

RESEARCH ARTICLE

Molecular and Histopathological Characterization of *Bacillus cereus* Infection in Rainbow Trout (*Oncorhynchus mykiss*)

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ABSTRACT

Bacillus species are widely used as probiotics in aquaculture due to their reported benefits, including growth promotion, immune enhancement, and water quality improvement. However, *Bacillus cereus*, a naturally occurring and potentially toxigenic microorganism, has been identified as a fish pathogen in several studies. In the present study, a mass mortality outbreak occurred at a rainbow trout farm in Isparta, Türkiye, where a daily mortality rate of 2% was recorded. Ten moribund fish (mean body weight: 15 g) were sampled for analysis. Bacterial identification was performed based on morphological characteristics, biochemical tests, and analyses of the 16S rRNA and *gyrB* genes. In addition, histopathological examination was carried out to assess the tissue alterations induced by the pathogen. Based on the antibiogram test using 12 antibiotic discs (Oxoid, Hampshire, UK), the isolate exhibited the highest susceptibility to florfenicol, erythromycin, and ofloxacin, while showing resistance to cefuroxime, amoxicillin, cefotaxime, ampicillin, and tetracycline. Histopathological examination revealed severe *B. cereus*-induced lesions, including necrosis and tissue damage in the spleen, kidney, liver, muscle, and skin. Melanomacrophage infiltration was observed in the kidney and spleen, while inflammatory cell infiltration was evident in the intestine and liver. This study concluded that the *B. cereus* strain isolated from a rainbow trout farm is pathogenic to fish. The combined use of molecular, microbiological, and histopathological methods improves diagnostic reliability and supports appropriate antibiotic selection for controlling *B. cereus* infections in aquaculture. Overall, the findings provide a valuable foundation for improving disease treatment and health management strategies in fish farming systems.

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INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is the most commercially important freshwater fish species cultured in Europe (FEAP, 2022). Among European countries, Türkiye is one of the leading producers of rainbow trout and makes a substantial contribution to the region's aquaculture industry (FAO, 2025). The farming of rainbow trout in Türkiye is typically carried out in freshwater systems such as rivers, lakes and seas in specially designed cages and ponds. However, several bacterial diseases can have a significant impact on rainbow trout farming, leading to significant losses in fish production. Infections are the most important risk factor causing economic losses in the

fish farming industry. The sustainable production of rainbow trout (*Oncorhynchus mykiss*) has become seriously threatened by bacterial infection outbreaks in aquaculture around the world (Duman *et al.*, 2023).

The most important bacterial pathogens isolated from rainbow trout were reported as *Aeromonas salmonicida* (Furunculosis), *Yersinia ruckeri* (Enteric redmouth), *Flavobacterium psychrophilum* (Coldwater disease), *Aeromonas hydrophila* (Motile Aeromonas septicemia), *Edwardsiella tarda* (Edwardsiellosis), *Piscirickettsia salmonis* (Piscirickettsiosis), *Vibrio anguillarum* (Vibriosis), *Lactococcus garvieae* (Lactococcosis) and *Streptococcus iniae* (Streptococcosis) (Toranzo *et al.*, 2009; Duman *et al.*, 2023; Irshath *et al.*, 2023). Numerous

attempts have been undertaken to reduce the incidence of fish infections, and one or more of the aforementioned pathogens have been linked to massive mortality events. At first, these initiatives involved the use of antibiotics, but this eventually backfired because of the problem of antibiotic resistance (Milijasevic *et al.* 2024, Mohammed *et al.* 2025a). In addition, vaccines, probiotics, prebiotics, and natural herbs are used to protect fish against diseases (Pérez-Sánchez *et al.*, 2013; Van Hai, 2015; Mohammed *et al.* 2025b).

Bacillus species are widely used as probiotic bacteria (Fachri 2024; Ghosh, 2025). Over the years, *Bacillus* as a probiotic has been experimentally proven to combat disease, improve feed utilization, resulting in increased growth, boosting immunity in aquaculture and improving the water quality, and reducing stress (Rodrigues *et al.*, 2021; Ke *et al.*, 2022; Zhang *et al.*, 2023). Many studies have been conducted on the presence of *Bacillus* species in the guts of different fish species, their pathogenicity, their tremendous potential as probiotics, and their effects on the fish general health, including immunological function (Kuebutornye *et al.*, 2019; Soltani *et al.* 2019; Ke *et al.*, 2022, Wang 2023; Ghosh, 2025).

