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Phenotypic Differences between *Lactococcus garvieae* Isolates Obtained from Rainbow Trout Farms in Turkey

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ABSTRACT

Lactococcus garvieae is one of the main pathogenic agents in rainbow trout farms in Turkey. Twenty-two *L. garvieae* isolates obtained from different regions in Turkey were evaluated phenotypically in the study. In all isolates, cream colored, bright, round and S-type colonies with smooth margins were seen in TSA medium. They were alive during native examination without movement. It was observed that morphologically all isolates were Gram (+), α-hemolytic (BA), oxidase and catalase negative and were reproduced under 21, 37 and 45 °C temperatures with 0–6.5 % NaCl salinity. As a result of the examination of biochemical properties with API Rapid ID 32 Strep test, it was observed that 2 *L. garvieae* isolates were different from other isolates in respect of sucrose utilization. 1 and 19 number isolates were negative for sucrose whereas other isolates gave positive results. 1 number isolate was different from other isolates based on maltose profile. While isolate 22 was maltose negative, the other isolates gave positive results. According to phenotypic differences, all isolates used in the study were classified as three different groups.

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ŞÖ did laboratory and statistical analysis and wrote the manuscript. MA revised the article.HÇ did Lab work and provided isolates.

Key words
Fish diseases, *Lactococcus garvieae*, Phenotyping, Rainbow trout, Fish farm

INTRODUCTION

The rainbow trout, which is the most cultured fish in the world, is preferred due to the shortness of incubation time, easy adaptation to environmental conditions, high ability to benefit from natural and artificial feed and resistance against diseases compared to other salmonid fishes (Edwards, 1978; Voorhees *et al*., 2019). In the rainbow trouts of North America, rootstock and business management, certification, hatchery, diseases and marketing are the main problems that challenge the culturing (Arabacı, 2007). Bacterial infections have an important place in fish diseases and Gram-positive cocci have been defined as important fish pathogens in the last decade. Several epidemic and sporadic cases of Gram-positive pathogens have been reported in various parts of the world (Arda *et al*., 2002). As in all the world, one of the main pathogenic factors frequently seen in Turkey in rainbow trout farms is *Lactococcus garvieae* (Çağırgan, 2009).

L. garvieae, the causative agent of lactococcosis infection, was first isolated in 1960 from yellowtail (*Seriola quinqueradiata*) fish (Kusuda *et al*., 1991) and the disease caused by it is called lactococcosis (Austin and Austin, 1999). In Turkey, the disease caused by these bacteria was seen for the first time in September 1992 in a small family business in Karacasu, Aydın, the same year five different epizooties were observed in the farm (Çağırgan, 2007). As of 2008, lactococcosis infection has spread to many regions in Turkey (Türe and Altınok, 2012). It is a septicemic disease that causes economic losses in many fish species especially in rainbow trout when water temperature reaches 15 °C in summer (Diler *et al*., 2002; Çağırgan, 2004). Sub-classification of the lactococcosis-agent *L. garvieae* can be performed separately by phenotypic, serotypic and genotypic techniques, and the differences should be evaluated comparatively to bring together the differences. The lack of information on the different subtypes of the agent reduces the efficacy of vaccines against the disease (Türe and Altınok, 2012). In the present study, the similarities and differences between the biochemical and phenotypic properties were examined in 22 *L. garvieae* isolates collected from different regions of Turkey at different times.

MATERIALS AND METHODS

This study was carried out with the permission of the Local Ethics Committee of Animal Experiments of Van Yüzüncü Yıl University on 18.11.2013 and with permission No. 27552122-341. The Study was carried out with a total of 22 isolates including 4 rainbow trouts isolated from farms located in Van, Bitlis, Muş and Hakkari and 18 *L. garvieae* isolates collected from different regions in Turkey.

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The rainbow trout samples were brought to the laboratory at 4 ºC on the same day and necropsy was performed under aseptic conditions. Inoculations made from kidney tissues of fish samples were incubated in TSA medium under aerobic conditions at 21°C for 24 h. The study was carried out with the bacteria that match the colony morphology of *L. garvieae* following the incubation period.

Gram staining of the isolates, a drop of 0.6% FTS was added on the slides from pure bacteria which were produced as a result of the 24-48-h incubation at 21 °C in TSA medium. Subsequently, the procedure was completed by treatment with crystal violet for 1 min, glycol for 1 min, alcohol for 15 sec and safranin for 1 min. Preparations were left to dry and then examined with binocular microscope (Leica ICC50 HD) with 4X, 10X, 40X and 100X magnifications, respectively. Following Gram staining, the isolates that were blue-violet color were evaluated as Gr (+) while red-pink color isolates were evaluated as Gr (-) (Beşe, 1974).

