



# Investigation of the effects of some phytochemicals on *Yersinia ruckeri* and antimicrobial resistance

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(With 6 figures)

## Abstract

In this study, it is aimed to investigate the effects of *Moringa oleifera* and *Sorbus domestica* plant extracts on bacterial disease agents *Yersinia ruckeri* in aquaculture. Morphological and biochemical properties of 2 different *Y. ruckeri* isolates were determined. Then, Real-Time PCR analysis and gene sequencing of the isolates were identified. Phytochemicals (*M. oleifera* and *S. domestica*) and antibiotics (Oxytetracycline (OX) and Enrofloxacin (ENR)) were used together in the antibiogram test of antibiotics compared to the effect status of antibiotics. Also, the effects of phytochemicals on *Y. ruckeri* growth was examined comparatively by spectrophotometrically measuring at 600 nm wavelength every 2 hours according to bacterial growth densities with 10 different groups formed on TSB medium. As a result of the study, it was observed that the isolates formed Gram negative, catalase positive, oxidase negative, mobile and typical *Y. ruckeri* colonies. After the biochemical tests performed with Microgen ID panel, 99.85% similarity was determined. The isolates overlap with the 16S rRNA gene region after sequence analysis, and 99% of the isolates were similar in phylogenetic analysis. After the antibiogram test, Oxytetracycline and Enrofloxacin antibiotics were resistant to *Y. ruckeri* but the effects of phytochemicals were less on solid medium (MHA). As a result of the measurements carried out in liquid medium (TSB), it was observed that phytochemicals such as *M. oleifera* and *S. domestica* inhibit the growth of bacteria by 40-50%. As the importance of antibiotic resistance is increasing day by day, we believe that these phytochemicals will give positive results in treatment instead of using antibiotics.

**Keywords:** Real-Time PCR, sequencing, antibiogram, fish diseases, phytochemical.

## Investigação dos efeitos de alguns fitoquímicos em *Yersinia ruckeri* e resistência antimicrobiana

### Resumo

Neste estudo, objetiva-se investigar os efeitos dos extratos de plantas de *Moringa oleifera* e *Sorbus domestica* sobre agentes bacterianos *Yersinia ruckeri* na aquicultura. Foram determinadas as propriedades morfológicas e bioquímicas de 2 isolados diferentes de *Y. ruckeri*. Em seguida, a análise de PCR em tempo real e o sequenciamento genético dos isolados foram identificados. Fitoquímicos (*M. oleifera* e *S. domestica*) e antibióticos (Oxitetraciclina e Enrofloxacina) foram usados juntos no teste de antibiograma dos antibióticos em comparação com o status de efeito dos antibióticos. Além disso, os efeitos dos fitoquímicos no crescimento de *Y. ruckeri* foram examinados comparativamente por medição espectrofotométrica no comprimento de onda de 600 nm a cada 2 horas de acordo com as densidades de crescimento bacteriano com 10 grupos diferentes formados no meio TSB. Como resultado do estudo, observou-se que os isolados formaram colônias Gram-negativas, catalase-positivas, oxidase-negativas, móveis e típicas de *Y. ruckeri*. Após os testes bioquímicos realizados com o painel Microgen ID, foi determinada uma similaridade de 99,85%. Os isolados se sobrepõem à região do gene 16S rRNA após a análise da sequência e 99% dos isolados foram semelhantes na análise filogenética. Após o teste do antibiograma, os antibióticos Oxitetraciclina e Enrofloxacina foram resistentes a *Y. ruckeri*, mas os efeitos dos fitoquímicos foram menores no meio sólido (MHA). Como resultado das medições realizadas em meio líquido (TSB), observou-se que os fitoquímicos inibem o crescimento de bactérias em 40-50%. Como a importância da resistência aos antibióticos está aumentando dia a dia, acreditamos que as plantas que são mais alternativas e mais adequadas para o uso de antibióticos hoje em dia darão resultados positivos no tratamento.

**Palavras-chave:** Real-Time PCR, sequenciamento, antibiograma, doenças de peixes, fitoquímicos.

### 1. Introduction

Seafood is an important food source with high protein value and necessary for healthy and balanced nutrition. It constitutes 17% of the animal protein consumed worldwide

and 6.5% of all protein sources (Naylor et al., 2000). In addition to water quality, appropriate environmental conditions, feed supply, marketing and labor problems

in aquaculture, the most important problem that causes economic losses in aquaculture in freshwater and seas is the existence of various diseases (Timur and Timur, 2003). Economic losses caused by fish diseases and infections are of great importance for the development of the fisheries sector (Pilarski et al., 2008; Ergönül et al., 2012).