B. cereus species belongs to the Bacillaceae family and is a Gram positive bacteria, spore-forming, aerobic/facultative anaerobic, motile, and rod-shaped organism (Ehling-Schulz *et al.*, 2011). This species was first isolated from the air in a cowshed (Frankland and Frankland, 1887). Approximately 120 years after this report, *B. cereus* was determined to cause food poisoning in humans (Bottone, 2010). It was exposed that it causes wounds, eye infections, and systemic infections in human (Ehling-Schulz *et al.*, 2011). The species *B. cereus* is actually an endemic species living in soil (Ehling-Schulz *et al.*, 2005). However, they are perfectly transported because they are very resistant to adverse environmental conditions. This situation is an obstacle to having full knowledge about the origin of the bacteria (Jensen *et al.*, 2003). *Bacillus* species have been reported on growth-enhancing, immune strengthening, stress-reducing and water quality-improving properties in aquaculture (Wang *et al.*, 2017; Rodrigues *et al.*, 2021). Previous studies documented that as a probiotic *B. cereus* boosted immune responses and the growth in the Nile tilapia (*Oreochromis niloticus*), carp (*Carassius auratus*) and shrimp (*Penaeus monodon*) (Wang *et al.*, 2017; Yang *et al.*, 2020). Dey *et al.* (2018) determined that *B. cereus* applied in an encapsulated live diet (chironomid larvae) increases disease resistance against *A. hydrophila* in *Clarias batrachus* juveniles.

In aquaculture, *Bacillus* species, including *B. cereus*, demonstrate a dual role as both probiotics and potential pathogens, depending largely on strain specificity, environmental conditions, and host susceptibility. On one hand, certain non-pathogenic *Bacillus* strains are widely used as probiotics to enhance fish and shrimp health by improving digestion, stimulating immunity, and producing antimicrobial compounds that inhibit pathogens such as *Vibrio spp.* and *Aeromonas spp.* (Zokaiefar *et al.*, 2012). These strains are spore-forming, making them stable in aquatic environments, and are known to secrete enzymes and bacteriocins that improve water quality and gut microbiota balance (Newaj-Fyzul *et al.*, 2014). However, some strains of *B. cereus* harbor virulence genes encoding

enterotoxins and cytotoxins which can cause opportunistic infections in aquacultural systems under stress or immunocompromised conditions (Tran *et al.*, 2022).

B. cereus has occasionally been mentioned as a fish pathogen (Pychynski *et al.*, 1981; Mondange *et al.*, 2022). It has been documented to cause branchionecrosis in common carp (Pychynski *et al.*, 1981) and African catfish (*Clarias gariepinus*) (Younes *et al.*, 2021). In 2015, it was reported that *B. cereus* was pathogenic and caused mass fish deaths in a catfish farm in India (Chandra *et al.*, 2015). In another study, it was determined that the combination of *B. subtilis* + *B. cereus toyoi* did not have a positive effect on trout species under the current experimental conditions, on the contrary, it had a harmful effect (Ramos *et al.*, 2017). In other research, typical hepatic hemorrhage symptoms were observed in the snakehead fish (*Ophiocephalus argus*) and the pathogen, *B. cereus*, was isolated (Wang *et al.*, 2023). In Türkiye, the first report about *B. cereus* as a disease agent, a case report was published on rainbow trout by Dinçtürk (2022).

Information on the pathogenicity, histopathological effects, and antimicrobial susceptibility of *B. cereus* in rainbow trout (*O. mykiss*) is still limited. The aim of this study was to investigate infection in naturally affected rainbow trout using molecular diagnostics, antibiogram profiling, and histopathological examination, with a focus on its implications for fish health and disease management in aquaculture. In addition, this study aimed to confirm *B. cereus* as the causative pathogen of the disease at the molecular level.

MATERIALS AND METHODS

Sampling of fish: This study investigated mortality observed in a rainbow trout farm in Isparta Province, Mediterranean region of Türkiye, where a daily 2% mortality rate was recorded. In February, ten diseased rainbow trout (mean body weight: 15 g) were collected alive and transported to the University laboratory in aerated, sealed plastic bags. The fish were examined macroscopically for external and internal clinical signs and pathological lesions. (Schäperclaus, 1992; Austin and Austin, 2016).

The isolation and identification of pathogen: Microbiological samples from the anterior kidney, liver, and spleen were inoculated onto Tryptic Soy Agar (TSA) for isolation and incubated at 25°C for 48 h. After incubation, bacterial isolates were purified and identified using conventional methods. Identification of *B. cereus* was based on colony morphology and standard biochemical tests, including Gram staining, catalase and oxidase activities, motility and oxidative/fermentative (O/F) reactions. For PCR analysis, the isolated strain as a pure culture was obtained. DNA isolation from the pathogen, a kit (GeneMATRIX Bacterial & Yeast Genomic DNA Purification Kit, Poland (<https://eurx.com.pl/docs/manuals/en/e3580.pdf>)) was used by following the manufacturer's instructions. The PCR study utilized the 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') primers as universal primers to amplify the 16S rRNA gene, yielding