Bactident Oxidase commercial kit was used for the oxidase test. After 24-48 h-incubation period at 21°C in TSA medium, samples from the formed colonies was taken using a sterile loop and spread on sterile filter paper was wetted with oxidase kit. The color changes on the paper surface within 30 sec were examined. Blue color formation was evaluated as positive, and no color formation was considered as oxidase negative (Çağırgan, 2007).

For the catalase test, 1-2 drops of 3% hydrogen peroxide was added to the slides and mixed with the sample taken using a sterile loop from the colonies grown on TSA medium at 21 °C for 24-48 h after incubation. The gas-forming isolates were determined as catalase-positive and the isolates that did not show any gas formation were evaluated as catalase negative (Austin and Austin, 1999).

As a result of the incubation of isolates at 21 °C for 24-48 h, the pure bacterial isolates were inoculated in sheep blood agar for hemolysis test. After the incubation period, bacteria were examined for their hemolytic properties. Hemolysis types were determined according to the green zone areas formed around the bacteria growing in the medium (Türe, 2011).

Tolerance to different salinity ratios were tested to determine the physiological characteristics of the isolates. Accordingly, sterile TSA media (pH: 7.4) containing 4% and 6.5% NaCl were prepared and incubated at 21 °C for 24-48 h (Beşe, 1974).

The bacteria that were inoculated in Petri dishes containing TSA medium prepared under sterile conditions were enumerated to determine their reproductive ability as a result of incubation at 21 °C, 37 °C and 45 °C for 24 h for temperature tolerance test (Kurtoğlu and Korun, 2018).

To determine the biochemical properties of isolates, API Rapid ID 32 Strep (Biomerieux, France) test was adopted. Pure *L. garvieae* isolates obtained in TSA medium were incubated in BA medium at 24 °C for 24 h and the colonies taken by the swap method and bacteria samples were added into tubes containing 2 mL sterile water until McFarland 4 turbidity was obtained. The suspension solution was prepared for immediate use in the ID 32 strep assay. Bacteria suspension was incubated by adding 55 ul to each well. The results were evaluated in accordance with the manufacturer's specifications.

The positive and negative data of all reagents in the API kit, in which the biochemical differences were determined, were evaluated using the SPSS 20 (IBM, Inc) package program as binomial data.

RESULTS AND DISCUSSION

It was seen that 24 of 54 rainbow trout farms that were visited for sampling were closed for various reasons in the summer of 2014 (June-July-August-September). The production capacity of the 30 rainbow trout farms was between 1 and 500 tons. During the summer months, the temperature during the sample collection procedure was between 19 °C and 38 °C, while the water temperature in rainbow trout farms where the samplings were carried out was between 13 °C and 24 °C. In the sampled fish, ascites, darkening of skin and exophthalmos were observed in fish. Four *L. garvieae* isolates from these fish farms were obtained. Also, 18 *L. garvieae* isolates were included in study from Turkey's various regions. This study was carried out with 22 isolates. Gram staining revealed that all isolates were coccus-shaped Gram positive. As a result of salinity tolerance tests, 22 isolates were able to grow in 4% and 6.5% salinity ratios in TSA medium.

To determine the phenotypic differences of isolates, API Rapid ID 32 Strep rapid diagnostic test was evaluated according to the evaluation table of the manufacturer (Biomerioux). As a result of API Rapid ID 32 Strep evaluation table, isolates 1 and 19 gave negative results in contrast to other isolates in terms of sucrose (red color; negative), other phenotypic features showed similar profiles with all isolates. The isolate 22 was separated from the other isolates (positive) by giving the maltose profile red-orange color (negative). Differences were observed in the phenotypic profiles of *L. garvieae* isolates obtained as a result of the API test. A total of three different phenotypic profiles were formed including API profiles "30321111020" for isolate 1 and isolate 19, API profile "30321110120" for Isolate 20, and API profiles for other isolates "30321111120" (Table I).

Table I. Biochemical properties and differences of *L.garvieae* isolates with API rapid ID 32 strep.

		Fenotip I	Fenotip III																	Fenotip I	Fenotip III		Fenotip II
Testin Kodu	**İzolat Adı / Testin Adı**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
1.0	ADH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.1	BGLU	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.2	BGAR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.3	BGUR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.4	aGAL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.5	PAL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.6	RIB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.7	MAN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.8	SOR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.9	LAC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.A	TRE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.B	RAF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.C	SAC	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
		🔴											🟡							🔴			
1.D	LARA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.E	DARL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.F	CDEX	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.0	VP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.1	APPA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.2	bGAL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.3	PyrA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.4	bNAG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	GTA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.6	HIP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.7	GLYG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.8	PUL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.9	MAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
		🟡																					🔴
0.A	MEL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.B	MLZ	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.C	M8DG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.D	TAG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.E	bMAN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.F	URE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*Red, negative results; Yellow, positive results; these results are differences between different *L. garvieae* isolates.