Enteric Red Mouth (ERM) created *Y. ruckeri* seen as intense Turkey and the world. The diseases, which is the most important problem of trout farms, causes serious economic losses. Yersiniozisin in all salmonids and catfish, goldfish, sturgeon fish such as non-salmonid disease is known to cause disease and cause great economic losses (Kahraman, 2013).

Although, *Y. ruckeri* can reproduce between 9-37 °C, the optimum breeding temperature was determined to be 20-25 °C (Austin and Austin, 1987). Studies are mostly focused on disease prevention because of the costly treatment, labor demand, antibiotic use causing resistance to pathogenic and non-pathogenic microorganisms and threatening human and animal health in this case (Timur and Timur, 1985).

Since fish pathogens can transfer resistance genes to bacteria that cause disease in humans, their use is limited (Uluköy et al., 2013). The unconscious use of antibiotics in the treatment of bacterial diseases in aquaculture can lead to the development of resistant bacteria and may have negative effects on the environment, humans and other living things. However, the use of alternative methods is also important due to reasons such as the inability to vaccinate after the disease has occurred and the resistance of the agents to antibiotics (Hatha et al., 2005).

## 2. Material and Methods

### 2.1. Supply of isolates

This study was carried out with the approval of the Animal Experiments Local Ethics Committee of Van Yuzuncu Yil University, dated 07/09/2018 and numbered 64096. ATCC 29473 reference *Y. ruckeri* strain and *Y. ruckeri* isolated at different times were used in the study. All bacteria were incubated in TSA and WS media at 37 °C for 24 hours. Typical colonies with morphological similarities were selected and identified. During the study, bacteria were stored at -80 °C in 15% glycerol, 85% medium (Austin, 1999).

### 2.2. Biochemical characterization

*Y. ruckeri* cultures isolated on medium and Gram stained, catalase and oxidase tests were carried out. After 24 h incubation at 37 °C in TSA medium, bacteria adjusted to McFarland 0.5 optical density were inoculated into the capsules in the kit and left to incubate at 37 °C for 24 hours. After incubation, the results were evaluated as positive and negative according to the Microgen company reading table. The results were matched with the Microgen website program and their biochemical identifications were performed (Gulaydin et al., 2018).

### 2.3. Molecular characterization

#### 2.3.1. DNA isolation

Genomic DNA of bacteria was isolated with GeneJet Genomic DNA isolation kit for the molecular identification of the isolates according to the manufacturer's instructions. The purity of the isolated DNAs was measured with a nanospectrophotometer (Thermo) at 260 nm wavelength. Isolated DNAs were stored at -20 °C during the study period (Önalán and Yavuz, 2019).

#### 2.3.2. Real-Time PCR

Isolated DNAs were adjusted to 25 µl total volume using *Y. ruckeri* specific Forward and Reverse primers (27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTGTACGACTT-3') (Lane, 1991)), 2X SYBRGreen master mix, DNase-RNase free water. After, 95 °C for 10 min pre-denaturation in Real-Time PCR, denaturation at 94 °C for 45 s, binding at 55 °C for 30 s and elongation at 72 °C for 45 s were completed in 45 cycles. As a final step, PCR was completed with a final extension at 72 °C for 7 min. Non-template control tubes were used as negative controls. Samples giving sigmoidal curves were evaluated as positive in RotorGene Q 9000 software. As a result of sequence analysis to be performed with PCR amplicons, the identification and similarity rates of *Y. ruckeri* isolates used in the study were determined (Önalán and Arabaci, 2016). Real-Time PCR amplicons were sent for sequence analysis and nucleic acid sequences were blasted on the NCBI website and the similarity rates of the isolates were obtained.

#### 2.3.3. Spectrophotometric determination of the effects of phytochemicals in liquid media

Moringa (*M. oleifera*) and Sorbus (*S. domestica*) phytochemicals were commercially purchased in liquid form. The purchased plant extracts were diluted in different dilutions and applied in solid (for antibiogram test) and liquid (for spectrophotometric measurement) media. To determine the effects of phytochemicals and antibiotics on bacterial cultures to be used in the study, suspension solutions consisting of 10 groups were prepared (Table 1). In this study, untreated groups with bacteria will be used as negative control. The growth of the reference bacteria was measured in the Tryptic Soy Broth (TSB) as a positive control. Measurements were taken every 2 hours (Bayaz, 2014).

#### 2.3.4. Antibiogram test

In the antibiogram test, 100 µl *Y. ruckeri* suspensions were spread with drigaski on Müller-Hinton Agar (MHA) medium. The antibiotic discs (OX and ENR) were then placed symmetrically in Petri dishes. The media were incubated at 37 °C for 24 hours. The zone diameters formed after the incubation period were measured in mm with the help of a ruler. The results were compared with Antibiotic Zone Diameter Table (Akhlaghi and Sharifi-Yazdi, 2008).

