World Journal of Engineering Research and Technology
WJERT
www.wjert.org
SJIF Impact Factor: 5.924

INVESTIGATION OF BACTERIAL DISEASES IN RAINBOW TROUT CAGES IN THE DOWN FIRAT BASIN

Mikail Özcan[1]* and Fatma Burcu Kuzucu[1]

[1]Kahramanmaraş Sütçü Imam University, Faculty of Agriculture, Department of Fisheries Kahramanmaras, 38000, TURKEY.

Article Received on 21/10/2019 **Article Revised on 11/11/2019** **Article Accepted on 01/12/2019**

***Corresponding Author**
Dr. Mikail Özcan
Kahramanmaraş Sütçü Imam University, Faculty of Agriculture, Department of Fisheries Kahramanmaras, 38000, TURKEY.

ABSTRACT

The aim of the study was to examine bacterial diseases frequently seen in the fish in cage production and aquaculture farms operating in Şanlıurfa and Gaziantep provinces in Lower Euphrates Basin and to determine effective antibiotics against these disease agents. For this purpose, samples were taken from the liver, spleen, kidney and intestines of a total 140 Rainbow trout with the weight range of 10-700 gr located in different cage culture facilities and 3 hatcheries between October 2018 and May 2019. For isolation of bacterial disease agents, Tryptic Soy Agar (TSA), Nutrient Broth (NB), Brain-Heart Infusion Agar (BHIA), KF Streptococcus Agar, Yersinia Selective Agar and Tryptone Yeast Extract Salt Agar (TYES-A) were used. These culture media were incubated at 15-24^{o}C for 24-72 hours in the incubator. Antibiogram sensitivity test on pure colonies was performed according to disc diffusion method. In addition to biochemical identification tests, Biolog System (The biolog GENIII micro plate) was applied to pure strains obtained from the samples taken from trout farms and their phenotypic properties were examined. As a conclusion, *Lactococcus garviae*, *Aeromonas hydrophila*-like DNA group 2, *Acinetobacter schindler*, *Lactococcus lactis ss lactis*, *Leuconostoc mesenteroides ss cremoris* and *Morexella lacunata* strains were isolated and identified according to the phenotypic and biochemical properties of the bacteria.

KEY WORDS: Bacterial Diseases, down Fırat Basin, Karkamış Dam Lake Rainbow trout, *Oncorhynchus mykiss*, Phenotypic, BIOLOG GEN III, Antibiotic disc.

INTRODUCTION

The need for protein is continuously increasing with the rapid increase of the world population. Aquaculture plays an important role in the elimination of protein deficit. Our country is one of the countries having rich resources and being suitable for fish farming. If these resources are used in a rational manner, an important contribution will be made to the economy through production with aquaculture. Aquaculture has developed rapidly in the last 30 years in our country. The number of enterprises based on the production of freshwater trout, sea bream and sea bass has been increasing. In our freshwater aquaculture, rainbow trout is mostly cultivated. Diseases have greatly increased with this rapid development. It is inevitable that enterprises will face great economic losses unless appropriate measures are taken on time (Akbulut and Keten 2001; Aydın et al., 2006).

People have to meet their metabolic activities with the nutrients. With increasing population density, food insufficiency is seen in various parts of the world. Fish meets a significant portion of our nutritional needs. For this reason, the importance given to intensive aquaculture is increasing all over the world. However, diseases on fish are becoming more and more problematic with intense aquaculture practices (Tekelioğlu et al., 2007).

Nearly 71% of the total surface area in the world is covered with water and a large part of these areas allow the life of various water species. Of the aquatic organisms, fish is the most commonly used food source by humans. For this reason, all the countries are looking for ways to use their water resources efficiently and increase their production (Tekelioğlu et al., 2007).

While the production of aquaculture was 20 million tons in the 1950s, it increased rapidly to 110 million tons in the early 1990s. The level of aquaculture production made by hunting has been stable in 2000s, and the production obtained by aquaculture has increased (FAO, 2004).

With the increase in industrialization around the world, pollution of water resources caused a decrease in the amount of aquaculture obtained by hunting from inland water and the sea, therefore the importance given to aquaculture has increased continuously both in the world and in our country (Tekelioğlu et al., 2007).

For the last 50 years, scientific and technological developments in the sector have been effective in terms of increasing the amount of production obtained by aquaculture (Bostock, 2011).

In the 1960s in Turkey, aquaculture beginning at inland waters was carried out by importing trout eggs from Europe to the farms in Bilecik and Akyazı in private sector (Memiş et al., 2002). Although the history of aquaculture sector in our country is new, it has shown a rapid development in recent years and 397.731 tons of sea products, 34.176 tons of fresh water products and 240.334 tons of aquaculture production, therefore a total of 672.240 tons of water products were realized in 2016 (Anonim, 2017).

The first type that comes to mind is the Rainbow trout both in the world and in Turkey. The reasons why Rainbow trout are preferred in aquaculture can be summarized as follows.

- Rapid adaptation to environmental conditions,
- Being able to feed easily due to active feed intake,
- Since the breeding has been carried out for more than 100 years, many aquaculture problems have been solved (Çelikkale et al., 1999).

In aquaculture, the negative change in the physical, chemical and biotic living conditions of the fish environment leads to many infectious diseases (Arda et al., 2005).

