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Research Article

Infection and treatment of *Vibrio aestuarianus* rainbow trout (*Oncorhynchus mykiss*) fry in Kahramanmaraş

Mikail Özcan[a]*, Yiğit Ökkeş Küçük[a], Feridun Özdemir[a], Berivan Özdemir[a], Yusuf Yılmaz Yıldız[a], Tuğçe Kıdık[a]

[a]*Kahramanmaraş Sutcu Imam University, Agriculture Faculty, Department of Animal Science, 46040-Kahramanmaraş, Türkiye*

Abstract: An infection with a high mortality rate was observed in fry weighing between 5-25 g in 10 different rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) farms in the Kahramanmaraş province. Liver, spleen, kidney, intestinal, and skin tissue samples were obtained from fish exhibiting symptoms of the disease and subsequently cultured on both general and special media. These samples were then incubated in an incubator at temperatures ranging from 15 to 24°C for a period of 24 to 72 hours. Subsequent biochemical identification tests were performed on pure strains obtained from these samples taken from trout farms, as well as 94 biochemical tests for the identification of Gram-negative and Gram-positive bacteria at the species level on The Biolog GEN III MicroPlate plates. The identification process yielded the detection of 10 strains of *Vibrio aestuarianus*, and subsequent antibiogram analysis was conducted.

Keywords: Rainbow trout, *Oncorhynchus mykiss*, *Vibrio aestuarianus*, The Biolog GEN III, Antibiogram

Mikail Özcan ORCID ID: 0000-0001-9032-0697
Yiğit Ökkeş Küçük ORCID ID: 0009-0005-8743-6417
Feridun Özdemir ORCID ID: 0009-0004-8495-8247
Berivan Özdemir ORCID ID: 0009-0003-0476-3750
Yusuf Yılmaz Yıldız ORCID ID: 0009-0004-8816-5164
Tuğçe Kıdık ORCID ID: 0009-0004-1033-1695

*Corresponding author: mikailozcan@ksu.edu.tr
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1. INTRODUCTION

In the context of aquaculture, bacterial diseases represent a significant challenge, with considerable ramifications for fish health and production efficiency. Such diseases are typically precipitated by a number of factors, including intensive production conditions, suboptimal water quality, stress, nutritional deficiens, and a lack of hygiene (Arda et al., 2005; Doğan & Önalan, 2023).

The first isolations and description of *Vibrio aestuarianus* were reported from seawater, oysters, clams and crabs from the Oregon and Washington coasts of the USA (Tison & Seidler 1983). The subsequent isolation of *Vibrio aestuarianus* was first documented in 2008 in an aquaculture facility in China, where *Cynoglossus semilaevis* flounder was cultured (Zhang et al., 2011).

Vibrio aestuarianus is a Gram-negative bacterium belonging to the Vibrionaceae family. It is particularly prevalent in marine environments. It has been observed to cause diseases in the Pacific oyster (*Crassostrea gigas*) and fish, acting as an opportunistic pathogen (Samain et al., 2004; Garnier et al., 2008; Saulnier et al., 2010; Austin & Austin, 2016; Karaton Kuzgun, 2023; Karaton Kuzgun, 2024). The bacterium is characterised by its fermentative, slightly curved rod oxidase-positive nature, and is motile, measuring 1.2-2.0 x 0.5-1.0 µm. Furthermore, the bacterium has been found to be catalase, oxidase, ßgalactosidase, arginine dihydrolase and indole positive, acetamidase, H_2S production and phenylalanine deaminase negative. The majority of strains utilise N-acetyl-D-glucosamine, and most produce indole. Furthermore, strains ferment sucrose, D-mannose, D-fructose, D-glucose, D-galactose, and D-trehalose (Garcia et al., 2021).

The observation of disease symptoms such as haemorrhages on the head, opercula and the base of the fins, dorsal fin rot, a swollen abdomen filled with acid fluid and intestinal herniation was made in cultured sole (Zhang et al., 2011).