### 3. Results

#### 3.1. Microbiological results

To isolate the bacteria, after the first planting in TSA and WS media, the bacteria were purified from single colonies selected according to colony morphology. Then, blood agar (BA) medium was carried out planting and hemolytic properties of the developing bacteria were determined (Figure 1).

Blood agar bacteria as a result of the tests performed Gram-negative, catalase positive, oxidase negative and were observed that they are mobile (Table 2).

Microgen’s GNA+B ID system was used to determine the biochemical properties of *Y. ruckeri* isolates. According

to color changes following bacterial incubation, biochemical test results (Table 3) are given below.

Biochemical test results were evaluated according to the color changes caused by the reaction between the reagents and the bacteria in the kit wells. By entering positive (+) and negative (-) results in the software of Microgen company, it was confirmed that 99.85% of the isolates were *Y. ruckeri* according to the results report obtained online. Also, there was no difference between the biochemical test results of 2 different *Y. ruckeri* isolates.

#### 3.2. Molecular characterization

The identification of the bacteria used in the study with different methods and whether there were differences between the isolates were used. Molecular methods to perform this process using the molecular identification of bacteria for universal bacterial primers performed using Real-Time PCR results are given below (Figure 2).

According to the results of Real-Time PCR analysis, *Y. ruckeri* reference and isolate used in the study yielded sigmoidal curves and were positive. Samples used as negative controls (NTC and Master mix Control) were found to be negative with a straight line below the threshold value. After Real-Time PCR analysis, sequencing of the 16S rRNA gene region was performed using PCR amplicons and primers 27F-1492R. The results of the sequence were confirmed to be *Y. ruckeri* in the gene bank by NCBI Blast application. 16S rRNA gene sequences of the bacteria used in the study are given below (Figure 3).

The sequence data and the data obtained after the blasting process was used in the CLC Main Workbench software to generate phylogenetic trees. The phylogenetic similarities of the isolates used in the study are given below (Figure 4). According to these results, *Y. ruckeri* isolates were similar with 99% similarity rate.

#### 3.3. Antibiogram test results

In the antibiogram test, 100 bacteria were grown in MHA medium (25 mL/Petri). Moringa and Sorbus solutions were added to the empty discs in the same amount as the antibiotic (ENR and OX) disc ratios and 15 µl empty discs. Zone diameters were calculated after a 24-hour incubation period at 37 °C. The antibiotic was evaluated within the limits specified in the Zone Diameter Table.

**Table 1.** Groups created for spectrophotometric measurements.

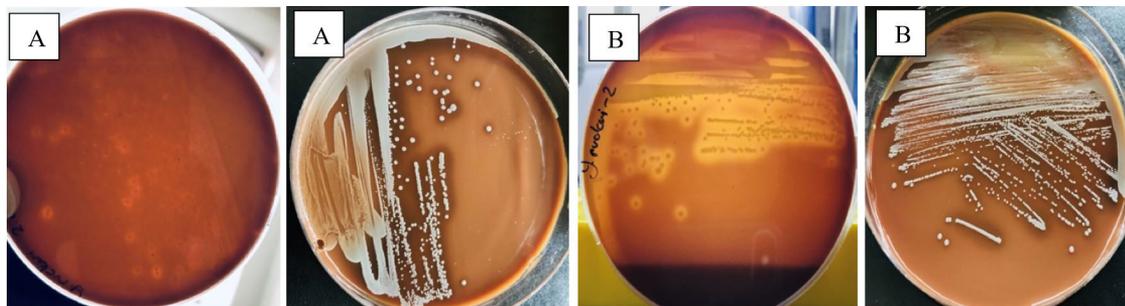
No	Group
1	10 mL TSB Control (10 mL TSB)
2	10 mL TSB+50 µl bacteria (Zero-reset with group 1)
3	10 mL TSB+400 µl Moringa
4	10 mL TSB+400 µl Moringa+50 µl bacteria (Zero-reset with group 3)
5	10 mL TSB+400 µl Sorbus
6	10 mL TSB+400 µl sorbus+50 µl bacteria (Zero-reset with group 5)
7	10 mL TSB+1-OX+50 µl bacteria (Zero-reset with group 9)
8	10 mL TSB+1-ENR+50 µl bacteria (Zero-reset with group 10)
9	10 mL TSB+1 Disk OX
10	10 mL TSB+1 Disk ENR

TSB: Tryptic soy agar, OX: Oxytetracycline, ENR: Enrofloxacin.

**Table 2.** Some microbiological test results of *Y. ruckeri* isolates.

Testin adı	<i>Y. ruckeri</i> reference strain	<i>Y. ruckeri</i> isolate
Gram staining	(-)	(-)
Catalase	(+)	(+)
Oxidase	(-)	(-)
Movement	(+)	(+)

\* (-): Negative, (+): Positive.