an expected amplicon size of approximately 1,470 bp. For species determination, the *gyrB* gene was amplified using the forward primer 5'-ATGGAACAAAAGCAAATGCA-3' and reverse primer 5'-GTGAAGAACCTTGATATTTA-3', producing an expected amplicon of approximately 1100 bp. Fragments of the 16S rRNA and *gyrB* gene were also amplified with these primers. PCR amplifications were initiated with 5 min denaturation at 95 °C. Each thermal cycle consisted of 45 s denaturation at 95 °C, 45 s annealing at 57 °C (for 16S rRNA gene) and 60 °C (for *gyrB* gene) and 60 s elongation at 72 °C. Additional extension was carried out for 5 min at 72 °C. The PCR reaction was performed utilizing the FIREPol® DNA Polymerase Taq polymerase enzyme from Solis Biodyne (Estonia). Obtained results deposited in GenBank.

Antimicrobial susceptibility test: Antimicrobial susceptibility of the *B. cereus* isolates was determined by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Oxoid, Hampshire, UK) according to Bauer et al. (1966) and Hudzicki (2009). Overnight cultures were adjusted to 0.5 McFarland standard, inoculated onto agar plates, and antibiotic discs were applied aseptically. Plates were incubated at 25°C for 18–24h. Inhibition zones were measured and interpreted as susceptible, intermediate, or resistant according to CLSI (2021) guidelines using *Staphylococcus aureus* disk diffusion breakpoints. Twelve antimicrobial agents were tested (Table 2).

Histopathological analysis: Fish were euthanized by concussion, a method approved under Directive 2010/63/EU. Necropsy was performed, and tissue samples from the liver, heart, spleen, kidney, muscle, intestine, and affected areas were collected in healthy and moribund fish samples. Samples were taken and fixed in 10% buffered formaldehyde. Tissues were processed using an automated tissue processor (Leica ASP300S), embedded in paraffin (Leica Histocore Arcadia H), and sectioned at 5 µm using a rotary microtome (Leica 2155) and stained with Hematoxylin and Eosin (H&E). Histological slides were examined under an Olympus CX41 microscope and photographed using the Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan) (Culling, 1963; Presnell et al., 1997; Mokhtar, 2021).

RESULTS

Post-mortem and clinical findings: At the farm, the fish were in spring water at 7°C, pH 7.4 and dissolved oxygen of 9 ppm at the time of sampling. The farm has an annual production capacity of 180 tons of rainbow trout, with a stocking density of 25 kg/m³. According to information obtained from the farmer, increased mortality was observed after the fish grading process. The clinical signs on the fish (15 g) were determined as balance disorder, darkening of the color, bilateral exophthalmos in the eyes, and hemorrhagic regions in the pelvic and anal fin base. Autopsy findings showed hepatomegaly and splenomegaly (Fig. 1.A), pale liver and enlargement in the kidney (Fig. 1.B), and ascites in the abdominal cavity were observed in sampled fish. Examined fish displayed similar clinical signs. In the farm, the mortality rate was determined as daily 2%.

Phenotypic characteristics of isolates: Gram staining revealed that isolated strains were Gram-positive. The specific physiological and biochemical characteristics are listed in Table 1 and were consistent with that of *B. cereus*. The bacteria samples, motile, short rods that were grouped in pairs or chains were the morphological characteristics of *B. cereus* isolate that was recovered. Biochemically, the bacteria tested as fermentative, catalase positive and oxidase negative. In addition, beta hemolytic zone formation was detected in blood agar (Fig. 2. A.B.C).



Fig. 1: The naturally infected *O. mykiss* with *B. cereus*. A. hepatomegaly (L) and splenomegaly (S), B. Pale liver (L) and enlargement in kidney (K).

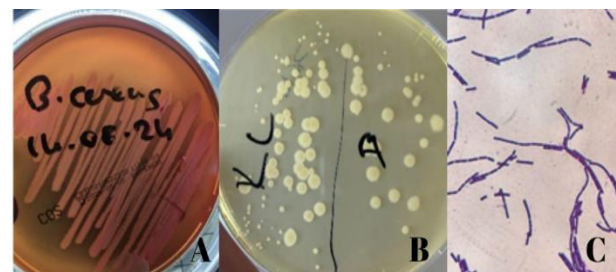


Fig. 2: A. Colony morphology of *B. cereus* on blood agar, B. Morphology of *B. cereus* on TSA, C. Gram stain image of *B. cereus* (1000X).

