,7]. In a study [19], 83.33% of all fish were infected by *P. damsela* subsp. *damsela*. In a different study, 77.56% of all sampled fish were infected by both subspecies of *P. damsela* [15]. Clinically, *P. damsela* caused skin lesions and darkening pigmentation [1,15,17] and tubercles in enlarged spleens of fish [17]. In the present study, similar results were observed. BD-Phoenix identification was utilized in several studies. In a study, BD-Phoenix had 82% true recognition in some Gram-positive cocci samples [12]. Another study [20] found 95.2% true BD-Phoenix identification with Gram-negative rods. In the current study, BD-Phoenix results were verified by polymerase chain reaction tests. The polymerase chain reaction technique has commonly been used for identification of *P. damsela* and its subspecies. In the present study, *P. damsela* isolation was confirmed in accordance with previous studies using the 16S rRNA [7,13] and the CPS gene [14,15]. Also, in subspecies differentiation, the urease gene was used in the current study, similar to previous studies [13,15]. The disc diffusion results differed depending on strains; *P. damsela* subsp. *piscicida* strains were reported as sensitive [9] and resistant [17] against sulfamethoxazole-trimethoprim in different studies. Some studies [9,10] reported sensibility against ampicillin and tetracycline on the same subspecies; on the other hand, in a study [3], resistancy was reported against ampicillin. Also, the same studies [9,10] reported susceptibility of *P. damsela* subsp. *damsela* strains against sulfamethoxazole-trimethoprim. Also, another study [2] reported resistance against the same antibiotic. There was a report [10] showing tetracycline resistance, and another one [19] reported resistance to cefatoxim on the same subspecies. In the present study, *P. damsela* subsp. *piscicida* showed resistance against sulfamethoxazole-trimethoprim. In addition, two subspecies' strains were intermediately resistant to tetracycline.

Conclusion. The findings of the study suggest that gilthead sea bream and European sea bass should be routinely observed by rapid diagnosis systems and molecular techniques to identify *P. damsela* outbreak in Mediterranean aquaculture.

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