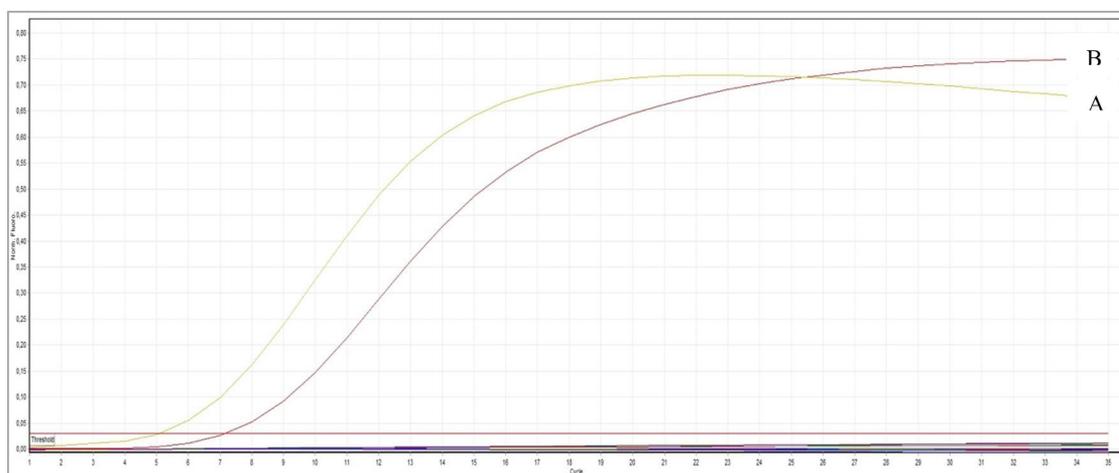


**Figure 1.** Image of *Y. ruckeri* isolates growing on blood agar medium (A) *Y. ruckeri* reference strain; (B) *Y. ruckeri* isolate).

**Table 3.** Biochemical test results of *Y. ruckeri* isolates.

Test	Explanation	Result	Test	Explanation	Result
INO	Acid from Inositol	+	TDA	Tryptophan Deaminase	-
LYS	Lysine Decarboxylase	+	GEL	Gelatine Liquefaction	+
ORN	Ornithine Decarboxylase	+	MAL	Malonate Utilization	-
H <sub>2</sub> S	Hydrogen Sulfide Production	-	SOR	Acid from Sorbitol	-
GLU	Acid from Glucose	+	RHA	Acid from Rhamnose	-
MAN	Acid from Mannitol	+	SUC	Acid from Sucrose	-
XYL	Acid from Xylose	-	LAC	Acid from Lactose	-
ONPG	O-Nitrophenyl-β-D-Galactopyranoside	+	ARA	Acid from Arabinose	-
IND	Indole	-	ADO	Acid from Adonitol	-
UR	Urea Hydrolysis	-	RAF	Acid from Raffinose	-
VP	Voges Proskauer	-	SAL	Acid from Arabinose	-
CIT	Citrate Utilization	+	ARG	Arginine Dihydrolase	-

\* (-): Negative, (+): Positive.

**Figure 2.** Real-Time PCR result of *Y. ruckeri* isolates (A) *Y. ruckeri* reference strain; (B) *Y. ruckeri* isolate).

ENR and OX antibiotics were found to have 24 mm zone diameter on MHA medium in the antibiogram test. In antibiotic Zone Diameter Table, this value was found to be resistant, while *M. oleifera* was 11 mm and *S. domestica* was 11 mm. With these values, *M. oleifera* and *S. domestica* (11, 9 mm) were found to be sensitive to ENR and OX (Figure 5).

#### 3.4. Spectrophotometric development results

To investigate the effects of antibiotics and phytochemicals on the growth of *Y. ruckeri* isolates in liquid medium, their concentrations (OD) at 600 nm were measured with 10 groups. The graph obtained using spectrophotometric development values are given below (Figure 6).

Moringa and Sorbus extracts were found to have more effects on the liquid medium than the solid medium (antibiogram test). In themselves, Moringa extract was found to be more effective than Sorbus extract. In the antibiogram test, it was observed that the sensitive phytochemicals inhibited the growth of bacteria by 50%, especially Moringa.

According to the obtained graph, it was observed that the bacterial growth in TSB medium was highest at 600 nm in the first 2 hour measurements, while the

effect of OX and ENR antibiotics levels did not begin at the beginning compared to the control group. Bacterial growth was found to be slower (40-50%) in Sorbus and Moringa groups. Bacterial growth was highest in the TSB+Yr group at the 14th and 16th hours, and the highest levels were observed at the 18th and 20th hours in the OX and ENR antibiotics groups.