Microbiological tests are generally utilised for the determination of the phenotypic characteristics of bacteria (Austin & Austin, 1987; Bernardet & Kerouault, 1989; Bernardet et al., 1996; Arda, 2000; Karaton Kuzgun & Gürel İnanlı, 2018; Karaton Kuzgun, 2019; Erecevit Sönmez et al., 2020).

The Biolog GEN III MicroPlate™ is a 96-well test panel that enables the identification of Gram-negative and Gram-positive bacteria by phenotypic analysis of microorganisms. The microplate contains a total of 94 unique biochemical tests, including 71 carbon source utilization tests and 23 chemical sensitivity tests (Singh et al., 2001).

The present study was conducted with the objective of ascertaining the causative agent of mass mortality in juvenile fish in several trout farms in Kahramanmaras and recommending the requisite treatment.

2. MATERIAL AND METHOD

In response to reports of mass mortalities in fry weighing between 5-25 g in 10 different rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) facilities in the Kahramanmaraş province between December 2014 and November 2015, on-site investigations were conducted. The owners were asked for information about the diseases the fish had been exposed to. Rainbow Trout fry (5-25 g) manifesting disease symptoms were transferred to the Fish Diseases Laboratory of Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Aquaculture. Upon arrival, the fish were subjected to a clinical examination and anaesthetised with 2-phenoxyethanol. The body surface of the fish was disinfected with 70% ethyl alcohol. In the laboratory environment, the fish were examined in a sterile cabinet in front of a burner flame using sterile scissors, forceps and scalpel according to the autopsy technique (Çolak, 1982; Frerichs & Millar, 1993; Timur & Timur, 2003; Arda et al., 2005). The preparations obtained from the internal organs and skin were examined under a microscope. Liver, spleen, kidney, intestine and skin samples from the fish exhibiting symptoms of the disease were collected and inoculated onto 6% blood agar and TCBS (Thiosulfate Citrate Bile Sucrose) Agar with 2% salt added. These media were then incubated at 24°C for a period of 48 hours.

Following this, pure colonies were obtained from the colonies in the growth media. A suspension was then prepared from these pure colonies using Biolog IF-A buffer solution. The bacterial concentration was then adjusted to between 92-98% by means of a turbidimeter. The concentration-adjusted bacterial samples were added to each well in the microplates (100 µl per well). These microplates were then subjected to an incubation period at 26°C for a duration of 24 hours. Following this, the microplate was subjected to analysis using a reader, and then compared with the system's database to identify the bacteria.

The isolates obtained were subjected to disk diffusion tests on Mueller-Hinton agar (Oxoid) with commercial impregnated disks (Oxoid) (NCCLS, 2001). The measurement of antibiotic zone diameters were evaluated according to the CLSI (Clinical and Laboratory Standards Institute), (CLSI, 2003), (Table 1)

Table 1. Antibacterial disks used in the study, their amounts and standard zone diameters

Antibiotic (µg /U)	Resistant ≤ mm (R)	Intermediate mm (I)	Susceptible ≥ mm (S)
Ampicillin (AMP, 10 µg)	13	14–16	17
Chloramphenicol (C, 30 µg)	12	13–17	18
Enrofloxacin (ENR, 5 µg)	16	16–20	21
Erythromycin (E, 15 µg)	11	14–22	23
Florfenicol (FFC, 30 µg)	14	15–18	19
Gentamicin (CN, 10 µg)	12	13–14	15
Oxytetracycline (OT, 30 µg)	15	15–18	26
Penicilin (P, 10 Ünite)	14	-	15

3. RESULTS

Anamnesis obtained from ten different enterprises operating in Kahramanmaraş province revealed that the fish exhibited a reluctance to feed, a decrease in movement, and a tendency to rub against the sides of the pools. A clinical examination of the fish in the establishments was conducted. The fish exhibited a general darkening of the skin colouration. A meticulous visual and magnifying glass examination of the body surface revealed the presence of water accumulation in the abdomen (ascites), haemorrhages at the base of the opercula and fins, and haemorrhages at the base of the fins.