In recent years, as in all living areas in the world, various problems have emerged in fish farming. Along with the pollution in water resources, bacterial fish diseases caused significant losses in the sector in terms of economy. While 15-20 bacteria species being pathogens in fish farming were shown to be effective 20 years ago, approx. 70 bacteria species were isolated in sick fish samples recently. 15-20 bacteria species being pathogens in fish farming were shown to be effective 20 years ago whereas approx (Munro, 1982). 70 bacteria types have been isolated in infected fish samples recently (Austin and Austin 1993). Quite common major pathogenic bacterial factors (*Aeromonas spp., Pseudomonas spp., Flexibacter spp., Vibrio spp., Yersinia spp., Renibacterium spp., Streptococcus spp*.,) have also been reported in enterprises in trout production in Turkey recently. Gram-positive cocci have been indicated that it was spread sporadic and endemic, and six of these different species (*Streptococcus parauberis, Streptococcus difficile, Streptococcus iniae, Vagococcus salmoninarum, Lactococcus piscium* and *Lactococcus garvieae*) were associated with fish diseases (Kum et al., 2004; Supriyadi and Rukyani, 1992; Egidius, 1987; Larsen et al., 1988; Eldar et al., 1999; Dalsgaard and Madsen, 2000; Roberts, 2001; Timur and Timur, 2003; Timur and Korun, 2004; Aydoğan, 2005; Avcı et al., 2010).

In our country, there are many studies on the isolation and diagnosis of bacterial pathogens that cause infection in rainbow trout (*Oncorhynchus mykiss*) cultivated in different geographical regions (Kum et al., 2004; Aydın et al., 2001; Sarıeyyüpoğlu, 1984; Timur et al., 1996; Aydın et al., 1997; Balta, 1997; Karataş and Candan, 1997; Diler et al., 2000a; Diler et al., 2000b; İspir et al., 2004; Altun et al., 2010).

In Rainbow trout, especially *Aeromonas salmonicida, Vibrio anguillarum, Lactococcus garviae* and *Yersinia ruckeri* infections are emphasized to cause serious mortality (Akınbowale et al., 2006). Furunculosis caused by *A. salmonicida* is a disease that can cause 90% mortality (sudden death, bleeding in fins' base and subcutaneous tissues). It could also be asymptomatic in addition to its chronic forms (blood boils, ulceration) (Kirkan et al., 2003). Streptococcosis, which is caused by *L. garvieae*, is generally defined as a type of infection showing exophthalmus, opacity, darkening of the skin, haemorrhage in the operculum and fins (Eldar and Ghittino, 1999).

In Rainbow trout aquaculture, Aeromonas is one of the major pathogens, also known as the causative agent of frunculosis disease, resulting in high mortality and economic losses (Rattanachaikunsopon and Phumkhachorn, 2012). As a secondary pathogen, *Aeromonas hydrophila* in fish and water causes haemorrhagic septicaemia in stressed fish (Austin et al., 1985; Erer, 2002). Necrotic lesions are seen in the skin of infected fish. Ulcers and bleeding in the fins and muscles, oedema in the body and exophthalmos are seen. Microscopically, necrosis and bleeding in the skin, muscle, kidney, liver and spleen are also caused by hyperaemia (Erer, 2002).

In recent years, Gram positive cocci have been identified as important fish pathogens. Sporadic and epidemic diseases caused by gram-positives have been reported by scientists in many countries around the world (Arda et al., 2005; Austin and Austin, 1993). There are six different types of Gram-positive cocci in taxonomic classification. The most important ones are classified as Lactococcus, streptococcus and Vagococcus (Eldar et al., 1999).

Lactococcosis caused by *Lactococcus garvieae* is an important disease. It is a form of streptococcosis in this disease. *Lactococcus garvieae* was first isolated from cattle in Britain. Later, it has been reported in countries such as Britain, Japan, Africa, Thailand, Australia and continues today (Vendrell et al., 2006). It is a type of pathogen that causes infections especially in summer with the rise of temperature (Sanchez et al., 2011).

Streptococcosis is known as pop-eye disease in fish and is one of the most important bacterial diseases in farms where all Rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) is grown. Diagnosis of this disease is only possible by isolation from the brain and internal organs of a fish infected by streptococci. *Streptococcus iniae, Streptococcus agalactiae, Streptococcus dysagalactiae, Streptococcus parauberis, Streptococcus feacalis, Streptococcus difficile, Lactococcus garvieae,* and *Lactococci* are known as the reasons of lactococcosis or streptococcosis from past to present (Haghighi et al., 2010).

Firstly, isolated from Atlantic salmon in Norway in 1978, Acinetobacter sp shows signs of disease that cause haemorrhage in the skin, large and small lesions in the liver pie and spleen, haemorrhage in the air sacs and hyperaemia in the dermal blood vessels (Roald and Hastein, 1980; Austin and Austin, 2007).

Among the members of the family *Moraxella Acinetobacter sp.* it is a motionless and gram-negative coccobacillus which can be easily isolated from human skin and certain nutrients in water, soil, and wastewater (Towner, 1996; Guardabassi et al., 2000). They are located in the gills, digestive tracts and skin. For this reason, they cause the disease to start very quickly (Horsley, 1973; Roald, 1977; Austin and Austin, 2007).

One of the methods commonly used in aquaculture is the use of probiotics in order to obtain high yields in aquaculture. Probiotics are living microorganisms and benefit the host when used correctly. Although the exact mode of action of probiotics has not yet been established in any animal, including fish, probiotics usually live as hosts and produce specific differences in their activity. The types, sources, doses, and times of supplementation of probiotics significantly affect the immunomodulatory activities of probiotics. Probiotics commonly used in aquaculture are Leuconostoc, Lactococcus, Aeromonas, Enterobacter species (Nayak, 2010).