#### 4. Discussion

As in many other areas in the world, it is known that the use and effects of plants on fish in the fisheries sector are investigated (Yigitarslan et al., 2011). In a limited number of studies conducted to determine the possibilities of using plants and active substances in aquaculture, it was reported that plant extracts added to food and water improved feed consumption, feed utilization, growth and carcass quality (Şimşek et al., 2005; Immanuel et al., 2009).

Medicinal plant extracts have some antimicrobial effects on some bacterial and fungal species that have been studied for many years (Kivanç and Akgül, 1986). In recent years, some researches have been conducted on the use of medicinal plants as feed additive in general

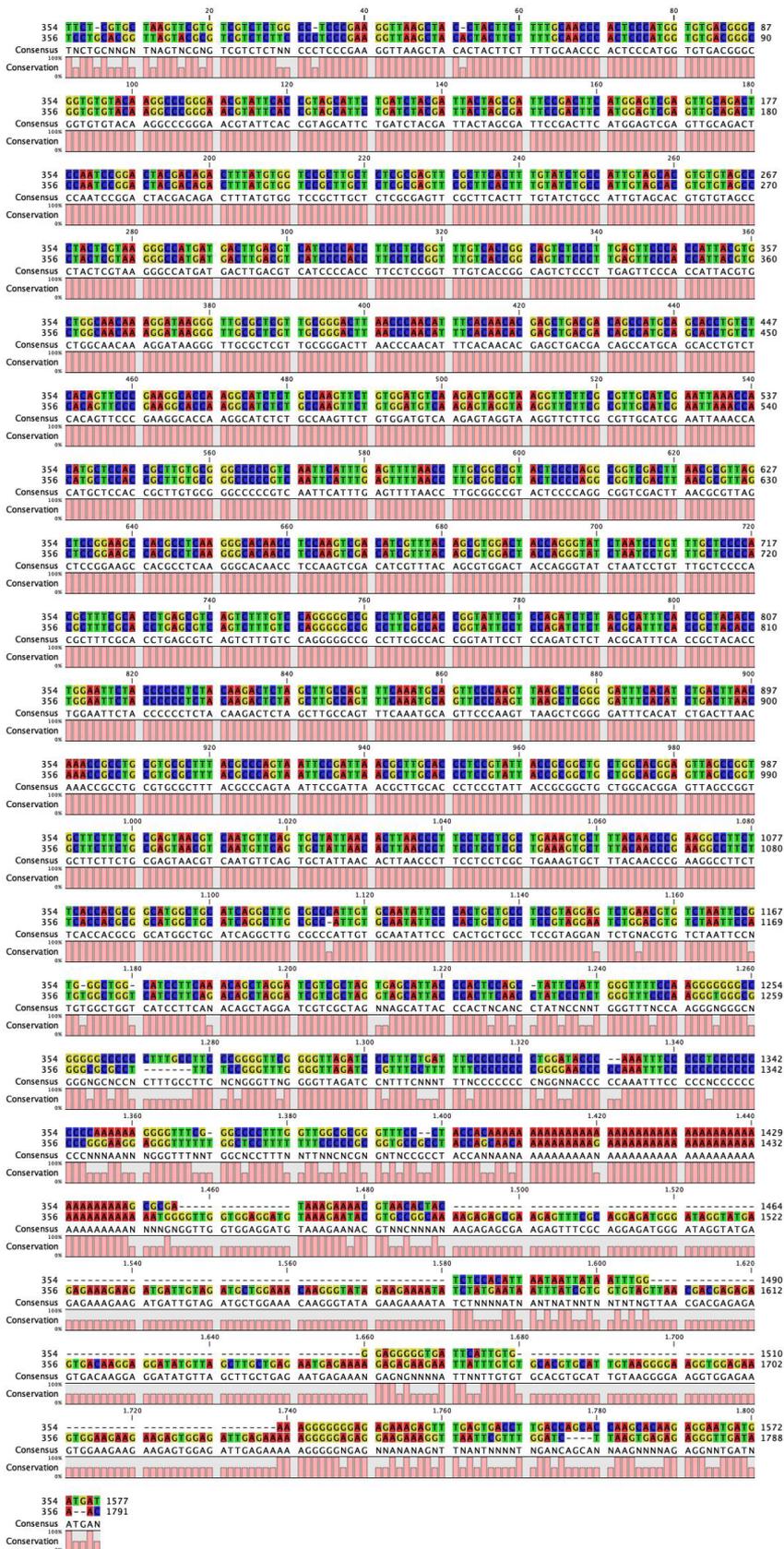
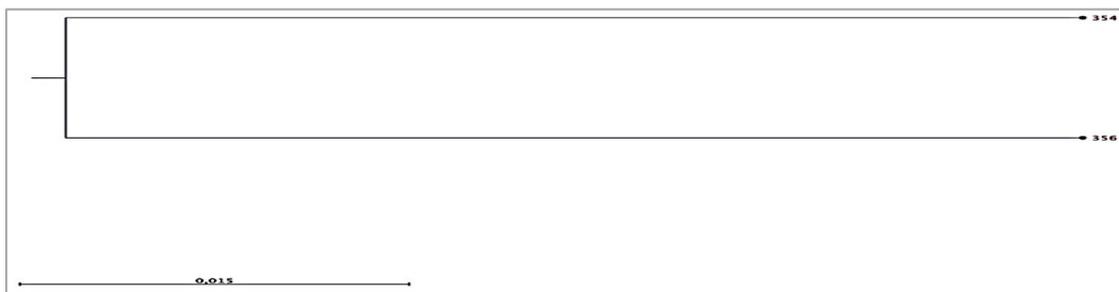
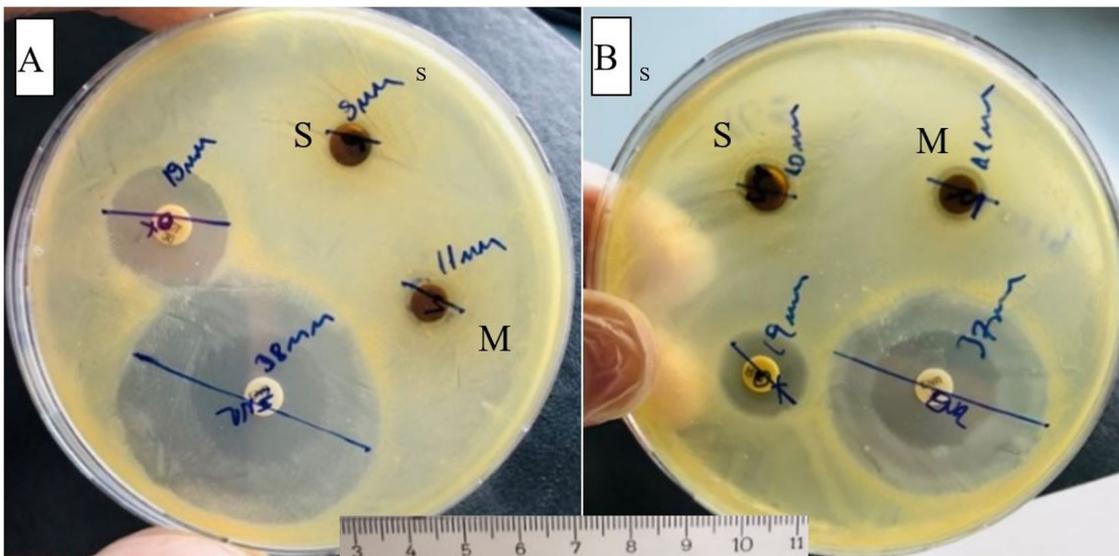