Subsequent to this preliminary examination, sick live fish were subjected to internal examination by autopsy under aseptic conditions. The examination of internal organs was conducted meticulously, employing both visual inspection and magnified observation. The presence of haemorrhage in all internal organs, pallor in the liver, haemorrhage in the air sac and blackening in the kidney was detected. The 10 isolates were identified as *Vibrio aestuarianus* according to their morphological and biochemical characteristics by performing classical culture tests (Table 2).

Table 2. Morphological and biochemical characteristics of 10 *Vibrio aestuarianus* isolated from rainbow trout

Biochemical Criterion	***Vibrio aestuarianus* (n:10)**
Gram Staining	-
Colony color	Yellow
Shape	Comma
Oxidase	+
Catalase	+
Motility	+
H_2S production	-
Metil Red	+
Voges Proskauer	-
Indole	+
Urease	+
O-F test	F
MacConkey Agar	+
Mueller-Hinton Agar	+
Growth at 0°C	-
Growth at 5°C	-
Growth at 15°C	+
Growth at 20°C	+
Growth at 25°C	+
Growth at 30°C	+
Growth at 37°C	+
Growth at % 0.0 NaCl	-
Growth at % 0.5 NaCl	-
Growth at % 1.0 NaCl	-
Growth at % 2.0 NaCl	+
Growth at % 6.5 NaCl	+

+: Positive reaction, -: Negative reaction, +/-: Variable, F: Fermentative

The Biolog System (The biolog GENIII micro plate) device was used to perform 94 biochemical tests for the identification of bacteria at the species level (Table 3).

Metabolic reaction profiles of the bacteria were obtained using the Biolog's Microbial Identification System software program. The metabolic profiles of known isolates in the system's library were then compared with those of the isolates obtained from diseased. This analysis revealed that three bacterial species (*Vibrio metschnikovii*, *Listonella anguillarum*, and *Vibrio cholerae* O1 (ATCC 25870)) exhibited similarity in different ratios, while one bacterial species (*Vibrio aestuarianus*) demonstrated similarity in percentage (Figure 1).

Table 3. The following section details the other phenotypic characteristics of *Vibrio aestuarianus* isolated from rainbow trout with the Biolog System (The Biolog GENIII micro plate) device

Biochemical criteria	Isolate reaction (n: 10)	Biochemical criteria	Isolate reaction (n: 10)
Negative Control	-	Gelatin	+
Dextrin	+	Glycyl-L-Proline	+
D-Maltose	+	L-Alanine	+/-
DTrehalose	Weak -	L-Arginine	+/-
DCellobiose	-	L-Aspartic Acid	+
Gentiobiose	-	L-Glutamic Acid	+
Sucrose	+	L-Histidine	+
D-Turanose	+/-	L-Pyroglutamic Acid	+/-
Stachyose	-	L-Serine	+
Positive Control	+	Lincomycin	-
pH 6	+	Guanidine HCl	+/-
pH 5	+/-	Niaproof 4	+
D-Raffinose	-	Pectin	+/-
α-D-Lactose	Weak -	D-Galacturonic Acid	+/-
DMelibiose	-	L-Galactonic Acid Lactone	+/-
β-Methyl-DGlucoside	+/-	D-Gluconic Acid	+/-
D-Salicin	+/-	D-Glucuronic Acid	-
N-Acetyl-D-Glucosamine	+	Glucuronamide	-
N-Acetyl-β-D-Mannosamine	+/-	Mucic Acid	-
N-Acetyl-D-Galactosamine	Weak -	Quinic Acid	+/-
N-Acetyl Neuraminic acid.	-	D-Saccharic Acid	-
1% NaCl	-	Vancomycin	+
4% NaCl	+	Tetrazolium Violet	-
8% NaCl	+/-	Tetrazolium Blue	+
α-D-Glucose	+	p-Hydroxy-Phenylacetic Acid	-
D-Mannose	+/-	Methyl Pyruvate	+/-
D-Fructose	+/-	D-Lactic Acid Methyl Ester	+
DGalactose	+	L-Lactic Acid	+
3-Methyl Glucose	-	Citric Acid	+
D-Fucose	-	α-Keto GlutaricAcid	+/-
L-Fucose	-	D-Malic Acid	-
L-Rhamnose	-	L-Malic Acid	+
Inosine	+	Bromo-Succinic Acid	+/-
1% Sodium Lactate	+	Nalidixic Acid	-
Fusidic Acid	+	Lithium Chloride	+/-
D-Serine	+/-	Potassium Tellurite	-
D-Sorbitol	+/-	Tween 40	+/-
D-Mannitol	+/-	γ-Amino-Butryric Acid	+/-
D-Arabitol	-	α-Hydroxy-Butyric Acid	+/-
Myo Inositol	-	β-Hydroxy-D,LButyricAcid	+/-
Glycerol	+/-	α-Keto-Butyric Acid	+/-
D-Glucose-6-PO_4	+	Acetoacetic Acid	
D-Fructose-6-PO_4	+	Propionic Acid	+/-
D-Aspartic Acid	+/-	Acetic Acid	+
D-Serine	-	Formic Acid	+/-
Troleandomycin	+/-	Aztreonam	+/-
Rifamycin SV	+	Sodium Butyrate	-
Minocycline	-	Sodium Bromate	-