Leuconostoc, Streptococcus, Lactobacillus and *Carnobacterium* strains were detected in the microflora of the gastrointestinal tract normally found in healthy fish. The amount and proportion of lactic acid bacteria in the digestive system affected by factors such as nutritional or environmental factors, salinity, stress is not known. In addition, it is stated in the sources that lactic acid bacteria with probiotic properties are isolated from the digestive systems of fish (Ringo and Gtesoupe, 1998).

Phenotypic method is used to reflect the genetic characteristics and this method is generally specific. The phenotypic trait, which is rarely found in a pathogen, may provide information about the pathway of this trait. On the other hand, subtypes should be considered if it is always a phenotypic trait (Arda, 1994).

The Biolog System (The biolog GENIII micro plate) is used to identify Gram-positive and Gram-negative bacteria using 94 biochemical tests. These tests are based on 71 carbon source usage tests and 23 chemical susceptibility tests. Biolog Microbial identification system (Micro Station Identification System) is a versatile system. It is used in the identification and characterization of environmental and pathogenic organisms in a wide range of microbiology (Singh et al., 2011; Bochner, 1989a; Bochner, 1989b).

In this study, the agents causing economic losses in the Rainbow trout farms in the Carchemish Dam Lake in Lower Euphrates Basin were identified by using the Biolog System (The biolog GENIII micro plate) to determine other phenotypic characteristics. The prevalence and distribution of diseases in the region is determined and chemotherapeutic and prophylactic measures against these infections are put into practice by the breeder to achieve healthy fish farming targets.

MATERIALS AND METHODS

Material

The Lower Euphrates Basin consists of Karkamış and Birecik Dam Lakes. Aquaculture production started in the Carchemish Dam Lake at the end of the 90s with the impoundment of the dam. In the Carchemish Dam Lake in Lower Euphrates Basin, there are a total of 28 farms operating in the net cages, 17 of which are registered to the Ministry of Food, Agriculture and Livestock and 17 of which operate in the provincial border of Gaziantep. The material of this research is created by all 28 enterprises and 3 hatcheries operating in the region.

For bacterial isolation and identification, Tryptic Soy Agar (TSA), Nutrient Broth (NB), Brain-Heart Infusion Agar (BHIA), KF Streptococcus Agar, Yersinia Selective Agar and Tryptone Yeast Extract Salt Agar (TYES-A) from Hugh - Leifson medium , Methyl Red-Voges Proskauer (MR-VP) medium, Simmons Citrate medium, Macconkey Agar, Dehydrated Growth Agar were used.

Between October 2018 and May 2019, 28 different cage enterprises operating in Lower Euphrates Basin in Şanlıurfa and Gaziantep were visited twice a month. Generally, in the research, 140 fish samples were collected from 28 fish farms routinely sampled from 3 to 15 fish weighing 10-700 g in each sampling.

Method

Live examinations of fish in terms of diseases were carried out in cage enterprises. Necessary disease information (anamnesis) was obtained from the business owner. While sampling, selection of fishes with disease symptoms were taken into consideration and fishes were taken from the ponds with the help of a ladle. In order to delay the autolysis of the fish collected from the enterprises, samples were transported to the laboratory environment in ice molds so as not to contact with ice.

Clinical (external) examination was performed on the fish brought to the laboratory. The body surface of the fish is disinfected with 70% ethyl alcohol. In the laboratory environment, it was performed by using sterile scissors, forceps and scalpel in front of the burner flame in the sterile cabinet according to the autopsy technique.

In the fish autopsy, samples were taken from the liver, spleen, kidney and intestines of the fish and sowings were made on Tryptic Soy Agar (TSA), Nutrient Broth (NB), Brain-Heart Infusion Agar (BHIA)**,** KF Streptococcus Agar, Yersinia Selective Agar ve Tryptone Yeast Extract Salt Agar (TYES-A) with the help of extract. The media were incubated at 15-24^{o}C for 48 hours. Pure colonies were obtained from colonies grown on the media.

Colony morphology, gram staining, gram reaction with Potassium hydroxide (KOH), oxidase, catalase, Methyl Red Voges Proskauer (MR –VP), Oxidation / Fermentation (O / F), indole and motile tests were performed for identification. Biolog System (The biolog GENIII micro plate) was also used to identify the metabolic activities of the bacteria. For the Biolog System, bacterial suspension was prepared from BIOLOG IF-A solution. The bacterial concentration was adjusted to 92-98% by turbidimeter. Bacteria samples with adjusted density were added to each well in the microplates to be 100 µl. These microplates were incubated for 24 hours at 26 ºC. Finally, the microplate was read in the reader and compared with the database of the system and the bacteria were identified (Bochner, 1989a; Bochner, 1989b; Anonim, 2008).

RESULTS AND DISCUSSION

It was found that 23 of the trout breeding enterprises are engaged in net cages and 5 of them in combined (fry and portion) production in Lower Euphrates Basin. Due to most enterprises of feedlots, seed fish need is available in different provinces of Turkey.

Wetlands, where local birds living in and around this ecosystem, which are allocated to this area and have a population of foreign species coming through migration at certain times, are the stations where birds stay and rest while migrating from one place to another.

In this study, samples taken from the enterprises operating in Lower Euphrates Basin are generally suspected of disease. The fish samples showing disease symptoms were found in farms while the weather and water temperatures increased. Generally, the fish which have darkened colour, bleeding in the mouths, operculum, external surface of the body and fins, bloating in the abdomen, exophthalmus in the eyes, and moving senselessly on the water surface of the cage were selected as samples by visual and manual examination.