In the present study, to isolate *L. garvieae* strains, inoculations were carried out from kidney tissues. Previous studies have also reported that they used TSA for isolation from kidney tissue samples in their studies on lactococcosis (Holt *et al*., 1994; Toranzo *et al*., 1994; Eldar *et al*., 1996, 1999; Buller, 2004; Cagirgan, 2004;

Vendrell *et al*., 2006). Some researchers have reported that they used BHIA, biliary agar (BA) and biliary eculin agar (BEA) media together with TSA for the isolation of *L. garvieae* (Türe, 2011; Ürkü, 2011). However, Ürkü (2011), in her study, examined the histopathological findings of spleen and liver tissues as well as kidney tissue and reported the same results on BHIA and TSA media. All of the *L. garvieae* strains were found to have a cream-colored, round shaped and S-type colony structure in terms of colony morphologies. It was determined that the results had characteristics of *L. garvieae* in terms of morphological properties. In parallel with the results of the present study, the previous researchers have reported that *L. garvieae* strains obtained from rainbow trout have cream-colored, round and S-type colony structure in TSA (Buller, 2004; Cagirgan, 2004; Ürkü, 2011; Vendrell *et al*., 2006; Kurtoğlu and Korun, 2018). All *L. garvieae* strains were found to be α-hemolytic in 5% sheep blood agar. In line with the results of the present study, previous studies have reported that *L. garvieae* strains obtained from rainbow trout had α-hemolytic structure (Buller, 2004; Cagirgan, 2004; Vendrell *et al*., 2006; Tanrıkul and Gültepe, 2011). Çağırgan (2007) have reported that some of the *L. garvieae* strains have α-hemolitik structure while some have non-hemolytic structure. The α-hemolytic structure of *L. garvieae* strains used in this study suggests that these strains may be more pathogenic. Some researchers described *L. garvieae* strains as β-hemolytic (Teixeira *et al*., 1996). Teixera *et al*. (1996) have reported different hemolytic properties in *L. garvieae* strains. This can be associated with different isolation sources (buffalo) of *L. garvieae* isolates. However, the differences between the methods used in the hemolysis test of streptococci are important. All of the *L. garvieae* strains used in the study showed salinity tolerance test in the salinity ratios of 4% and 6.5% NaCl in TSA.

Previous researchers have also reported that *L. garvieae* strains obtained from rainbow trout showed growth in media containing 4% or 6.5% NaCl (Eldar *et al*., 1999; Vela *et al*., 2000; Buller, 2004; Kawanishi *et al*., 2005; Vendrell *et al*., 2006; Kurtoğlu and Korun, 2018). As a result of the study, the salinity tolerance rates of *L. garvieae* strains were found to be similar with those previously reported. All of the *L. garvieae* strains obtained in the study were observed could grow in TSA at temperatures 21, 37 and 45 °C. The strains exhibited a lesser growth at 37 °C and 45 °C in TSA compared to that at 21 °C. The optimum temperature for the API (Biomerioux) test is reported in the package insert at 37 °C. Previous researchers have shown that *L. garvieae* strains show growth at 21 °C, 37 °C and 45 °C, in parallel with the results of the present study (Kusuda *et al*., 1991; Prieta *et al*., 1993; Eldar *et al*., 1996; Fortina *et al*., 2007) whereas some researchers have reported that *L. garvieae* strains showed poor growth at 45 °C (Teixeira *et al*., 1996). To determine the phenotypic properties of *L. garvieae* strains, API Rapid ID 32 Strep test was used by many researchers (Eldar *et al*., 1999; Vela *et al*., 2000; Baeck *et al*., 2006; Altun *et al*., 2007; Türe and Altınok, 2012; Altun *et al*., 2013). Following the API Rapid ID 32 Strep test to determine biochemical properties and differences between isolates, different phenotypic profiles were formed due to the reaction of sucrose (SAC) and maltose (MAL) reagents. Considering these differences, *L. garvieae* strains were found to form three different phenotypic groups. These groups were evaluated as those using sucrose (+) and not using maltose (-) phenotype-1, non-sucrose (-) and maltose (+) phenotype-2 and phenotype-3 using both reagents. In parallel with the results of the present study, Eldar *et al*. (1999) reported that *L. garvieae* strains obtained from rainbow trout showed differences in terms of tagatose (TAG) and sucrose (SAC) tests as a result of API Rapid ID 32 Strep test. Eldar *et al*. (1999) have grouped the strains obtained from rainbow trout farms in Italy and ATCC reference strains as phenotype-1 (TAG: -, SAC: -), the only strain from the rainbow trout farm in Australia as phenotype-2 (TAG: +, SAC: -), and strains obtained from rainbow trout farms in Spain (TAG: +, SAC: +) as phenotype-3. The phenotype-3 group obtained in this study was similar to phenotype-3 group reported by Eldar *et al*. (1999), while phenotype-2 group was similar to phenotype-2 group. Eldar *et al*. (1999), similar to the results obtained in the present study, have reported that they obtained three phenotypic groups, however only two groups (phenotype-2 and phenotype-3) were found to have phenotypically common properties between the two studies. This difference can be associated with collecting strains from different locations and the difference between the number of strains studied. Çağırgan (2004) performed API CH 50 test at 24 °C to determine phenotypic differences between 20 isolated *L. garvieae* strains from rainbow trout farms located in various regions of in Turkey. Çağırgan (2004) have reported that phenotypic differences in *L. garvieae* strains in terms of acid formation from lactose and, as a result of these differences, *L. garvieae* strains produced 2 different phenotypic profiles. Çağırgan (2004) compared the results they obtained to those by Eldar *et al*. (1999) and reported that phenotype-2 group was phenotypically similar to Spanish strains. The phenotype-2 obtained by Çağırgan (2004) and the phenotype-3 group obtained from the present study were similar, therefore the phenotype-3 group obtained in the present study was similar to the Spanish strains. Altun *et al*. (2004) collected 4 *L. garvieae* strains from rainbow trout farms in Turkey,

