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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Photobacteriosis in farmed Atlantic bluefin tuna (*Thunnus thynnus)*: The first comprehensive study of the Aegean Sea

Yetiştiricilikte Atlantik mavi yüzgeçli orkinosunda (*Thunnus thynnus*) fotobakteriyozis: Ege Denizi'ne ait ilk kapsamlı çalışma

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Abstract: *Photobacterium damselae* subsp. *damselae* is a significant pathogen that cause severe bacterial septicemia in a broad range of hosts. This study presents the first comprehensive analysis of *Photobacterium damselae subsp. damselae* affecting farmed Atlantic bluefin tuna (*Thunnus thynnus*) stocks in Aegean Sea, Türkiye. The total mortality rate of the farm was calculated as 18% exhibiting clinical signs of the disease, characterised by lethargic swimming and loss of equilibrium in bluefin tuna cages. Although external clinical signs were not determined, hemorrhages were observed in liver, swim bladder and heart. Microbiological examinations resulted in the isolation of a total of four bacterial strains, including two isolates from the summer sampling period and two isolates from the autumn sampling period. Among these, representative strains designated as THAS1 and THAS2 were selected for further biochemical and molecular characterization, through which *Photobacterium damselae* subsp. *damselae* was identified. Histopathological examinations of liver, heart, spleen, and kidney tissues associated with the infection revealed the presence of melanomacrophage centers in histological sections of the spleen and kidney. Consequently, this study reveals the epizootic and impact of *P. d.* subsp. *damselae* infection in farmed Atlantic bluefin tuna with clinical and microbiological results associated with pathological changes. Further research on the epidemiology and epizootiology of this disease is essential for sustainable aquaculture of tuna fish stocks, especially in Mediterranean.

Keywords: Photobacterium, marine cage culture, tuna, bacteriology, histopathology

Öz: *Photobacterium damselae* subsp. *damselae*, birçok deniz balığı türünde şiddetli bakteriyel septisemiye neden olan önemli bir patojendir. Bu çalışma, Türkiye'nin Ege Denizi'nde yetiştiriciliği yapılan Atlantik mavi yüzgeçli orkinos (*Thunnus thynnus*) stoklarını etkileyen *Photobacterium damselae* subsp. *damselae*'nin ilk kapsamlı analizini sunmaktadır. Hastalığa ait klinik bulguların gözlendiği işletmede toplam mortalite oranı %18 olarak hesaplanmış olup, mavi yüzgeçli orkinos kafeslerinde letarjik yüzme davranışı ve denge kaybı ile karakterize edilmiştir. Dış bakıda klinik bulgular tespit edilmemekle birlikte, karaciğer, yüzme kesesi ve kalpte hemorajiler gözlenmiştir. Mikrobiyolojik incelemeler sonucunda, yaz örnekleme döneminden iki ve sonbahar örnekleme döneminden iki olmak üzere toplam dört bakteriyel suş izole edilmiştir. Bunlar arasından THAS1 ve THAS2 olarak adlandırılan temsilci suşlar ileri biyokimyasal ve moleküler karakterizasyon analizleri için seçilmiş ve bu analizler sonucunda *Photobacterium damselae* subsp. *damselae* tanımlanmıştır. Enfeksiyonla ilişkili olarak karaciğer, kalp, dalak ve böbrek dokularında yapılan histopatolojik incelemelerde, dalak ve böbreğe ait histolojik kesitlerde melanomakrofaj merkezlerinin varlığı ortaya konulmuştur.Sonuç olarak bu çalışma, yetiştiriciliği yapılan Atlantik mavi yüzgeçli orkinoslarda *P. d.*subsp. *damselae* enfeksiyonunun epizootik seyri ve etkilerini, klinik ve mikrobiyolojik bulgular ile patolojik değişiklikler eşliğinde ortaya koymaktadır. Özellikle Akdeniz bölgesinde orkinos yetiştiriciliğinin sürdürülebilirliği açısından, bu hastalığın epidemiyolojisi ve epizootiyolojisine yönelik ileri araştırmalar büyük önem taşımaktadır.