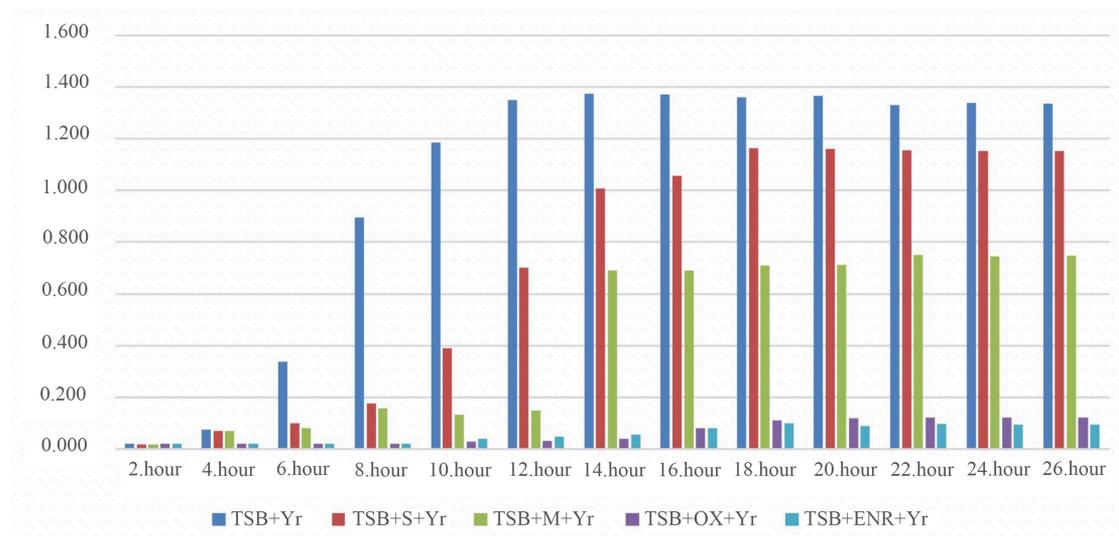
Figure 3. 16S rRNA gene sequences of *Y. ruckeri* isolates used in the study (354: *Y. ruckeri* reference strain, 356: *Y. ruckeri* isolate).