2 from rainbow trout farms in Spain, 1 from rainbow trout farms in England and 1 reference strain (ATCC 43921), a total of 8 strains, and determined their phenotypic differences with API Rapid ID 32 Strep test at 37 °C. Altun *et al*. (2004) reported that mannitol (MAN), tagatose (TAG) and sucrose (SAC) results were similar and positive in all strains after phenotypic examinations. Evaluating the phenotypic results, it was seen that phenotypic properties of strains obtained from rainbow trout by Altun *et al*. (2004) were similar to the phenotype-3 group obtained in the present study. Türe (2011) phenotypically examined a total of 41 *L. garvieae* strains, 3 obtained from rainbow trout, Black Sea trout and sea bass samples from Turkey, and 38 obtained by different researchers Italy, France and Japan. The phenotypic differences between the *L. garvieae* strains were investigated at 30 °C using API 20 Strep kit. As a result of the API 20 Strep test, differences were reported in terms of Voges Proskauer (VP), hippurate (HIP), pyroglutamic acid arylamidase (PyrA), α-galactosidase (α–GAL), ß-galactosidase (b-GAL), arginine dhidrolase (ADH), ribose (RIB), mannitol (MAN), lactose (LAC) and trehalose (TRE) reagents. Türe (2011) have reported that *L. garvieae* strains formed 8 different phenotypic groups. The phenotype-2 obtained by Türe (2011) and the phenotype-3 group obtained in the present study had similar properties. Differences in the phenotypic groups between those reported by Türe (2011) and the present study was associated with the different types of API kits used and *L. garvieae* strains isolated from different sources (rainbow trout, sea bass and Black sea trout). Türe (2011) used API 20 Strep tests at 30 °C incubation temperature whereas, API Rapid ID 32 Strep tests was used at 37 °C incubation temperature in the present study. Vela *et al*. (2000) phenotypically examined *L. garvieae* strains they obtained from rainbow trout in Portugal, France and Italy, from buffalo and humans in Brazil, from humans in the USA, from yellowtail fish in Japan, and from cattle and rainbow trout in Spain. Unlike the present study, Vela *et al*. (2000) used API Rapid ID 32 Strep at 30 °C, and reported that there were differences between the *L. garvieae* strains in terms of sucrose (SAC), tagatose (TAG), mannitol (MAN) use and cyclodextrin (CDEX), pyroglutamic acid arylamidase (PyrA) and N-acetyl-β-glucosaminidase (βNAG) reagents. As a result of these differences, it was reported that *L. garvieae* strains formed 13 phenotypic groups. The phenotype-I group obtained from rainbow trout by Vela *et al*. (2000) were similar to phenotype-1 and phenotype-3 groups obtained in the present, furthermore phenotype-3 group obtained by the researchers were similar to phenotype-2 group obtained in the present study. The other 11 phenotypic groups obtained by Vela *et al*. (2000) were not similar to those obtained in the present study. This difference was associated with the fact that Vela *et al*. (2000) isolated *L. garvieae* strains that form 13 different phenotypes from different sources (water, human, cattle, fish and buffalo) and used API Rapid ID 32 Strep tests at 30 °C. In the present study, *L. garvieae* strains were isolated only from rainbow trout and incubated in API Rapid ID 32 Strep test at 37 °C.