Molecular Detection by PCR: Following PCR amplification, nucleotide sequencing was conducted using the Illumina iSeq 100 platform (Illumina Inc., San Diego, CA, USA). The PCR amplicons of the 16S rRNA and *gyrB* genes were sequenced on this next-generation sequencing platform. The obtained sequences were subjected to homology analysis via the NCBI BLAST tool to determine species-level similarity. Results revealed that the bacterial isolates shared 98.5% sequence identity with *B. cereus*. To further elucidate phylogenetic relationships, the *gyrB* gene sequences were analyzed and visualized in a dendrogram. Phylogenetic analysis based on the *gyrB* gene sequence revealed that the isolate obtained in the present study clustered together with the reference *B. cereus* sequence (GenBank accession number: AB190226.1), forming a highly supported clade with a bootstrap value of 100% (Fig 3). The short branch distance observed between the isolate and the reference strain indicates a high degree of genetic similarity and confirmed the identification of the isolate as *B. cereus*. In contrast, other bacterial species included in the analysis, such as *Pseudomonas fluorescens*, *Aeromonas hydrophila* subsp. *hydrophila*, *Vibrio anguillarum*, *Flavobacterium psychrophilum*, and *Lactococcus garvieae*, formed

distinct phylogenetic groups separated by longer branch lengths, reflecting greater genetic divergence from the isolate. These findings demonstrate that the *gyrB* gene provides sufficient discriminatory power for reliable identification and phylogenetic placement of the isolated *B. cereus* strain (Fig. 3). The dendrogram construction was performed using the Neighbor-Joining (NJ) method, with evolutionary distances calculated according to the Jukes-Cantor model. The dendrogram was constructed using CLC Main Workbench 14.0 (Qiagen, Aarhus, Denmark), and the type strain *B. cereus* ATCC 14579 (16S rRNA GenBank accession number: NR_074540) was included as the reference strain. The robustness of the clustering was assessed using 100 bootstrap replicates. The resulting dendrogram demonstrated a close genetic relationship between the isolates and *B. cereus*, supporting the BLAST findings. Moreover, both 16S rRNA and *gyrB* gene sequences (GenBank accession number: PQ643766 for the 16S rRNA gene and PZ530880 for the *gyrB* gene) exhibited the highest degree of homology (98.5%) with *B. cereus*. Phylogenetic analysis based on 16S rRNA sequences of *Bacillus* species and other aquatic pathogens further confirmed this identification, as the isolates clustered within the same branch as *B. cereus* in the dendrogram. The polymerase chain reaction (PCR) products were visualized on a 1% agarose gel (Fig. 4). As a result of the 16S rRNA and *gyrB* gene analysis, the pure bacterium from sick fish was identified as *B. cereus*.

Antibiogram test to pathogen *B. cereus* isolates: The antibiogram results obtained using the disc diffusion method demonstrated that the recovered *B. cereus* isolates were highly susceptible to florfenicol (FFC, 30µg), with an inhibition zone diameter of 34mm, and erythromycin (E, 15µg), with an inhibition zone diameter of 31mm (Table 2). In contrast, the isolates exhibited resistance to cefuroxime (30µg), amoxicillin (30µg), cefotaxime (30µg), ampicillin (10µg), and tetracycline (30µg). Based on the

antimicrobial susceptibility profile, florfenicol was selected for therapeutic intervention and administered orally at a dosage of 10mg/kg body weight/day for 10 consecutive days. Mortality was no longer observed by the end of the treatment period.

Table 1: Physiological and biochemical characteristics of isolated bacteria from diseased fish, *B. cereus*.

Characteristics	Response
Growth temperature and time	25°C, 48h
Colony pigment	White
Gram Stain	Positive
Morphology	Thick and long rods
Motility	Motile
Oxidation-Fermentation(O/F)	Fermentative
Catalase	Positive
Citrate	Positive
Oxidase	Negative
Indole	Negative
Voges Proskauer	Positive
Arabinose	Negative
Fructose	Positive
Maltose	Positive
Glucose	Positive
Sucrose	Positive
Gelatin hydrolysis	Positive
Starch	Positive
Growth on Trypticase Soy Agar	Positive
Growth on 5% Sheep Blood Agar	Beta-hemolytic

Table 2: Antibiotic sensitivity patterns of the pathogen *B. cereus* strain.

Antibiotic	Zone size (mm)	Sensitivity
Florfenicol (30 µg)	34	S
Erythromycin (15 µg)	31	S
Ofloxacin (5 µg)	25	S
Clindamycin (2 µg)	24	S
Flumequine (30 µg)	21	S
Gentamicin (10 µg)	20	S
Doxycycline (30 µg)	16	S
Cefuroxime (30 µg)	0	R
Cefotaxime (30 µg)	0	R
Tetracycline (30 µg)	0	R
Amoxicillin (30 µg)	0	R
Ampicillin (10 µg)	0	R