Anahtar kelimeler: Fotobakteriyozis, deniz kafes yetiştiriciliği, orkinos, bakteriyoloji, histopatoloji

INTRODUCTION

Tuna aquaculture is a key sector in global seafood production, responding to growing consumer demand and the decline in wild tuna stocks. *Thunnus thynnus* (Atlantic Bluefin Tuna), *Thunnus orientalis* (Pacific Bluefin Tuna), *Thunnus maccoyii* (South Asian Bluefin Tuna) and recently *Thunnus albacore* are highly valued meat and preferred for capture-based aquaculture (Ottolenghi, 2008). In the Mediterranean, the extensive coastline of Türkiye and the favourable marine environment provide adequate conditions for tuna aquaculture. The production of bluefin tuna is continuously increasing, with a growth of 12.9% between 2022 and 2023, reaching a total of 2,587 tons in 2023 (TÜİK, 2025). However, intensive farming practices, high stocking densities, and environmental stressors have also increased the susceptibility of cultured fish to infectious diseases. As a result, bacterial pathogens have become an important challenge for the sustainability and economic viability of aquaculture systems.

Among these pathogens, Photobacterium is a Gram-negative bacterial genus that includes several species that affect marine organisms, especially in aquaculture. Originally, *Photobacterium damselae* subsp. *damselae (P. d.* subsp. *damselae)* was referred to as *Vibrio damselae* (*V. damselae*), named after the damselfish from which it was isolated (Love et al., 1981). However, following additional research on its genetic and phenotypic traits, the name *P. damselae* was suggested (Rivas et al., 2013; Mohamad et al., 2019). The species includes two subspecies; *P. d.* subsp. *damselae* and *P. Damselae* subsp. *piscicida (P. d.* subsp. *piscicida)*. Both path ogens cause acute fish photobacteriosis/pseudotuberculosis in various fish species (Austin and Austin, 2016). *P. d.* subsp.

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damselae is considered an emerging pathogen of marine fish significant to aquaculture, with a considerable expansion in its geographical distribution over the past few years (Terceti et al., 2016, Trevisani et al., 2017). This mesophilic bacterium is increasingly reported from a diverse variety of organisms, including cetaceans, reptiles, molluscs, crustaceans, and various species of wild and cultivated marine fish around the world (Rivas et al., 2013).

Several studies have documented *P. d.* subsp. *damselae* isolation from diseased fish such as Asian seabass (*Lates calcarifer*) (Weawsawang et al., 2024), silver pomfret (*Pampus argenteus*) (Tao et al., 2018; Zhang et al. 2024), yellowtail kingfish (*Seriola lalandi*) (Gupta et al., 2021), parrotfish (*Scarus globiceps*) (Petchimuthu et al., 2021), mullets (*Mugil cephalus*, *Liza aurata*, *Liza ramada* and *Liza saliens)* (Trevisani et al., 2017) cultured redbanded seabream (*Pagrus auriga* Valenciennes) (Labella et al., 2006), and big-eye trevally (*Caranx sexfasciatus*) (Hassanzadeh et al., 2015) caught in the Persian Gulf, Iran. Recent studies revealed the *P. d.* subsp. *damselae* disease from shrimp species as well; Pacific white shrimp (*Litopenaeus vannamei*) (Wang, 2020; Chuchird et al., 2024), and Kuruma shrimp (*Penaeus japonicus*) (Alolod et al., 2024). This broad host distribution suggests that the pathogen has low host specificity and is capable of infecting phylogenetically diverse fish species. Therefore, the presence of this pathogen in Atlantic bluefin tuna in the present study is consistent with its known ecological adaptability and opportunistic nature.

In Türkiye, *Photobacterium damselae* infections have previously been reported in several cultured marine fish species, indicating the presence and potential impact of this pathogen in regional aquaculture systems. However, information regarding Atlantic bluefin tuna remains limited. This study presents the first report of *P. d.* subsp. *damselae* infection in bluefin tuna with detailed diagnostic methods used to identify the disease, along with the biochemical and molecular characterisation of the pathogen. Additionally, the histopathological findings associated with the infection contribute to a deeper understanding of its impact on bluefin tuna health and aquaculture.