Program	MicroLog 3/5.2.01 33
Project	ML5
File Name	balık23.1.2014.D5E
User	biolog
Instrument	MicroStation 2 Reader
Instrument S/N	0
Incubation Hours	21.00
Plate Number	1
Plate Type	GEN III
Protocol	A
İzolat No	M.Aç Böbrek
İzole Edildiği Yer	Balık Kahramanmaraş
İzole Eden	Mikail
Tanılayan	Mustafa KÜSEK
Tarih	23/01/2014
Date & Time of Read	Oca 23 2014 10:51 AM
Biolog ID DB	Biolog GEN III 2_6_1_08.I5G

Result	Species ID: Vibrio aestuarianus
Comment	
Notice	

Rank	PROB	SIM	DIST	Organism Type	Species
1	0.555	0.555	6.569	GN-Nent	Vibrio aestuarianus
2	0.100	0.100	7.866	GN-Nent	Listonella anguillarum
3	0.064	0.064	8.418	GN-Nent	Aeromonas hydrophila DNA group 1
4	0.059	0.059	8.522	GN-Nent	Vibrio parahaemolyticus

Key: <x: positive, x: negative, <x-: mismatched positive, x+: mismatched negative, {x: borderline, -x: less than A1 well

Well Color Values

Plate	1	2	3	4	5	6	7	8	9	10	11	12
A	108	< 270	< 250	{ 155 +	{ 180	137	< 259	{ 171	{ 140	< 286	< 291	75
B	{ 156	{ 164 +	{ 182	< 245	{ 175	< 255	< 216	91 +	{ 174	{ 181	< 267	{ 199
C	< 244	< 238	< 243	< 255	{ 210	{ 191 -	{ 187	{ 185	< 246	< 318	< 265	{ 196
D	< 230	< 240	{ 196	< 224	< 252	< 263	< 232	{ 162	105	< 258	< 276	54
E	< 252	< 247	< 254	< 232	< 264	< 257	< 251	{ 172	< 253	75	< 268	< 278
F	< 243	{ 173	< 214	< 244	{ 155	131	123	{ 168	69	< 268	{ 122	< 402
G	43	< 229	< 246	< 250	57	{ 182	95	< 250	< 232	49	< 286	69
H	< 231	136	< 234 -	{ 150	136	{ 152	76	< 261 -	{ 182 -	{ 236	{ 140	82

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Figure 1. Output of the biolog diagnostic system of *Vibrio aestuarianus* isolated from rainbow trout (*Oncorhynchus mykiss*)

The antibiogram test revealed that the isolated bacteria were susceptible to ampicillin (AMP, 10 µg) and chloramphenicol (C, 30 µg). Conversely, the bacteria exhibited resistance to erythromycin (E, 15 µg), gentamicin (CN, 10 µg), and oxytetracycline (OT, 30 µg) (Table 3).