"S" = Susceptible; "R" = Resistant

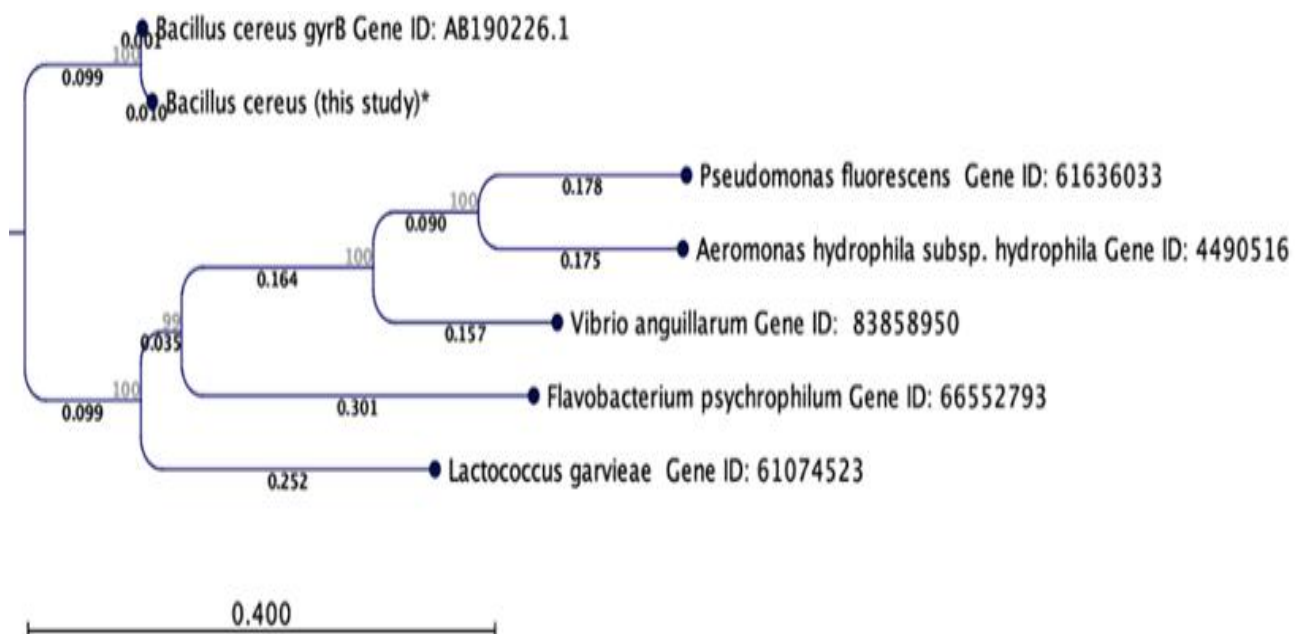


Fig. 3: Dendrogram showing the similarity rates of the isolated *B. cereus* strain with other species in the study.

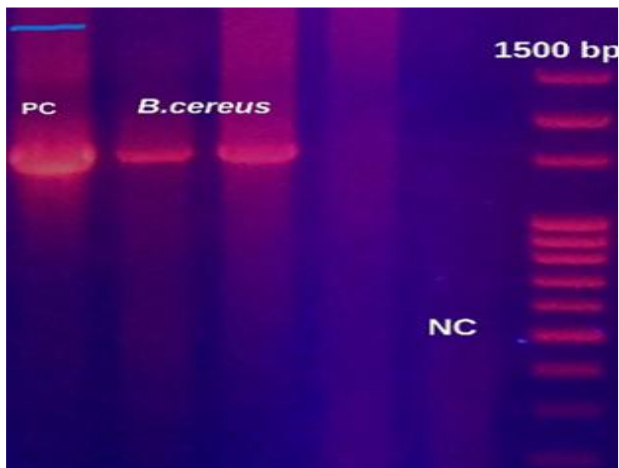


Fig. 4: *B. cereus* gene in the agarose gel (PC:positive control; NC:negative control).

Histopathological findings: The organs sampled (kidney, liver, heart, spleen, intestine, muscle) from healthy and diseased fish were prepared for histological analysis. Healthy fish showed normal tissue histology in the skin, muscle, and visceral organs (Fig. 5, 6). Fish that were naturally infected with *B. cereus* showed signs of liver and kidney enlargement and congestion, as well as an accumulation of serous fluids in the body cavity. Significant melanomacrophage infiltrations were observed in the kidneys of fish. All layers of the intestines exhibited inflammatory cell infiltrations of different intensities, with the serosa displaying the most prominent. There were significantly common melanomacrophage centers present in the spleen slides. The liver contained a significant inflammatory cell (Fig. 7). Necrosis and the infiltration of inflammatory cells were frequently observed in the visceral organs, muscles, and skin of fish (Fig. 8).