MATERIALS AND METHODS

Sample collection and study area

Daily mortalities were recorded from a commercial tuna farm in the Aegean Sea, İzmir, Türkiye. The mortalities started in August 2024 and continued until the end of October 2024. During this period, two moribund fish from the summer sampling period and two moribund fish from the autumn sampling period (each approximately 50–60 kg in weight and 1.6–1.7 m in length) were individually sampled from the cages and transported by the farm personnel to the İzmir Katip Çelebi University Faculty of Fisheries, Fish Diseases and Biotechnology Laboratory within two hours of death under cold chain conditions. Clinical signs were recorded, and comprehensive parasitological and bacteriological examinations were performed. The sample size was limited due to field conditions, the large body size of Atlantic bluefin tuna, and the restricted availability of moribund individuals during mortality events.

Bacteriological and parasitological examination

After dissection, comprehensive parasitological and bacteriological examinations were performed. Parasitological analysis included the inspection of external surfaces, gills, and internal organs for the presence of parasites. For bacteriological examination, samples were aseptically collected from the anterior kidney and spleen tissues. A total of two bacterial isolates were obtained from the summer and autumn sampling periods. The isolates were streaked on different culture media, including marine agar (MA, Merck, Germany), tryptic soy agar (TSA, Merck, Germany) supplemented with 2% NaCl, thiosulfate citrate bile sucrose agar (TCBS, Merck, Germany), and TSA (Merck, Germany) supplemented with sheep blood, to isolate the causative bacterial agent. The inoculated plates were incubated at 24 °C for 24–48 h. Colony morphology was subsequently examined, and the isolated colonies were re-streaked on TSA for further identification tests. Isolates were selected for further analyses based on consistent colony morphology, repeated isolation from target organs, and their presumed association with systemic infection. Only representative isolates showing dominant growth characteristics were subjected to biochemical and molecular identification.

Biochemical characterization

Motility, oxidase and catalase activity tests and Gram staining were implemented due to the standard procedures (Austin and Austin, 2016). The biochemical characteristics of the isolated bacteria were detemined with API 20E tests (BioMerieux S.A.)

Molecular characterization

Molecular characterization of the isolated bacterial strains was performed based on 16S rRNA gene sequencing. Genomic DNA was extracted using the EurX GeneMATRIX Bacterial & Yeast DNA Isolation kit (Poland), following the protocol rcommended by the manufacturer. DNA concentration and ourity were evaluated using a Thermo Scientific Nanodrop 2000 spectrophotometer (USA).

Amplification of the target gene region ws performed using the universal primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTTACGACTT 3'). PCR amplification was conducted under the following cycling conditions: an initial denaturation at 95 °C for 5 min; 30 amplification cycles consisting of denaturation at 95 °C for 45 s, primer annealing at 57 °C for 45 s, and extension at 72 °C for 60 s; and a final elongation step at 72 °C for 5 min. The reaction mixture was then maintained at 4 °C. PCR products were separated on a 1.5% agarose gel prepared with 1× TAE buffer and electrophoresed at 100 V for 90 min. After staining with ethidium bromide, DNA fragments were visualized under UV light. A fragment of approximately 1470 bp was successfully amplified using FIREPol® DNA Polymerase (Solis

Biodyne, Estonia). Successful amplification was confirmed by the presence of distinct bands.

Amplicons exhibiting a single, well-defined band were purified using the MAGBIO HighPrep™ PCR Clean-up System according to the manufacturer's instructions. Purified products were sequenced using an ABI 3730XL Genetic Analyzer (Applied Biosystems, USA) with the BigDye Terminator v3.1 Cycle Sequencing Kit. Forward and reverse reads obtained with 27F and 1492R primers were assembled into consensus sequences using the CAP algorithm implemented in BioEdit software.

The obtained 16S rRNA gene sequences were compared with closely related sequences available in the GenBank database using the BLASTN (version 2.6.1) algorithm. Sequence alignment and phylogenetic analyses were conducted using MEGA 11 software. The phylogenetic tree was constructed based on the Neighbor-Joining (NJ) method, and the robustness of the tree topology was evaluated by bootstrap analysis with 1000 replicates.