**Figure 4.** Phylogenetic similarity results of *Y. ruckeri* isolates used in the study (354: *Y. Ruckeri* reference strain, 356: *Y. ruckeri* isolate).



**Figure 5.** Antibiogram test results of antibiotics and phytochemicals used in the study (A) *Y. ruckeri* reference strain; (B) *Y. ruckeri* isolate, M: *Moringa oleifera*, S: *Sorbus domestica*).



**Figure 6.** Spectrophotometric development rates of *Y. ruckeri* isolates in antibiotic and phytochemical supplemented medium (“TSB+Yr”: Tryptic Soy Broth+*Y. ruckeri*, “TSB+S+Yr”: Tryptic Soy Broth+Sorbus+*Y. ruckeri*, “TSB+M+Yr”: Tryptic Soy Broth+Moringa+*Y. ruckeri*, “TSB+OX+Yr”: Tryptic Soy Broth+Oxytetracycline+*Y. ruckeri*, “TSB+ENR+Yr”: Tryptic Soy Broth+Enrofloxacin+*Y. ruckeri*).

animal and aquaculture production, increase in growth, positive results in feed evaluation and use as preventive products (Görmez, 2012). In addition to being easier to obtain and inexpensive, herbal products are preferred because some plants act against bacterial, viral, fungal and parasitic agents (Cihangir and Diler, 2016).

*M. oleifera* (Moringaceae) is a very valuable plant, distributed in many tropical and subtropical countries. It has a high nutritional value and an impressive range of medical uses. Different parts of this plant contain a profile of important minerals and are good sources of protein, vitamins, carotene, amino acids and various phenolic. In addition to its water treatment forces and high nutritional value, *M. oleifera* is very important for its medicinal value. Various parts of this plant, such as leaves, roots, seeds, bark, fruit, flowers and immature bark, serve as stimulating the heart and circulation. It has anti-diabetic, hepatoprotective, anti-bacterial and anti-fungal activities and is used especially for the treatment of different diseases in the native drug system in South Asia (Anwar et al., 2007). Medicinal plants and the essential oils obtained from them have anti-bacterial, anti-viral, anti-fungal, anti-inflammatory, antiseptic, antioxidant, anti-parasitic, anti-toxicogenic and insecticidal properties have been reported to be effective on microorganisms resistant to antibiotics in the studies (Yigitarslan et al., 2011).

*Y. ruckeri* isolates used in this study were used in microbiological tests; In terms of gram staining, *Y. ruckeri* isolate was found to be Gram negative in the form of red-pink bacteria seen on microscope as a result of exposure to crystal violet, lugol alcohol and saffron. Similarly, some researchers have stated that Gram staining results are negative in their studies (Kubilay, 1997; Okka, 2009). In this study, oxidase reagent kit was poured onto sterile filter paper which was placed in a Petri box to determine the oxidase properties of the bacteria. Many studies conducted with *Y. ruckeri* have reported the same results (Tiravoglu-Demirtas, 2006). The catalase tests of the isolates used in the study were carried out by adding a colony with the aid of hydrogen peroxide which was poured a few drops on the slide. Foam catalase formed due to O<sub>2</sub> deficit from the presence of catalase was observed as positive. It was reported by some researchers that the catalase test was performed with the same method and that the same results were obtained on *Y. ruckeri* (Korun et al., 2019). To determine the motility of the isolates, *Y. ruckeri* isolate which was applied on a drop of PBS dropping slide was examined under a microscope and the bacteria showed Glayding movement. Similarly, some researchers reported that they obtained similar results in their studies on the same factor (Koyun and Şeker, 2017). GNA+B (Microgen) kits were used to evaluate the isolates for biochemical tests. *Y. ruckeri* isolates in suspension were incubated at 37 °C for 24 hours after inoculating the wells in the kit and then the results were evaluated as positive and negative according to the Microgen company reading table. The results were matched with Microgen web site program and it was concluded that *Y. ruckeri* was 99.85% in terms

of biochemical identification. Biochemical properties and differences between bacteria are of great importance in microbiological studies. In-species differences of bacteria cause different results in biochemical reactions of the same bacteria (Önalın, 2019).