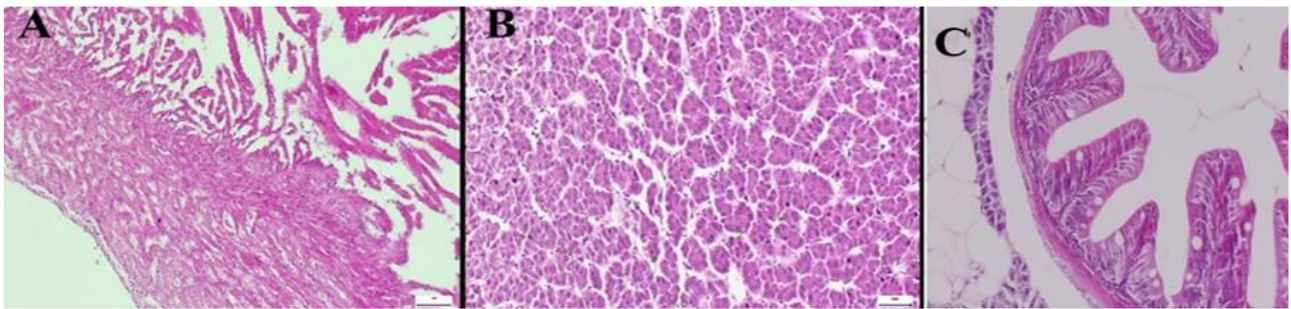


Fig. 5: Healthy rainbow trout tissues, heart (A), liver (B) and intestine (C) appearance. H&E, Scale bars=50µm.

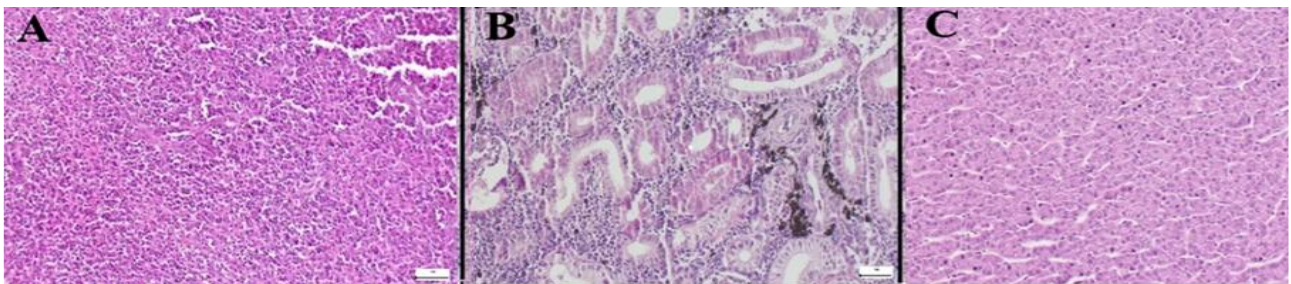


Fig. 6: Healthy rainbow trout tissues, spleen (A), kidney (B) and liver (C) appearance. H&E, Scale bars=50µm.

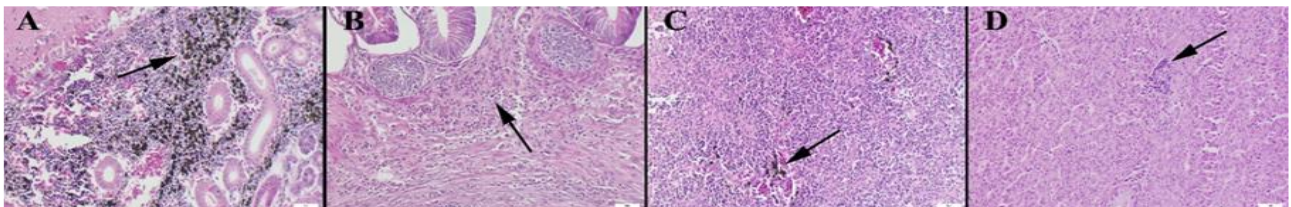


Fig. 7: Histopathological appearance in the organs of diseased rainbow trout. Intense melanomacrophage infiltration (arrow) in the kidneys (A), inflammatory cell infiltration (arrow) in the submucosa of the intestines (B), severe hyperemia (arrow) and melanomacrophage infiltrations in the spleen (C), and inflammatory cell infiltrations (arrow) in the liver (D), H&E, Scale bars=50µm.

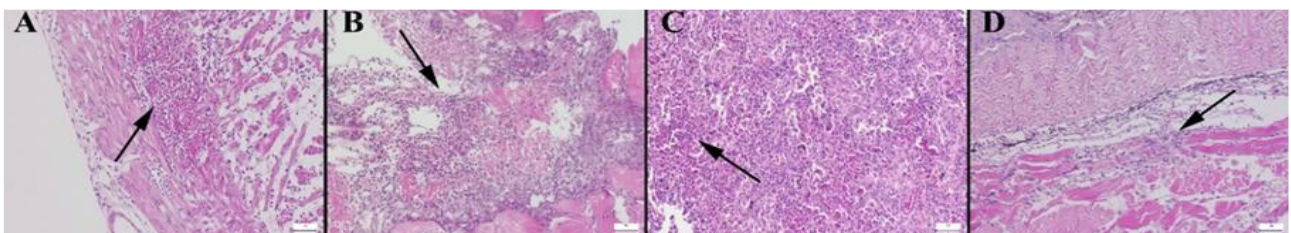


Fig. 8: Representative microphotographs in diseased fish. Marked inflammatory cell infiltrations (arrows) in the heart (A), Necrosis and severe inflammatory cell infiltrations (arrow) in muscle (B), Slight inflammatory cells (arrow) in the spleen (C), Inflammatory cell infiltrations (arrow) in subdermal tissue (D), H&E, Scale bars=50µm.