Histopathological examination

Histopathological examinations were performed on all sampled fish, and tissue sections from each examined organ were evaluated for histopathological alterations. Liver, kidney, spleen, and heart tissue from infected tuna samples were fixed in 10% neutral buffer formalin solution. After fixation, the standard ethanol dehydration protocol was performed according to Roberts (2012). The processed and blocked tissues were sectioned at a thickness of 5 µm using a soft tissue microtome. The samples were then prepared for staining with Hematoxylin-Eosin (H&E) stain according to the modified method of Roberts (2012). The stained samples were examined with a light microscope (Olympus, CX22RFS1) to determine histopathological changes. Histopathological alterations were semi-quantitatively evaluated using a lesion severity scoring system. Each lesion (degeneration, vacuolisation, inflammation, and necrosis) was scored as follows: 0 = absent, 1 = mild, 2 = moderate, and 3 = severe.

RESULTS

This study investigated the epizootic and impact of *P. d.* subsp. *damselae* infections in farmed bluefin tuna with clinical and microbiological results associated with pathological changes. The molecular results confirmed the identification of the pathogen together with the biochemical characterisation of the isolated pathogens.

Lethargic swimming and loss of equilibrium followed by death were observed in blufin tuna fish cages. An 18% mortality rate was recorded in the cages of farmed bluefin tuna. The affected fish weighed approximately 50–60 kg and measured 1.6–1.7 m in length, while the sea temperature was 23 °C and the salinity was 38‰. Although no clinical findings were observed on external examination, haemorrhagic septicaemia in liver, swim bladder and heart, reddish fluid in the peritoneal cavity, petechial haemorrhages in the viscera were detected at autopsy (Figure 1). Parasitological examination of the sampled fish revealed no detectable findings.

Figure 1. A: The sampled bluefin tuna, B: the clinical resuls (arrrow: haemorhagic septisemia in the visceral fat

Two distinct bacterial strains, designated as THAS1 and THAS2, were isolated from the summer and autumn sampling periods, respectively. The isolates were recovered from the anterior kidney and spleen tissues of moribund Atlantic bluefin tuna. Following primary isolation, colonies showing consistent morphological characteristics were subcultured to obtain pure cultures. Representative isolates demonstrating dominant and uniform colony morphology were selected for further biochemical, molecular, and phylogenetic analyses. Both strains were found to be Gram-negative, motile and rod-shaped (Figure 2). The biochemical characteristics of the isolates obtained using the API 20E system are summarized in Table 1.

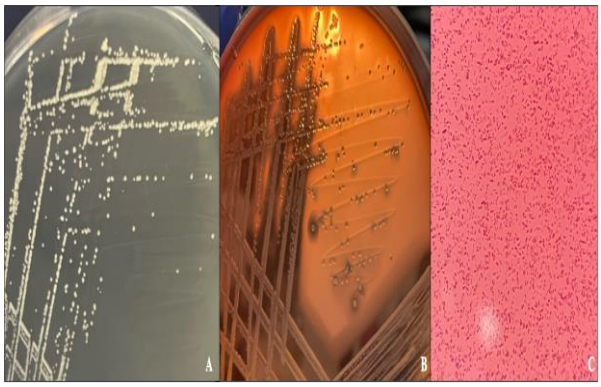

Figure 2. A: Colony morphology of *P. d.* subsp. *damselae* on TSA in 2% NaCl, B: Colony morphology of *P. d.* subsp. *damselae* on TSA supplemented with sheep blood and β-hemolysis, C: Gram staining of isolated bacteria

Table 1. The morphological and API 20E biochemical properties *P. d.* subsp. *damselae* strains (THAS1 and THAS2) isolated from *Thunnus thunnus*

Tests	THAS1	THAS2
Gram stain	-	-
Shape	Rod-shaped	Rod-shaped
Catalase	+	+
Oxidase	+	+
ONPG	-	-
ADH	+	+
LDC	+	+
ODC	-	-
CIT	-	-
H_2S	-	-
Urease	+	+
TDA	+	+
IND	-	-
VP	+	+
GEL	+	+
GLU	+	+
MAN	-	-
INO	-	-
SOR	-	-
RHA	-	-
SAC	-	-
MEL	-	-
AMY	-	-
ARA	-	-