After morphological examination of fish suspected of disease, internal organs were examined by autopsy. Abdominal dropsy, liver and spleen discolorations and growth, swelling kidney, haemorrhages in internal organs were observed.

The Biolog System device was used to validate biochemical tests and determine other phenotypic properties (Table 1). With this system, bacteria were identified according to their phenotypic characteristics (Table 2).

Table 1: phenotypic properties by Biolog System of bacteria isolated from *Oncorhynchus mykiss.*

Biochemical criteria	***Lactococcus garvieae***	***Aeromonas hydrophila***	***Acinetobacter schindleri***	***Lactococcus lactis***	***Leuconostoc mesenteroides***	***Morexella lacunata***
pH 5	-	+/-	-	-	-	-
pH 6	+	+	+	+	+	-
Positif Kontrol	+	+	+	+	+	+
Stachyose	Weak +	+/-	-	+/-	Weak +	-

D- Turanose	+	+/-	-	+/-	+	+/-
Sucrose	Weak +	+	-	+	Weak +	+/-
Gentiobiose	+	+/-	+/-	+	+	+/-
D-Cellobiose	+	+/-	-	+	+	+/-
D-Trehalose	+	+	-	+	+	+/-
D-Maltose	+	+	-	+/-	+	-
Dextrin	+	+	+/-	+/-	+	-
Negatif Kontrol	-	-	-	-	-	-
D-Serine	-	+	-	-	-	-
Fusidic Acid	-	+/-	-	-	-	-
%1 Sodium Lactate	+	+	+	+/-	+	+
I Nosine	+	+	-	+/-	+	-
L-Rhamnose	+/-	+/-	+/-	-	+/-	-
L-Fucose	+/-	+/-	+/-	+/-	+/-	-
D-Fucose	+/-	+/-	+/-	+/-	+/-	-
3-Methyl Glucose	+	+/-	+/-	+/-	+	-
D-Galactose	+	+/-	+/-	+/-	+	-
D-Fructose	+	+	-	+/-	+	+/-
D-Mannose	+	+	-	+/-	+	-
α-D-Glucose	+	+/-	-	+/-	+	-
Niaproof 4	-	+	+/-	-	-	-
Guanidine HCl	+/-	+	-	+/-	+	-
Lincomycin	+/-	+	-	-	-	+/-
L-Serine	-	+	-	-	-	+/-
L-Pyroglutamic Acid	-	+/-	-	-	-	-
L-Histidine	-	+/-	-	-	-	+/-
L-Glutamic Acid	-	+	-	-	-	+
L-Aspartic Acid	-	+	-	-	-	+/-
L-Arginine	+/-	+/-	-	+/-	+/-	-
L-Alanine	-	+/-	+	-	-	+/-
Glycyl-L-Proline	+/-	+/-	-	+/-	+/-	+/-
Gelatin	-	+/-	-	-	-	-
Potassium Tellurite	+	+/-	+	+	+	+/-
Lithium Chloride	-	+/-	-	-	-	+/-
Nalidixic Acid	+	+/-	-	+	+	-
Bromo-Succinic Acid	-	+/-	-	-	-	-
L-Malic Acid	-	+	-	-	-	+/-
D-Malic Acid	-	+/-	-	-	-	+/-
α-Keto-Glutaric Acid	-	+/-	-	-	+/-	+/-
Citric Acid	-	+/-	-	-	-	-
L-Lactic Acid	-	+/-	+	-	-	+/-
D-Lactic Acid Methyl Ester	-	-	+/-	-	-	+/-
Methyl Pyruvate	+/-	+/-	+	-	-	-
p-Hydroxy-Phenylacetic Acid	-	+/-	-	-	-	-
%8 NaCl	Weak +	+/-	-	+/-	+	+/-
%4 NaCl	+	+/-	-	+/-	+	+/-
%1 NaCl	+	+	+	+	+	+
N-Acetyl NeuraminicAcid	-	+/-	-	-	-	-

N-Acetyl-D-Galactosamine	+	Weak +	-	+	+	+/-
N-Acetyl- β-D-Mannosa-mine	+	+/-	-	+	+	-
N-Acetyl-D-Glucosamine	+	+	-	+	+	+/-
D-Salicin	+	+/-	-	+	+	-
β- Methyl-D-Glucoside	+	Weak -	-	+	+	-
D-Melibiose	+/-	+/-	-	-	+/-	-
α-D-Lactose	+/-	+/-	-	+/-	+/-	+/-
D-Raffinose	+/-	-	-	+/-	+/-	+/-
Minocycline	-	+/-	-	-	-	-
Rifamycin SV	+	+	+	+	+	-
Troleando-mycin	-	+	-	-	-	-
D-Serine	-	+/-	-	-	-	-
D-Aspartic Acid	-	+/-	-	-	+/-	-
D-Fructose-6- Phosphate	+/-	+/-	+/-	-	+/-	-
D-Glucose-6- Phosphate	+/-	+	-	+/-	+/-	-
Glycerol	+	+/-	-	+	+	-
myo-İnositol	+/-	+/-	-	+/-	+/-	+/-
D-Arabitol	-	+/-	-	-	+/-	+/-
D-Mannitol	+	+	-	+/-	+	-
D-Sorbitol	+	+/-	-	+/-	+	+/-
Tetrazolium Blue	+/-	+	+	-	-	+/-
Tetrazolium Violet	+/-	+	+/-	-	+/-	-
Vanco-mycin	-	+	+	-	-	-
D-Saccharic Acid	-	+/-	-	-	+/-	-
Quinic Acid	-	+/-	-	-	-	-
Mucic Acid	+/-	-	-	+/-	+/-	-
Glucoronamide	+/-	+/-	+/-	-	+/-	-
D-Glucoronic Acid	+/-	+/-	+/-	-	+/-	-
D-Gluconic Acid	+	+	-	+/-	+	-
L-Galactonic Acid Lactone	-	+/-	-	-	+/-	-
D-Galacturonic Acid	+	+/-	-	+/-	+	-
Pectin	+	+/-	-	+/-	Weak +	-
Sodium Bromate	Weak +	+/-	-	+/-	+/-	-
Sodium Butyrate	+	+/-	-	+	+	+/-
Aztreonam	+	+/-	+/-	+	+	-
Formic Acid	-	+/-	-	-	-	-
Acetic Acid	+/-	+/-	+	+/-	+/-	+
Propionic Acid	-	+/-	+	-	+/-	+/-
Acetoacetic Acid	+/-	+/-	+/-	+/-	+	+/-
α-Keto- Butyric Acid	+/-	+/-	-	+/-	+/-	+/-
β- Hydroxy-D,L-Butyric Acid	-	+/-	+/-	-	-	+/-
α-Hydroxybutyric Acid	+/-	+/-	-	-	+/-	+/-
γ-Amino-Butryric Acid	-	+/-	-	-	-	+/-
Tween 40	+/-	+/-	+	-	+/-	Weak +