Table 3. The following ten samples of *Vibrio aestuarianus* were isolated from rainbow trout and the results of the antibiotic tests are shown below.

Antibiotic (µg /U)	Sensitivity
Ampicillin (AMP, 10 µg)	S (20)
Chloramphenicol (C, 30 µg)	S (50)
Enrofloxacin (ENR, 5 µg)	S (30)
Erythromycin (E, 15 µg)	R (2)
Florfenicol (FFC, 30 µg)	S (25)
Gentamicin (CN, 10 µg)	R (0)
Oxytetracycline (OT, 30 µg)	R (0)

S: Sensitive, R: Resistant

4. DISCUSSION AND CONCLUSION

In this study, the isolation and identification of pathogens were conducted on tissue samples from 60 juvenile rainbow trout (*Oncorhynchus mykiss*) measuring between 5-25 g. These samples were collected from 10 different trout farms located in the Kahramanmaraş province between December 2014 and November 2015. Pathogen isolation was performed using the well-established classical culture technique, and identification was carried out using the Biolog System (Biolog GEN III MicroPlate). According to the results obtained from the BIOLOG GEN III automated identification system, *Vibrio aestuarianus* was identified as the causative agent.

Vibrio aestuarianus has been recognized as a pathogenic bacterium in the cultivated *Crassostrea gigas* species and has been shown to cause massive mortality in summer (Garnier et al., 2007; Garnier et al. 2008). On a farm in

China, a high mortality rate in cultured sole was consistently observed. The identification of *Vibrio aestuarianus* was confirmed by the authors through a combination of phenotypic, morphological, physiological and biochemical characteristics of the isolates (Zhang et al., 2011). In this study, *Vibrio aestuarianus* was isolated and identified from diseased rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) fry. To the best of our knowledge, *Vibrio aestuarianus* has not been previously reported as a pathogen in rainbow trout.

The following clinical findings were observed in sick fish: darkening of the skin colour, abdominal water retention (ascites), haemorrhage at the base of the opercula and fins, pallor of the liver, haemorrhage in the air sac and blackening of the kidneys. These clinical findings were similar to those reported in studies conducted in different countries (Zhang et al., 2011).

Scarano et al. (2014), *Vibrio aestuarianus* bacteria were treated with oxolinic acid (OXA), ampicillin (AM), amoxicillin (AMX), cephalothin (CF), erythromycin (E), florfenicol (FF), flumequine (FM), and streptomycin (S). Sulfadiazine (SZ). The bacteria exhibited sensitivity to tetracycline (TE) and trimethoprim (TMP) antibiotics and resistance to cloramphenicol (CL), gentamicin (GM), kanamycin (K), and oxytetracycline (OT) antibiotics. *Vibrio aestuarianus* isolated in this study demonstrated comparable susceptibility to ampicillin (AMP), florfenicol (FFC), gentamicin (CN) and exhibited divergent susceptibility to erythromycin (E) (15 µg) and chloramphenicol (C) (30 µg).

Authors' Contributions

MÖ: Manuscript design, writing, statistical analyses
YÖK: Laboratory experiments, reading, draft checking
FÖ: Laboratory experiments, reading, draft checking
BÖ: Laboratory experiments, reading, draft checking
YYY: Laboratory experiments, reading, draft checking
TK: Laboratory experiments, reading, draft checking
All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest for this study.

Statement on the Welfare of Animals

Ethical approval: All animal studies were approved by the Animal Ethics Committee of Kahramanmaraş Sütçü Imam University, Faculty of Agriculture (KSÜZİRHADYEK) and Research Institute (Protocol number: 2014/03-04).