In the application of biochemical tests, many researchers have obtained different kits and methods. Some researchers have performed biochemical tests with API kits (Santos et al., 1993), some researchers from automated systems BD Phoenix ID (Muraleedharan et al., 2019) and some researchers VITEK (Arias et al., 2007; Crowley et al., 2012) reported that they investigated the biochemical properties of *Y. ruckeri* isolates by automated system. Recently, it has been reported by some researchers that the identification of *Y. ruckeri* with MALDI\_TOF\_MS, which is a new and widespread method in bacterial identification, is very high (Popovic et al., 2017). In addition to the many studies that carried out biochemical tests with different methods, many studies have also been reported by *Y. ruckeri* biochemical properties and differences using GNA+B (Microgen) kits used in this study and performed by manual system (Lutwyche et al., 1995; Gulaydin et al., 2018).

Although, morphological and biochemical tests can be used to identify and characterize bacteria, nowadays, they are the most sensitive method. These studies performed on DNA and RNA interactions and sequences are frequently used in microbiological studies to reveal identification differences. In this study, Real-Time PCR analysis was performed for the molecular identification of bacterial isolates. Universal primers (27F-1492R) specific to the 16S rRNA gene, which are frequently used for identification in bacteria, were used for this purpose. PCR amplicons were confirmed from the gene bank after sequencing. Although, it has been reported by some researchers that the 16S rRNA gene is used for the identification of bacteria (Altun et al., 2013), some researchers used ompTS, gyrA, Yer, n-DNA and p57 gene in the PCR method for the test of molecular identification (Bastardo et al., 2011; Altun et al., 2013).

Comparisons of the phytochemicals used in the study with antibiotic discs were made with the antibiogram test. The antibiogram test of ENR and OX antibiotics showed a zone diameter of 24 mm in MHA medium. In the Antibiotic Zone Diameter Table, this value was found to be resistant, while *M. oleifera* was 11 mm and *S. domestica* was 11 mm. With these values, *M. oleifera* and *S. domestica* (11, 9, 8 mm) were found to be sensitive to ENR and OX. In line with these results, antibiotics used were resistant to *Y. ruckeri*. It was observed that *M. oleifera* and *S. domestica* extracts were sensitive to antibiotics, when considering the dilution of liquid solution and concentration ratio 10 times, it was observed that the plant extracts to be prepared in the same manner as the antibiotic powder forms could have the same effect.

As a result of the spectrophotometric measurements evaluating the bacterial growth in the liquid medium, the control group showed the highest bacterial growth at 600 nm in the first 2 hour measurements while the effect of OX and ENR antibiotics levels did not begin at

the beginning of the control group. Bacterial growth was found to be slower in Sorbus and Moringa groups. Bacterial growth was highest in the TSB + Yr (*Yr: Y. ruckeri*) group at the 14th and 16th hours, and the highest levels were observed at the 18th and 20th hours in the OX and ENR antibiotics groups. In previous studies with *Y. ruckeri*, OX and ENR were reported to be resistant to bacteria in the antibiogram test (Akhlaghi and Sharifi-Yazdi, 2008; Bastardo et al., 2011). Because of their potential to be an alternative to chemical preservatives and antibiotics against fish pathogens, many researches have been conducted on the antibacterial effects of plant essential oils (Chiarello, 1995; Metin et al., 2017). In recent years, inadequate drugs and therapeutically effective substances, which are synthetic against bacterial disease agents, have led to studies on the use of different treatment methods (Toroglu and Çenet, 2006). In previous studies, it has been reported that sulfonamides and OXs are resistant to *Y. ruckeri* (Post, 1987). It was observed that the effects of *M. oleifera* and *S. domestica* extracts against the bacteria in the liquid medium (spectrophotometric test) were higher than the effects on the solid medium (antibiogram test). In themselves, Moringa extract was found to be more effective than Sorbus extract. In the antibiogram test, it was observed that the phytochemicals, which are susceptible, affect the growth of bacteria, especially Moringa, at a rate of 50%.

As a result, it was observed that the isolates formed Gram negative, catalase positive, oxidase negative, mobile and typical *Y. ruckeri* colonies. After the biochemical tests performed with Microgen ID panel, 99.85% similarity was determined. The isolates were 100% overlapping with the 16S rRNA gene region after sequence analysis, and 99% of the isolates were similar in phylogenetic analysis. After the antibiogram test, it was observed that ENR and OX antibiotics were resistant to *Y. ruckeri*, but the effects of phytochemicals were less on solid medium. As a result of measurements carried out spectrophotometrically to evaluate the effects of bacterial growth on TSB medium, phytochemicals inhibited the growth of bacteria by 40-50%. As the importance of antibiotic resistance is increasing day by day, we believe that the plants that are more economical and alternative to antibiotic use will give positive results in the treatment.

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