Altun *et al*. (2013) identified the biochemical properties of *L. garvieae* strains with API Rapid ID 32 Strep, similar to the method used in the present study. In terms of phenotypic features the researchers have reported that they obtained positive results from β-glucosidase (b-GUL), ribose (RIB), sorbitol (SOR), lactose (LAC), raffinose (RAF), Voges Proskauer (VP), alanyl-phenylalanyl-proline arylamidase (APPA), pyroglutamic acid arylamidase (PyrA), hippurate (HIP) and urease (URE) reagents. Although the sucrose (SAC) and mannitol (MAN) reagent results obtained by Altun *et al*. (2013) as a result of API Rapid ID 32 Strep test was similar to the results of the study, no similar groups were determined in terms of phenotypic groups. API kits used in the present study and by Altun *et al*. (2013) and were the same, however different incubation temperatures may be the reason why different phenotypic groups were obtained. Altun *et al*. (2007) incubated API Rapid ID 32 strep test at 37 °C and reported similar groups with phenotypic groups obtained in the present study. However, the groups obtained by Altun *et al*. (2013) by using the API Rapid ID 32 strep test at 35 °C were not similar to those obtained in the present study. Detailed research is needed to clarify the reasons for these differences. In this study, similar groups were observed between phenotypic groups formed by *L. garvieae* strains in the present study and the previous studies. It was found that phenotype-2 group obtained by Eldar *et al*. (1999) from rainbow trout was similar to phenotype-2 obtained in the present study while phenotype-3 group obtained by Eldar *et al*. (1999) also from rainbow trout was similar to phenotype-3 group obtained in the present study. The phenotype-I group obtained from rainbow trout by Vela *et al*. (2000) were similar to phenotype-1 and phenotype-3 groups obtained in the present study. Furthermore, phenotype-3 group obtained by the researchers were similar to phenotype-2 group obtained in the present study. It was seen that phenotype-1 group of strains obtained from rainbow trouts by Altun *et al*. (2007) were similar to the phenotype-3 group obtained in the present study. phenotype-2 group obtained from rainbow trout by Çağırgan (2004) was similar to the phenotype-3 group obtained in the present study. The phenotype-2 group obtained from *L. garvieae* strains by Türe (2011) was similar to the phenotype-3 group obtained in the present study. Comparing the phenotypic test results

obtained in the present study with those obtained in the previous studies, the differences can be associated with different sources (human, bovine, buffalo, cat, chicken, water, Black sea trout and sea bass) and the application of tests used to characterize the biochemical characteristics of strains under different environmental conditions (incubation temperature). Studies on the effects of different environmental conditions on results are needed. It is thought that evaluating all of the *L. garvieae* strains obtained by the previous studies together and under same conditions will yield better results in the determination of the phenotypic differences in *L. garvieae* strains.

The results of the present study showed that *L. garvieae* is one of the main pathogenic agents in rainbow trout farms in Turkey. Twenty two *L. garvieae* isolates obtained from different regions in Turkey were evaluated phenotypically in the study. In all isolates, cream colored, bright, round and S-type colonies with smooth margins were seen in TSA medium. They were alive during native examination without movement. It was observed that morphologically all isolates were Gram (+), α-hemolytic (BA), oxidase and catalase negative and were reproduced under 21, 37 and 45 °C temperatures with 0–6.5 % NaCl salinity. As a result of the examination of biochemical properties with API Rapid ID 32 Strep test, it was observed that 2 *L. garvieae* isolates were different from other isolates in respect of sucrose utilization. One and 19 number isolates were negative for sucrose whereas other isolates gave positive results. 1 *L. garvieae* isolate was different from other isolates based on maltose profile. While isolate 22 was maltose negative, the other isolates gave positive results. According to phenotypic differences, all isolates used in the study were classified as 3 different groups (Table II).

Table II. Phenotypic groups of *L. garvieae* isolates and their properties.

	SAC	MAL	Isolate number
Phenotype-1	-	+	1, 19
Phenotype -2	+	-	22
Phenotype -3	+	+	Other 19 isolates

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Statement of conflict of interest

The authors declare there is no conflict of interest.

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