DISCUSSION

Numerous bacterial species, known as Bacilli, are present in nature. They may thrive in a variety of settings, including soil, plants, and the gastrointestinal tract of animals, and they are extremely tolerant of environmental changes. *Bacillus* strains are increasingly applied in aquaculture due to their positive effects on host health, water quality and disease resistance. These bacteria enhance digestive efficiency by producing extracellular enzymes and contribute to improved growth and feed conversion rates. They also modulate the host immune system by increasing antioxidant activity and promoting immune gene expression, which boosts resistance to bacterial infections (Eissa *et al.*, 2024). In addition, *Bacillus* probiotics improve water quality by reducing nitrogenous waste, including ammonia and nitrites, through microbial bioremediation processes (Naiel *et al.*, 2022). Their high stability in feed and aquatic environments, allowing for consistent application across the systems. These properties make *Bacillus* an effective tool for promoting sustainability and reducing antibiotic dependence in modern aquaculture practices (Fachri *et al.*, 2024). But in some unfavorable circumstances, the bacteria turn harmful, and severe systemic infections begin to happen.

B. cereus, a common bacteria of public health concern, has just been identified as a fish-threatening infection, causing the aquaculture industry to suffer economical losses (Chandra *et al.*, 2015). *B. cereus* was associated with disease outbreaks in rainbow trout, suggesting a likely involvement in the observed changes. It has been reported in fish species similar to this study, such as common carp (*Cyprinus carpio*) (Pychynski *et al.*, 1981), African catfish (*Clarias gariepinus*) (Younes *et al.*, 2021), European seabass (*Dicentrarchus labrax*) (Yiagnisis and Athanassopoulou, 2011; Aboyadak *et al.*, 2016) white seabream (*Diplodus sargus*) (Ali *et al.*, 2019), stinging catfish (*Heteropneustes fossilis*) (Chandra *et al.*, 2015) and mullet (*Mugil seheli*) (Algammal *et al.*, 2022) may become unwell due to *Bacillus* species, especially *B. cereus*.

In the current study, clinical signs of the diseased *O. mykiss* had balance disorder, darkening of color, bilateral exophthalmos in the eyes, lesions, and hemorrhagic regions in the pelvic and anal fins base. Autopsy findings revealed pale liver and fluid accumulation in the abdomen. Seabass (Aboyadak *et al.*, 2016), white seabream (Ali *et al.*, 2019), stinging catfish (Chandra *et al.*, 2015) and mullet (Algammal *et al.*, 2022) infected with *B. cereus* have shown similar outcomes. An earlier study found that experimentally infected African catfish with *B. cereus* had hemorrhagic patches on their skin and gills, along with popeyes, abdominal distension, and apparent skin lesions (Younes *et al.*, 2021). The signs of a *B. cereus* infection in white seabream were almost the same both clinically and after fish mortality (Ali *et al.*, 2019). Our results are quite similar to a prior work by Younes *et al.* (2021) that fish showed hemorrhagic areas on the skin and visible fluid in the abdominal cavity naturally infected with *B. cereus*. We found slightly variable results, which could be related to fish species, water temperature, and other environmental factors. Similar to Bottone (2010), Ali *et al.* (2019) and Aboyadak *et al.* (2016), Pakhira *et al.* (2025) descriptions, *B. cereus* colonies were big, white, granular, irregularly rimmed, and measured as 1.5–2.5 mm in diameter in a petri

dish. They looked to be Gram-positive and were grouped in short or long chains. According to the bacteriological study, every isolate that was recovered from sick fish displayed the characteristic phenotypic characters of *B. cereus*, which are in line with Osman *et al.* (2018) and Algammal *et al.* (2022). The molecular profiles and morphologies of the isolates were identical. Additionally, the isolated bacterium induced mortality and reproduced clinical signs and pathological lesions similar to those observed during the farm outbreak. The occurrence of comparable symptoms and lesions across different fish species suggests common pathogenic mechanisms of *B. cereus*, including toxin production and tissue damage. Variations in disease severity may be related to host susceptibility, immune status, environmental conditions, and bacterial virulence. These findings emphasize the pathogenic potential of *B. cereus* in aquaculture and the importance of effective monitoring and management strategies.