ONPG: β-galactosidase, ADH: Arginine dihydrolase, LDC: Lysine decarboxylase, ODC: Ornithine decarboxylase, CIT: Citrate, H_2S: hytdrogen sulfide, TDA: Tryptophan deaminase, IND: Indole , VP: Voges-Proskauer, GEL: Gelatin, GLU; Glucose, MAN: Mannose, INO: Inositol, SOR: Sorbitol, RHA: Rhamnose, SAC: Sucrose, MEL: Melibiose, AMY: Amygdalin, ARA: Arabinose.

The isolates demonstrated hemolytic activity (β –hemolysis) on blood agar, gelatinase activity, and motility, which are key distinguishing characteristics of *P. d.* subsp. *damselae*, unlike the non-hemolytic, non-motile, and gelatinase-negative phenotype generally observed in *P. d.* subsp. *piscicida.* These biochemical and morphological observations, along with the 16S rRNA gene sequence analysis exhibiting 100% identity with *P. d.* subsp. *damselae* in the BLASTN database, facilitated the identification of the isolates as *P. d.* subsp. *damselae*. The sequences designated as THAS1 and THAS2 have been registered in GenBank under accession numbers PQ633205 and PQ633217, respectively. A phylogenetic tree that includes these strains and homologous sequences from GenBank is illustrated in Figure 3.

Histological sections of diseased bluefin tuna liver showed cellular degeneration, vacuolisation, inflammation and necrosis within the liver parenchyma (Figure 4 – A, B, C). These lesions were predominantly scored as moderate to severe (scores 2–3). Cardiac tissue examination showed focal cardiomyopathy, characterized by disorganised cardiac muscle fibers and necrotic areas (Figure 4-D), with lesion severity generally ranging from mild to moderate (scores 1–2). The examination of the spleen tissue sample revealed melanomacrophage centers and presented in Fig. 4 – E, F indicating activation of the immune response, with moderate lesion scores (score 2). Inflammatory cells, tubular necrosis and melanomacrophage centers were identified, suggesting the presence of a possible renal parenchymal damage within the kidney (Figure 4 - G, H). These alterations were mostly moderate to severe in intensity (scores 2–3).

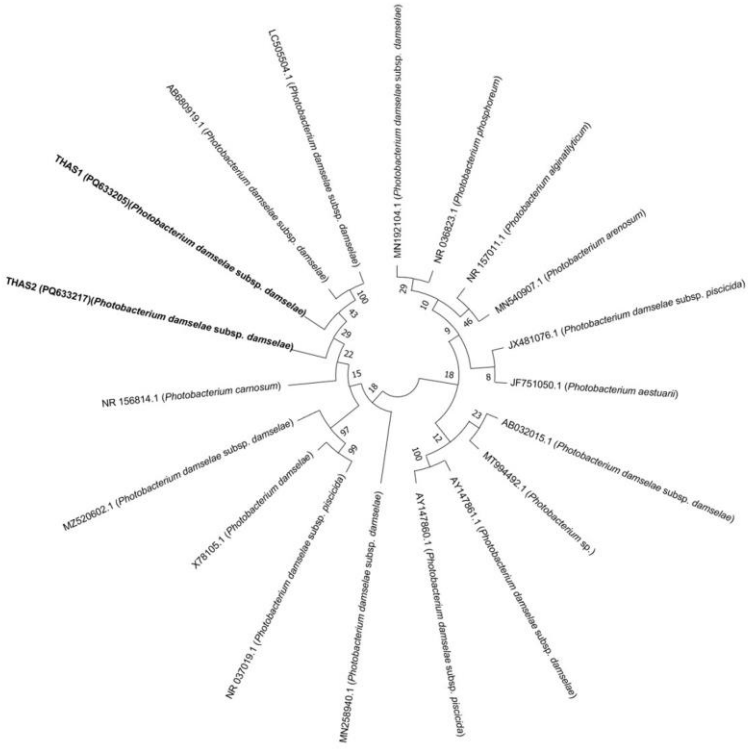

Figure 3. Phylogenetic tree of *P. d.* subsp. *damselae* strains PQ633205 (THAS1) and PQ633217 (THAS2) (in bold) depicts the evolutionary relationships between related *Photobacterium* sequences obtained from GenBank (NCBI). The Maximum Composite Likelihood Method was accomplished with Mega 7 software with using 1000 bootstrap replicates.