Table 2: Distribution of identified bacteria in sick fish.

Bacteria type	Liver	Spleen	Kidney	Intestine	Total N (%)
Lactococcus garvieae	1	0	1	0	2 (22.5)
Aeromonas hydrophila-like DNA group 2	0	0	0	1	1 (11.1)
Leuconostoc mesenteroides ss cremoris	1	1	1		3 (33.7)
Lactococcus lactis ss lactis	0	0	0	1	1 (11.1)
Moraxella lacunata	0	0	1	0	1 (11.1)
Acinetobacter schindleri	0	0	0	1	1 (11.1)

In this study, samples taken from the enterprises operating in Lower Euphrates Basin are generally suspected of disease. The fish were examined for disease symptoms while the weather and water temperatures increased. In the samples taken; the fish which have darkened colour, bleeding in the mouths, operculum, external surface of the body and fins, bloating in the abdomen, exophthalmus in the eyes, and moving senselessly on the water surface of the cage were selected as samples by visual and manual examination. Isolation and identification of selected samples by classical culture method was performed and confirmation of bacterial samples with Biolog System was performed successfully. As a result, definitions of 2 *Lactococcus garvieae* strains, 1 *Aeromonas hydrophila*-like DNA group 2 strains, 3 *Leuconostoc mesenteroides ss cremoris* strains, 2 *Lactococcus lactis ss lactis* strains, 1 *Acinetobacter schindleri* strain and 1 *Moraxella lacunata bacteria* were made according to the phenotypic properties of the bacteria.

Today, the consumption of white meat has increased gradually due to many reasons such as reduced agricultural areas because of the increase in population density, economic conditions and food deficiencies. A rapid development has been achieved in also intensive fish culture techniques due to the high-quality protein deficit, which is important especially for physical and mental development, and the lack of natural resources to meet the needs. In an intensive aquaculture, naturally higher level of farming, reduced water quality, inadequate diet and manipulations also cause disease problems more frequently (FAO, 1995).

Sowings were made to the media such as Tryptic Soy Agar (TSA), Nutrient Broth (NB), Brain-Heart Infusion Agar (BHIA)**,** KF Streptococcus Agar, Yersinia Selective Agar and Tryptone Yeast Extract Salt Agar (TYES-A) from the liver, spleen, kidney and intestines of the fish by the help of extract in a sterilized manner (Grizzle et al., 2003). Sowing results were monitored at 24^{o}C for 24 and 48 hours, while cultivation for *Flavobacterium*

psychrophilum was kept at 15^{o}C for 7 days. The bacteria isolated on the media were stored at -84 ° C in tubes containing 15% glycerol in pure form for further study.

Although it is known that 15 - 20 bacterial species have shown pathogenic effects for fish until recent past (Munro, 1982), more bacterial species have been isolated in naturally infected fish nowadays (Austin and Austin 1993). As a result of the identification results of bacterial isolates (Table 1) and bacteriological verification tests of the agents, the agent *L. garvieae,* which causes significant economic losses in bacterial species, was isolated (Austin and Austin 1993; Çağırgan, 2004; Woo and Bruno 2003). It is stated that isolated bacterial agents can be found in the normal microflora of the water, but the incidence of diseases is increased especially in cases of sudden changes in water temperature values due to the seasons, that leads to increased stress (hygienic defects, dense fish in production area, lack of clean barriers) (Austin and Austin 1993; Cabello, 2006). The fact that the isolated disease agents were observed more frequently in the months when the water temperature increased, supports these findings.

The presence of erosive and ulcerative lesions in the skin, fins and tail were found to be consistent with the literature in the cases isolated from *A. hydrophila* (Austin et al., 1985; Mancini et al., 1997).

Motile aeromonas has been reported to cause high mortality and cause more than 50% loss in offspring in 2-3 weeks (Özer et al., 2008; Cipriano, 2001). In our study, it was also found that *A. hydrophila* caused 11.1% of death in rainbow trout.

Lactococcus lactis, which has been reported to cause disease in rainbow trout (Bragg and Broere, 1986), shows that it is also present in Rainbow trout of our region. *L. lactis subsp. Lactis* (Bascomb and Manafi, 1998) used in the production of fermented foods has been reported to cause infection in waterfowl (Goyache et al., 2001). The Lower Euphrates Basin serves as the station where the birds migrate from one place to another. Therefore, *L. Lactis* bacteria can always infect fish in this basin.