The bacterium obtained from the sick fish was confirmed by 16S rDNA sequencing and biochemical analysis. In Türkiye, the first report about *B. cereus* as a disease agent was deposited in GenBank by Dinçtürk (2022). Also other studies obtained the molecular identification (Chandra, 2015; Algammal *et al.*, 2022) that was the same with our strain results. Consistent molecular findings support the reliability of 16S rDNA-based identification and indicate that *B. cereus* is widely distributed among fish hosts. However, differences in clinical outcomes suggest that host factors and environmental conditions may influence disease expression. The spread of antibiotic-resistant bacteria in aquaculture poses a growing threat to aquaculture and public health. Extensive antibiotic use promotes the emergence of resistant bacteria and resistance genes; therefore, prudent antibiotic use, strengthened biosecurity, and routine susceptibility testing are essential for infection control and resistance management (Mohammed *et al.*, 2025a).

According to a prior investigation, *B. cereus* isolates resisted ampicillin, kanamycin and oxytetracycline but were sensitive to erythromycin, ciprofloxacin, and norfloxacin. Except for OTC, this result agreed with that of Chandra *et al.*, (2015). Additionally, Weber *et al.* (1988) found that *B. cereus* was susceptible to ciprofloxacin, tetracycline, and chloramphenicol. The presence of certain antibiotic resistance genes in *B. cereus* isolates may be the cause of variation in antibiotic sensitivity profiles (Ali *et al.*, 2019).

In the current study, regarding the antibiogram pattern, florfenicol (30µg), and erythromycin (15µg) exhibited promising results in antibacterial activity against the recovered *B. cereus* isolates. For the treatment of sick fish, florfenicol was used at 10 mg/kg body weight for 10 days. Mortality stopped at the end of the treatment period. There were some variations in the drug sensitivity findings of *B. cereus* isolated from various locations which were documented. The present findings affirm the emergence of multidrug resistance to isolates of *B. cereus* in aquaculture sectors. Differences in antimicrobial susceptibility among studies may be attributed to variations in antibiotic use, local selective pressures, and the acquisition of resistance determinants. Nevertheless, the frequent occurrence of

multidrug-resistant *B. cereus* isolates highlights their adaptive capacity and potential to persist in aquaculture environments, posing challenges for disease control and treatment.

Histopathological examination revealed degenerative and inflammatory lesions in multiple tissues, including the kidney, liver, heart, spleen, intestine, and muscle. The intestine showed marked leukocyte infiltration, while necrosis and inflammatory cell accumulation were evident in hepatic, splenic, muscular, and cutaneous tissues. Melanomacrophage centers observed in the spleen indicated immune activation. Similar findings, including inflammation, congestion, and necrosis in the liver and kidney, have also been reported by Nuryati et al. (2023). According to Ali et al. (2019), the posterior kidney displayed inflammatory cell aggregation together with deteriorated glomeruli and renal tubules. Austin and Austin (2016) corroborated these results by observing petechial hemorrhages and localized necrosis in the liver and kidney of fish infected with *B. cereus*. In this study, in the kidneys of sick fish, we saw significant melanomacrophage infiltrations. In the spleen tissue, there were also noticeable melanomacrophage centers. Additionally, there were a lot of inflammatory cells in the liver. In the skin, muscle, and visceral organs of sick fish, necrosis and inflammatory cell infiltrations were prevalent. These findings are consistent with previous studies reporting hepatic and renal inflammation, congestion, and necrosis in bacterially infected fish, indicating a conserved pathological response to systemic bacterial infections in aquaculture species. The recurrence of similar lesions across studies further supports the pathogenic role of *B. cereus*, while variations in lesion severity may reflect differences in host immunity, bacterial virulence, and environmental conditions.

This study identified *B. cereus* in diseased rainbow trout and demonstrated its association with severe systemic pathology and mortality. Histopathological examination revealed necrosis and inflammatory changes in multiple organs. The isolate showed high susceptibility to florfenicol, erythromycin, and ofloxacin, while resistance to several commonly used antibiotics was detected. These findings emphasize the importance of accurate diagnosis, antimicrobial susceptibility testing, and effective health management practices for disease control in aquaculture.

Conclusions: Effective control of *B. cereus* infections in rainbow trout farms relies on biosecurity measures, water quality management, regular health monitoring, and prudent antimicrobial use based on susceptibility testing. This study represents the first report of *B. cereus* in naturally infected rainbow trout in Isparta, Türkiye, highlighting its potential threat to aquaculture. The findings demonstrate the value of integrated phenotypic, molecular, and histopathological approaches for pathogen identification and emphasize the need for further research on the virulence and pathogenesis of *B. cereus* in fish.

Competing of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflict of interest statement: The authors declare no conflict of interest.

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