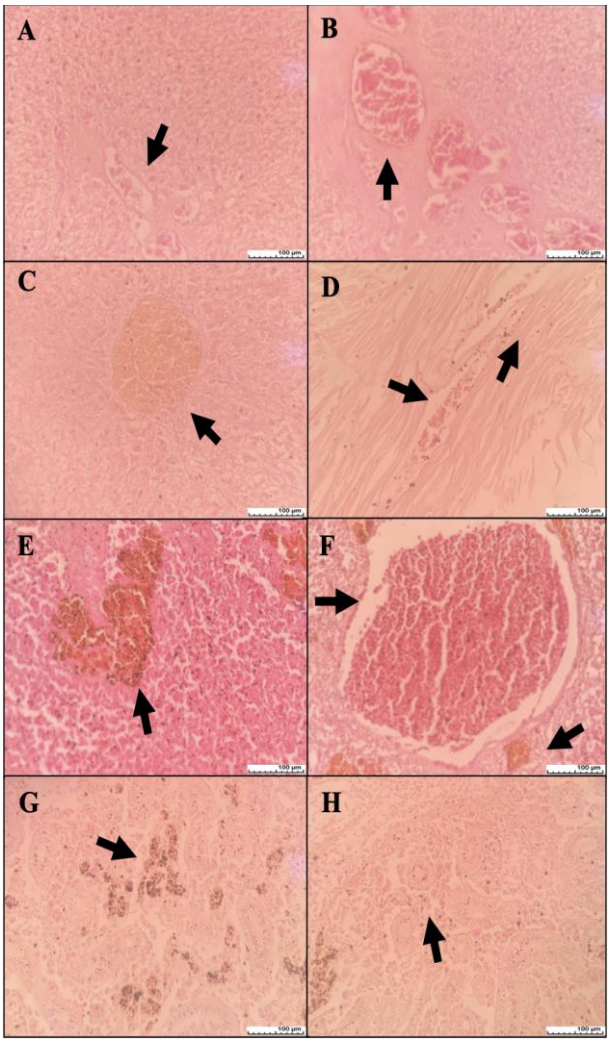

Figure 4. Hematoxylin and Eosin (H-E) stain of *P. d.* subsp. *damselae* infected tissues; A-B-C: liver tissue with vacuolization, inflammation, cellular degeneration and necrosis, D: focal cardiomyopathy and necrotic areas along with disorganised cardiac tissue, E-F: melanomacrophage centers in spleen tissue, G-H: kidney tissue with tubular necrosis, inflammatory cells and melanomacrophage centers

DISCUSSION

In the present study, *P. d.* subsp. *damselae* infection was detected in Atlantic bluefin tuna, representing the first report in this species. Previous studies on *P. d.* subsp. *damselae* have demonstrated that this pathogen is prevalent across a variety of marine fish hosts, both in wild populations and cultured species. The health status of the tuna population, whether in aquaculture or in wild fisheries, is the main concern given the significant economic value of the tuna species. This study provides a comprehensive analysis of *P. d.* subsp. *damselae* disease impacting tuna stocks in Türkiye, presenting detailed molecular and histopathological findings.