Data Availability Statements

The authors confirm that the data supporting the findings of this study are available within the article.

REFERENCES

Arda, M. (2000). *Basic Microbiology*. 2nd ed. Medisan Publishing House Series, Ankara.

Arda, M., Seçer, S., & Sarıeyyüpoğlu, S. (2005). *Fish Diseases*. Medisan Publication, Ankara.

Austin, B., & Austin, D. A. (2016). *Bacterial Fish Pathogens: Disease of Farmed and Wild Fish*. Springer, UK.

Austin, B., & Austin, D.A. (1987). *Bacterial Fish Pathogens: Disease in Farmed and Wild Fish*. Ellis Horwood Ltd. Chichester.

Bernardet, J. F., & Kerouault, B. (1989). Phenotypic and genomic studies of ''*Cytophaga psychrophila*'', isolated from diseased rTrout (*Oncorhynhus mykiss*) in France. *Applied and Environmental Microbiology*, *55*(7), 1796–1800. https://doi.org10.1128/aem.55.7.1796-1800.1989

Bernardet, J. F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K., & Vandamme, P. (1996). Cutting a Gordian Knot: Emended classification and description of the Genus Flavobacterium, emended description of the Family Flavobacteriaceae, and proposal of *Flavobacterium hydatis* nom. nov. (Basonym, *Cytophaga aquatilis* Strohl and Tait 1978). *International Journal of Systematic and Evolutionary Microbiology*, *46*(1), 128-148. https://doi.org/10.1099/00207713-46-1-128

CLSI. (2003). Performance standards for antimicrobial disk susceptibility test; 8th ed., Approved standard M2- A8., CLSI, Wayne, PA, USA.

Çolak, A. (1982). *Handbook of Fish Diseases*. Esnaf Matbaası, İstanbul.

Doğan, S., & Önalan Ş. (2023). Determination of antimicrobial resistance gene variations using Tet and Str genes in fish species. *Cellular and Molecular Biology*, *69*(1), 150-155. http://dx.doi.org/10.14715/cmb/2022.69.1.26

Erecevit Sönmez, P., Karaton Kuzgun, N., & Kırbağ, S. (2020). Quality changes and storage life of common carp (*Cyprinus carpio*) with the use of Ginger (*Zingiber officinale*) essential oil. *Progress in Nutrition*, *22*(4). https://doi.org/10.23751/pn.v22i4.10516

Frerichs, G. N., & Millar, S. D. (1993). Manual for The Isolation and Identification of Fish bacterial Pathogens. Institute of Aquaculture, University of Stirling, Pisces Pres, Stirling. 60p.

Garcia, C., Mesnil, A., Tourbiez, D., Moussa, M., Dubreuil, C., Gonçalves de Sa, A., Chollet, B., Godfrin, Y., Dégremont, L., Serpin D., & Travers, M. A. (2021). *Vibrio aestuarianus* subsp. cardii subsp. nov., pathogenic to the edible cockles *Cerastoderma edule* in France, and establishment of *Vibrio aestuarianus* subsp. *aestuarianus* subsp. nov. and *Vibrio aestuarianus* subsp. francensis subsp. nov. *International Journal of Systematic and Evolutionary Microbiology*, *71*(2), 004654. https://doi.org/ff10.1099/ijsem.0.004654

Garnier, M., Labreuche, Y., & Nicolas, J. L. (2008). Molecular and phenotypic characterization of *Vibrio aestuarianus* subsp. francensis subsp. nov., a pathogen of the oyster *Crassostrea gigas*. *Systematic*

and Applied Microbiology, *31*, 358-365. https://doi.org/10.1016/j.syapm.2008.06.003

Garnier, M., Labreuche, Y., Garcia, C., Robert, M., & Nicolas, J. L. (2007). Evidence for the involvement of pathogenic bacteria in summer mortalities of the Pacific oyster *Crassostrea gigas*. *Microbial Ecology*, *53*, 187-196. https://doi.org/10.1007/s00248-006-9061-9

Karaton Kuzgun, N., & Gürel İnanlı, A. (2018). The investigation of the shelf life at 2 ± 1°C of *Luciobarbus esocinus* fillets packaged with films prepared with the addition of different essential oils and chitosan. *Journal of Food Science and Technology*, *55*, 2692–2701. https://doi.org/10.1007/s13197-018-3191-1

Karaton Kuzgun, N. (2019). Fatty acid composition of fish crackers produced from *Luciobarbus esocinus*. *Advances in Food Sciences*, *41*, 1, 16-20.