260 fish samples and 56 water samples of seven different trout farms in Mersin were examined for Gram-positive bacterial flora for a year by classical isolation and identification methods. *Leuconostoc* spp. was found (%1.6) (Özer et al., 2008). In this study (33.7%) was determined.

In autopsy of fish infected with *Acinetobacter* sp, it has been reported that hyperaemia in dermal blood vessels, haemorrhage in the skin, oedema in the base of the fins, lesions in the liver, kidney and spleen, small haemorrhages in the air sac and peritoneum dermal may occur (Roald and Hastein, 1980; Austin and Austin, 2007). In this study, the lesions observed in liver and kidney seems to support the findings of the researchers.

CONCLUSIONS

In this study, sowing was made from liver, spleen, kidney and intestines of live fish weighing 10-700 g showing signs of disease in 28 farms registered in Gaziantep and Sanliurfa provinces in Carchemish Dam Lake, and culture method was used for identification of bacterial disease agents in the basin. In addition, biochemical identification tests and Biolog System (The biolog GENIII micro plate) were applied to examine the phenotypic properties of pure strains obtained from infected fish samples.

ACKNOWLEDGEMENTS

This study is summary of the second author's master thesis. This work was presented as a oral presentation at International Congress on Domestic Animal Breeding Genetics and Husbandry– 2019 (ICABGEH-19), September 11-13, 2019, Prag- Czech Republic.

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: 2017/01).

REFERENCES

1. Akbulut, S., Keten, A. 2001. Düzce Yöresindeki Alabalık Yetistiriciligi Üzerine Bir Çalısma, Süleyman Demirel Üniversitesi Orman Fakültesi Dergisi, 2: 49-60.
2. Akinbowale, O.L., Peng, H., and Barton, M.D. 2006. Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. J. Appl. Microbiol., 100: 1103-1113.
3. Altun, S., Kubilay, A., Diler, Ö. 2010. *Yersinia ruckeri* Suşlarının Fenotipik ve Serolojik Özelliklerinin İncelenmesi. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 16(B): 223-229.
4. Anonim, 2008. Biolog Gen III Microplate Instruction for Use.
5. Anonim, 2017. Su Ürünleri İstatistikleri 2018. TÜİK. Ankara.

6. Arda, M. 1994. Biyoteknoloji: Bazı Temel İlkeler. Kükem Derneği Bilimsel Yayınları No:2 İkinci Baskı.
7. Arda, M., Seçer, S., ve Sarıeeüpoğlu, M., 2005. Balık Hastalıkları. Medisan Yayın serisi: 61, II Baskı Ankara 230pp.
8. Austin, B., and Austin, D.A. 1985. Bacterial pathogens of fish. J.Apl. Bacteriol.58,483-506.
9. Austin, B., and Austin, D.A. 1993. Bacterial fish pathogens disease in farmed and wild fish, Ellis Horwood, London.
10. Austin, B., and Austin, D.A. 2007. Bacterial Fish Pathogens Disease in Farmed and Wild Fish, 4 th edition, Praxis Publishing, Chichester, 594 pp.
11. Avcı, H., Aydoğan, A., Tanrıkul, T.T., and Birincioğlu, S.S. 2010. Pathological and microbiological investigations in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) naturally infected with *Lactococcus garvieae*. Kafkas Univ. Vet. Fak. Dergisi, 16: 313-318.
12. Aydın, F., Köksal, G., Demir, N., Bekcan, S., Kırkagaç, M., Gözgözoglu, E., Erbas, S., Deniz, H., Matlas, Ö., and Arpa, H. 2006. Su ürünleri yetistiriciligi ve politikalar, Erisim:[http://www.zmo.org.tr/etkinlikler/6tk05/039fikriaydin.pdf], Erisim Tarihi: 15.12.2006.
13. Aydın, S., Çiltaş, A., and Akyurt, İ. 1997. Investigations on systemic *Aeromonas hydrophila* infactions appeared in rainbow trout (*Oncorhynchus mykiss* Walbaum) in 1991–1995. Akdeniz Balıkçılık Kongresi, 359-369, 9–11 Nisan 1997, İzmir.
14. Aydın, S., Erman, Z., and Bilgin, Ö.C. 2001. Investigations of *Serratia liquefaciens* in rainbow trout (*Oncorhynchus mykiss* Walbaum). Turkish Journal of Veterinary and Animal Sciences, 25: 643-650.
15. Aydoğan, A. 2005. *Aeromonas salmonicida* ile Enfekte Edilen Gökkuşağı Alabalıklarında (*Oncorhynchus mykiss* Walbaum, 1792) Patolojik Bulguların İncelenmesi. Yüksek Lisans Tezi, Adnan Menderes Üniversitesi, Sağlık Bilimleri Enstitüsü, Aydın.
16. Balta, F. 1997. Kültürü yapılan alabalıklarda (*Oncorhynchus mykiss*) görülen *Flexibacter psychrophila* enfeksiyonu. IX. Ulusal Su Ürünleri Sempozyumu. 641- 648. 17–19 Eylül 1997, Eğirdir/Isparta.
17. Bascomb, S., and Manafi, M. 1998. Use of enzyme tests in characterization and identification of aerobic and facultatively anaerobic Gram-positive cocci, *Clinical Microbiology Reviews*, 11(2): 318-340.
18. Bochner, B.R. 1989a. Sleuthing out Bacterial Identities. Nature 339, 157-158.