There is limited information about nutritional, environmental, and microbial diseases on the contrary of parasitic cases of tuna fish diseases (Munday et al., 2003). Environmental factors such as water parameters and the presence of stressors can affect the virulence of pathogens, especially in aquaculture facilities. Additionally, there is a notable lack of comprehensive information on bacterial diseases that affect tuna stocks. Further studies focusing on virulence gene profiling and whole genome sequencing are required to better understand strain-specific pathogenicity. The opportunistic bacterial infection agents are listed as *Aeromonas* sp., *Vibrio* spp., *P. d.* subsp. *piscicida*, *Photobacterium phosphoreum* and *Mycobacterium marinum* for tuna fish (Munday et al., 2003; Mladineo et al., 2006). This study is the first known report of *P. d.* subsp. *damselae* infection of atlantic Bluefin tuna and provides important information on the epidemiology of *P. d.* subsp. *damselae* in tuna stocks. Disease outbreaks could be related with suppression of immune systems due to increased water temperature, nitrite, ammonia, copper, iron, and salinity associated with reduced dissolved oxygen (Plumb and Hanson, 2010; Essam et al., 2016). Comparably, Fouz et al. (1992) revealed that *V. damsela* infection in turbot developed due to the sudden increase in water temperature which causes multiplication and rapid growth of the pathogen. Taking into account all these data, the elevated water temperature may enhance the proliferation of *P. d.* subsp. *damselae*. Although environmental parameters were not directly analyzed in this study, previous studies have suggested that elevated water temperatures may contribute to the proliferation of opportunistic bacterial pathogens in aquaculture systems.

Common clinical signs of *P. d.* subsp. *damselae* were assigned as abdominal distention, bleeding at the base of the fins, around the mouth and eyes, and ulcerative lesions (Austin and Austin, 2016; Gouife et al., 2022). According to previous studies, the typical symptoms of the disease were detected in several species of fish. Jun et al. (2010) observed *P. d.* subsp. *damselae* case in giant grouper with clinical symptoms of lethargy, anorexia and depression for two weeks and at necropsy, hemorrhages on the skin, presence of yellowish fluid in the abdominal cavity and severe hemorrhages inside the intestine, pale, enlarged liver were detected from diseased individuals. Tao et al. (2018) detected *P. d.* subsp. *damselae* infection in cultured silver pomfret (*Pampus argenteus*), with observation of hemorrhages in the fins and petechial hemorrhages in the liver with tissue necrosis. Zhang et al. (2019) reported extensive skin ulcers on the body surface of net-cage cultured black rockfish (*Sebastes schlegeli*) with exophthalmos, and orbital, whitening ulceration. Mladineo et al. (2006) reported *P. d.* subsp. *piscicida* infection in Atlantic bluefin tuna with no external clinical signs except atypical swimming and changed coloration. In this study, while clinical findings exhibited similarities with Mladineo et al. (2006), the common symptoms of the disease were not observed in the sampled fish. These results indicate a potential variability in symptomatology between different species, and further research is warranted to clarify the underlying factors, including host immune responses, pathogen virulence factors, and environmental stressors.

API 20E standardized conventional biochemical test strips are generally used for the presumptive identification of Gram-negative rods, including *Photobacterium* (Essam et al., 2016). Although *Photobacterium* is evolutionarily related to *Vibrio*, it exhibits certain distinguishing phenotypic properties that are commonly found in *Photobacterium* species but are infrequently observed in *Vibrio* species such as degradation of gelatin, positive glucose, VP, LDC and ADH, and inability to utilize mannitol (Urakawa et al. 1999; Alolod et al., 2024). The biochemical results of the API 20E profile of the strains isolated in this study demonstrate compatibility and provide accurate test results for the presumptive identification of *Photobacterium*. These results were supported with 16s rRNA sequence results, and the strains were compared with related Photobacterium strains (Figure 3). A comprehensive genomic analysis of *P. d.* subsp. *damselae* strains isolated from various origins are suggested to demonstrate a more detailed understanding of the virulence and aetiology of disease (Rivas et al., 2013). Although 16S rRNA gene sequencing supported the identification of the isolates as *Photobacterium damselae*, it should be noted that this method has limited resolution at the subspecies level. Therefore, the identification of *P. d*. subsp. *damselae* should be interpreted with caution, and further confirmation using multilocus sequence analysis or whole genome sequencing would be beneficial.