Karaton Kuzgun, N. (2023). Defne yaprağı esansiyel yağının +4°C'de muhafaza edilen gökkuşağı alabalığı (*Oncorhynchus mykiss* Walbaum,1792) filetolarının raf ömrü üzerine koruyucu etkisi [Protective effect of bay leaf essential oil on shelf life of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) stored at +4°C]. *International Journal of Pure and Applied Sciences*, *9*(1), 107-114. https://doi.org/10.29132/ijpas.1191629

Karaton Kuzgun, N. (2024). Defne ve limon esansiyel yağlarıyla hazırlanan balık (*Luciobarbus esocinus* (Heckel, 1843)) köftelerinin +4°C'de kimyasal ve duyusal özellikleri [The chemical and sensory properties at +4°C of fish (*Luciobarbus esocinus* Heckel, 1843) balls prepared with bay and lemon essential oils]. *Dünya Sağlık ve Tabiat Bilimleri Dergisi*, *7*(2), 180-189. https://doi.org/10.56728/dustad.1586597

NCCLS. (National Commi_ee for Clinical Laboratory Standards of Antimicrobial Susceptibility Testing) (2001). Performance Standards for Antimicrobial Susceptibility Testing; Eleventh Information Supplement. NCCLS document M100-S11 NCCLS, West Walley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

Samain, J. F., Boudry, P., Degremont, L., Soletchnik, P., Ropert, M., Nicolas, J. L., Le Roux, L. Renault, T., Burgeot, T., & Bacher, C. (2004). Summer mortality in the Pacific Oyster *Crassostrea gigas*, overview of 3-year results of the cooperative "MOREST" Project. *Journal of Sellfish Research*, *23*, 309-310.

Saulnier, D., De Decker, S., Haffner, P., Cobret, L., Robert, M., & Garcia, C. (2010). A large-scale epidemiologicval study to identify bacteria pathogenic to Pacific oyster *Crassostrea gigas* and correlation between virulence and metalloprotease-like activity. *Microbial Ecology*, *59*, 787- 798. https://doi.org/10.1007/s00248-009-9620-y

Scarano, C., Spanu, C., Ziino, G., Pedonese, F., Dalmasso, A., Spanu, V., Virdis, S., & De Santis, E. P. L. (2014). Antibiotic resistance of Vibrio species isolated from *Sparus aurata* reared in Italian mariculture. *New Microbiol*, *37*(3), 329-337.

Singh, J., Batra, N., & Sobti, R. C. (2001). Serine alkaline protease from a newly isolated Bacillus sp. SSR1. *Process Biochemistry*, *36*, 7824-3085. https://doi.org/10.1016/S0032-9592(00)00275-2

Timur, G., & Timur, M. (2003). *Fish Diseases*. Istanbul University Rectorate Publication No: 4426, Istanbul.

Tison, D., & Seidler, R. (1983). *Vibrio aestuarianus*: a new species from estuarine waters and shellfish. *International Journal of Systematic Bacteriology*, *33*(4), 699-702.

Zhang, X. J., Qin, G. M., Bing, X. W., Yan, B. L., & Liang, L. G. (2011). Molecular and phenotypic characterization of *Vibrio aestuarianus*, a pathogen of the cultured tongue sole, *Cynoglossus semilaevis* Günther. *Journal of Fish Diseases*, *34*(1), 57-64. https://doi.org/10.1111/j.1365-2761.2010.01212.x