19. Bochner, B.R. 1989b. "Breathprints" at the Microbial Level. ASM News 55, 536-539.
20. Bostock, J. 2011. Foresight Project on Global Food and Farming Futurs, The Application of Science and Technology Development in Shaping Current and Future Aquaculture Production Systems, Journal of Agricultural Science, 149: 133-141.
21. Bragg, R.R., Broere, J.S.E. 1986. *Streptococcosis* in rainbow trout in South Africa. Bulletin of the European Association of Fish Pathologists, 6(3): 89-91.
22. Cabello, F.C. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environmental Microbiology, 8: 1137-1144.
23. Cipriano, RC. 2001. *Aeromonas hydrophila* and motile aeromonad speticemias of fish. Fish Diseases Leaflet. US Fish and Wild Wife Service; 68: 1–25.
24. Çağırgan, H. 2004. Biotyping of *Lactococcus garvieae Isolated from Turkey,* EU Journal of Fisheries and Aquatic Sciences, 21: 267-269.
25. Çelikkale, M.S., Düzgüneş, E. and Okumuş, İ. 1999. Türkiye Su Ürünleri Sektörü: Potansiyeli, Mevcut Durumu, Sorunları ve Çözüm Önerileri, İstanbul Ticaret Odası Yayın No: 1999-2, İstanbul. Lebib Yalkın Yayımları ve Basım İşleri A.Ş., İstanbul,s. 53: 413 pp.
26. Dalsgaard, I., Madsen, L. 2000. Bacterial pathogens in rainbow trout, *Oncorhynchus mykiss* (Walbaum), reared at danish freshwater farms. J Fish Dis, 23: 199-209.
27. Diler, Ö., Altun, S., Çalıkuşu, F., Diler, A. 2000b. Gökkuşağı alabalığı (*Oncorhynchus mykiss*)'nın yaşadığı ortam ile ilişkili kalitatif ve kantitatif bakteriyel florası üzerine bir araştırma. Turkish Journal of Veterinary and Animal Sciences, 24: 251–259.
28. Diler, Ö., Altun, S., Diler, A., Işıklı, B. I., Gürcan, O.C. 2000a. Bazı balık çiftliklerinde gökkuşağı alabalıklarının (*Oncorhynchus mykiss*) mikroflorasının tespiti ve kontrolü üzerine bir çalışma. Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 4: 58–69.
29. Egidius, E., 1987. *Vibriosis:* pathogenicity and pathology. A review. Aquaculture, 67: 15-28.
30. Eldar, A., and Ghittino, C., 1999. *Lactococcus garvieae* and *Streptococcus iniae* infections in rainbow trout *Oncorhynchus mykiss*:similar, but different diseases. *Dis. of Aquatıc Organıs. Dıs.*, 36: 227-231.
31. Eldar, A., Goria, M., Ghittino, C., Zlotkin, A., and Bercovier, H. 1999. Biodiversity of *Lactococcus garvieae* strains isolated from fish in Europe, Asia and Australia. Appl. Environ. Microbiol., 65: 1005-1008.

32. Erer, H. 2002. Balık Hastalıkları. 2. baskı, Selçuk Üniv. Basimevi, Konya.
33. FAO, 1995. The State of the World Fisheries and Aquaculture, Rome, 27s.
34. FAO, 2004. Global Production Statistics. http://www.fao.org/figis/servlet/static.html
35. Goyache J., Vela, A.I., Gibello, A., Blanco, M.M., Briones, V., González, S., Téllez, S., Ballesteros, C., Domínguez, L., and Fernández-Garayzábal, J.F. 2001. *Lactococcus lactis* subsp. *lactis* infection in waterfowl: First confirmation in animals, CDC (Centers for Disease Control and Prevention) Emerging Infectious Diseases, 884. 7(5).
36. Grizzle, J.M., Altinok, I., and Noyes, A.D. 2003. PCR method for detection of large mouth bass virus. Dis. Aquat. Org., 54: 29-33.
37. Guardabassi, L., Dijkshoorn, L., Collard, J.M., Olsen, J.E., and Dalsgaard, A. 2000. Distribution and in-vitro transfer of tetracycline resistance determinants in clinical and aquatic Acinetobacter strains, Journal of Medical Microbiology, 49: 929-936.
38. Haghighi, K.S., Soltani, M., Nikbakhat, B.G., Ghasemi, M., and Skall, H.F. 2010. Molecular epidemiology of zoonotic *Streptococcosis/Lactococcosis* in rainbow trout (*Oncorhynchus mykiss*) aquaculture in Iran. Iranian J. of Microbiol. 2(4): 198-209.
39. Horsley, R.W. 1973. The bacterial flora of the Atlantic salmon (*Salmo salar L.*) in relation to its environment. Journal of Applied Bacterology, 36: 377-386.
40. İspir, Ü., Şeker, E., Sağlam, N., Dörücü, M. 2004. Doğu Anadolu Bölgesinde Bazı Gökkuşağı alabalığı (*Oncorhynchus mykiss*) İşletmelerinde görülen *Flavobacterium psychrophilum* Enfeksiyonunun Araştırılması. Fırat Üniversitesi, Fen ve Mühendislik Bilimleri Dergisi, 16(4): 718–724.
41. Karataş, Düğenci, S., and Candan, A. 1997. Marmara bölgesindeki bir gökkuşağı alabalığı (*O. mykiss*) işletmesinde *Yersiniosis* vakası. IX. Ulusal Su Ürünleri Sempozyumu. 17 – 19 Eylül 1997, Eğirdir/Isparta. 641–648.
42. Kirkan, S., Goksoy, O.E., and Kaya, O. 2003. Isolation and antimicrobial susceptibility of *Aeromonas salmonicida* in rainbow trout (*Oncorhynchus mykiss*) in Turkey hatchery farms. *J. Vet. Med. B*, 50: 339-342.
43. Kum, C., Gökbulut, C., Akar, F., Kırkan, S., and Sekkin, S. 2004. Gökkusagı Alabalıklarında (*Oncorhynchus mykiss*) *Enterococcus seriolicida* izolasyonu ve etkili Antibakteriyel Sagaltım Seçeneginin Belirlenmesi, Veteriner Hekimler Dernegi Dergisi, 75(3): 47-53.
44. Larsen, J.L., Rasmussen, H.B., and Dalsgaard, I. 1988. Study of *Vibrio anguillarum* strains from different sources with emphasis on ecological and pathological properties. Appl Environ Microbiol, 54: 2264-2267.