One of the virulence factors associated with *P. d.* subsp. *damselae* is hemolytic activity. The correlation of virulence and haemolytic activity of *P. d.* subsp. *damselae* was divided into two groups: Group 1 demonstrating strong haemolytic activity and has all virulence genes, and Group 2 demonstrating low haemolytic activity and has only one virulence gene. Haemolytic activity against fish or sheep erythrocytes has been associated with a toxin called damselysin (Dly) (phospholipase-D), porogenic toxin (PhlyP), chromosomal toxin (PhlyC), and phospholipase PlpV activity (Rivas et al., 2011; Rivas et al., 2013; Vences et al., 2017, Osorio et al., 2018; Gouife et al., 2022). It is established that, β-hemolytic bacteria exhibit greater pathogenic potential than their α-hemolytic counterparts (Hasssanzadeh et al., 2015). In this study, β -hemolysis was observed in the THAS1 and THAS2 isolates. With regard to previous research and the course of the current case of the disease, it is clear that the virulence factors of *P. d.* subsp. *damselae* should be investigated in detail to clarify the effects of the pathogen in different species in the Mediterranean area.

Zhang et al. (2024) observed histopathological evaluations of experimentally *P. d.* subsp. *damselae* infected silver pomfret (*Pampus argenteus)* and multiorgan bleeding, histopathocyte vacuolation and inflammatory infiltration were reported as the main pathological changes. Tao et al. (2018) determined cytoplasm vacuolization, cellular swelling, cyolysis of erythrocytes and pleomorphic nuclei of hepatic cells in the liver tissue of *P. d.* subsp. *damselae* infected silver pomfret. Shao et al. (2019) reported the presence of diffuse necrosis in liver tissue of naturally diseased fish. Petchimuthu et al. (2021) suggested *P. d.* subsp. *damselae* are more virulent in wild caught fish than in culture fish on the basis of the severe tissue damage observed in histopathological results. In the current study, necrosis and inflammation were consistently observed as common histopathological results in the sampled tissues. Additionally, melanomacrophage centres were observed in spleen and kidney samples, indicating an immune response to infection. The presence of these centers suggests a heightened state of immune activity, which may reflect the fish's attempt to combat the pathogen. The presence of melanomacrophage centres in spleen and kidney tissues indicates activation of the innate immune response, which is commonly associated with chronic or systemic infections. This observation is critical because it emphasises the role of the immune system in responding to *P. d.* subsp. *damselae* infection and highlights the potential for developing immunological interventions to improve disease resistance. The histopathological alterations observed in different studies point to the significant impact of the pathogen on fish health. These findings align with recent studies that *P. d.* subsp. *damselae* infection leads to systemic physiological disturbances, not only localised damage such as ulcerations, that warrant further investigation into the mechanisms of virulence and host-pathogen interactions.

CONCLUSION

In conclusion; this study provides detailed information on *P. d.* subsp. *damselae* disease in Atlantic bluefin tuna with clinical, microbiological, molecular, and pathological studies. *P. d.* subsp. *damselae* are considered a severe disease that causes bacteria in wild and commercially important aquaculture species. However in the tuna aquaculture, due to the high economic value of the species and the limited ability to easily transfer a large number of samples from the farms to the laboratory based on the physical characteristics of the fish, the sampling numbers are restricted. This limitation of the present study was the restricted sample size, which was associated with the economic value of fish and the limited availability of moribund fish during the mortality period. As a result, detailed studies on the diseases of tuna fish, similar to those conducted on other cultured fish species, cannot be readily carried out. The findings of this study highlight the importance of regular health monitoring, early pathogen detection, and effective disease management strategies to reduce the potential impact of bacterial infections in Atlantic bluefin tuna farming systems. More research is necessary to demonstrate the transmission of the pathogen between aquaculture facilities and asymptomatic carriers, clarify the virulence factors and the relationship between environmental factors in the Mediterranean area.

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AUTHORSHIP CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ezgi Dinçtürk and Tevfik Tansel Tanrıkul. The first draft of the manuscript was written by Ezgi Dinçtürk and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors have no relevant financial or non-financial interests to disclose.

ETHICAL APPROVAL

This study did not require ethical approval as no live vertebrate animals or human subjects were involved.

DECLARATION OF AI USE

ChatGPT (version 5.0) was used for language editing and improving readability during the preparation of this manuscript. All scientific content, analysis, and interpretations were developed by the authors.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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