45. Munro, A. 1982. The pathogenesis of bacterial diseases of fish, Microbial Disease of Fish, Academic Press, London, p: 131–149.
46. Nayak, S.K. 2010. Probiotics and immunity; A Fish Perspective. *Fish & Shellfish Immunology*. 29: 2-14.
47. Özer, S., Demirel, M., Us, M., Yıldırım, S. 2008. Mersin İli Çağlarca Köyündeki Gökkuşağı Alabalığı (*Oncorhynchus mykiss, Walbaum*) Kuluçkahanlerinin Mikrobiyal Florası. *Journal of Fisheries Sciences,* 2(3): 261-271.
48. Rattanachaikunsopon, P., and Phumkhachorn, P. 2012. Detection of *Aeromonas salmonicida* by reverse transcription-multiplex polymerase chain reaction. *Journal Bioscience, Biotechnology, and Biochemistry*. 76(4): 665-670.
49. Ringo, E., Gatesoupe, F.J. 1998. Lactic acid bacteria in fish, review, Aquaculture, 160: 177-203.
50. Roald, S.O. 1977. Effects of sublethal concen-trations of lignosulphonates on growth, in-testinal microffora and some digestive enizymes of rainbow trout (*Salmo gairdneri*). Aquaculture, 12: 327-335.
51. Roald, S.O., and Hastein, T. 1980. Infection with an acinetobacter-like bacterium in Atlantic salmon (*Salmo salar*) broodfish, in Ahne, W. eds. Fish Disease. Third COPRAQ Session, Berlin, Springer-Verlag, pp:154-156.
52. Roberts, J,R. 2001. Fish Pathology, 3rd ed., W.B. Saunders, USA.
53. Sanchez, T.P., Balcazar, J.L., Merrifield, D.L., Carnevali, O., Gioacchini, G., DeBlas, I., and Zarzuella, I.R. 2011. Expression of immune-related genes in rainbow trout (*Oncorhynchus mykiss*) induced by probiotic bacteria during *Lactococcus garvieae* infection. Fish & Shellfish Immunol., 31: 196-201.
54. Sarıeyyüpoğlu, M., 1984. Gökuşağı Alabalıklarında (*S. gairdneri*) Mide-Bağırsak Bakteriyel Florasının Aerobik Yönden İncelenmesi. Doğa Bilim Dergisi, Seri: 1(8): 3, 281-287.
55. Singh, J., Batra, N., and Sobti, R.C. 2001. Serine Alkaline Protease from a Newly Isolated Bacillus sp. SSR1. Process Biochem., 36: 781-785.
56. Supriyadi, H., and Rukyani, A., 1992. The use of chemotherapeutic agents for the treatment of bacterial disease of fish and shrimps in Indonesia. Disease in Asian Aquaculture I, p: 515–517.
57. Tekelioğlu, N., Kumlu, M., Yanar, M., and Erçen, Z. 2007. Türkiye'de Su Ürünleri Üretim Sektörünün Durumu ve Sorunları, *Ulusal Su Günleri Şurası*, 16-18 Mayıs 2007, Ankara.

58. Timur, G., Karataş, S., Çolak, S., and Akaylı, T. 1996. Gökkuşağı alabalık (*Oncorhnchus mykiss*, W, 1792) yavrularında görülen furunkulosis hastalığı üzerine bir çalışma. II. International Symposium on Aquatic Products. İstanbul, Türkiye.
59. Timur, G., and Korun, J. 2004. First Outbreak of Vibriosis in Farmed Rainbow Trout (*Oncorhynchus mykiss*) in Turkey. *Istanbul University Journal of Fisheries*, 18: 1-9.
60. Timur, G., and Timur, M. 2003. Balık Hastalıkları. İstanbul Üniversitesi Rektörlük Yayın No: 4426, İstanbul. 588 pp.
61. Towner, K.J. 1996. Biology of *Acinetobacter spp*. in Bergogne-Bérézin, E., Joly-Guillou, M. L., Towner, K. J. eds. Acinetobacter: Microbiology, Epidemiology, Infections, Management, CRC Press, Boca Raton, *FL*, 13-36.
62. Vendrell, D., Balcazar, J.L., Zarzuela, I.R., DeBlas, I., Girones, O., and Muzquiz, J.L. 2006. *Lactococcus garvieae* in fish: A review. Comp. Immunol. Microbiol.&Infect. Dis., 29: 177–198.
63. Woo, P.T.K., and Bruno, D.W. 2003. Fish disease and disorders, Volume 3: Viral, bacterial and fungal infections. CABI Publishing, Oxfordshire, UK.