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Determination Biochemical Differences and Rapid Identification of *Yersinia ruckeri* and *Lactococcus garvieae* Isolated from Aquaculture

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Abstract

Aquaculture has growing much more than 100% at the last decade. Rainbow trout is the most economically important fish among the aquatic species. Bacterial agent, especially, *Yersinia ruckeri* and *Lactococcus garvieae* have limited freshwater fish production all over the world. There are many reports about identification of bacterial pathogens in aquaculture but most of them should not supported with detailed biochemical features. Therefore, we aimed in this study, determination of biochemical differences and identify two bacteria in a short time by using rapid test kits. 30 isolates of each *Y. ruckeri* and *L. garvieae* that are represent different regions of Turkey were used and biochemical characteristics were determined by conventional microbiologic test and API RAPID test kits. *Y. ruckeri* is the more heterogenetic biochemical structure comparing to *L. garvieae*. All isolates were successfully identified within 5-7 hours with rapid test kits while there are some differences between each other. The comprehensive biochemical differences of *Y. ruckeri* and *L. garvieae* were shown in the present study.

Keywords: *Yersinia ruckeri, Lactococcus garvieae*, API RAPID Test Kits, biochemical differences

Introduction

Y. ruckeri, *L. garvieae* and motile Aeromonads are the infectious disease that causes outbreaks and cumulative mortalities in rainbow trout farming and other aquatic species. Into these bacterial species, *Y. ruckeri* was first isolated from diseased rainbow trout cultured in Mediterranean Sea region (Timur and Timur, 1991). The infectious agent was identified in Malatya, Elazığ, Bursa and Yalova and outbreaks were reported in the other countries (Savas and Ture, 2007; Ozer et al., 2008; Altun et al., 2010; Seker et al., 2012). The recovering of agent from different region revealed that *Y. ruckeri* has a high spreading potentially between aquaculture.

Y. ruckeri isolates were reported as biochemically heterogenic according to the different isolation source and also determined as four different group by outer membrane protein profiles and isolates have 86,03% similarities between each other (Altınok et al., 2016; Duman et al., 2017a). Altun et al., (2013a) reported that there were many biochemical differences, and also 17 *Y. ruckeri* isolates were diverged as five genotype by working on phenotypic and biochemical studies. The researcher found that aquaculture farms were infected as 52,9% with *Y. ruckeri* or fish found as carrier for this agent (Şeker et al., 2012). The reports showed highest prevalence of *Y. ruckeri* infection in Turkey. In addition, *Y. ruckeri* diverged as 18 genotype by further molecular phylogenies working on Turkey more recently (Duman et al., 2017a). Beside of phylogenetic studies, sorbitol was detected mainly biochemical feature

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showing differences in addition to other enzyme and carbon utilizing (Wortberget et al., 2012; Altun et al., 2013).
Comparing to *Y. ruckeri*, when water temperature as higher than 15°C, L. garvieae was recovered from aquaculture as primarily agent of mortalities. *L. garvieae* most important disease that could affect in humans causing primarily endocarditis and also urinary, circulatory, skin and respiratory disease in immunosuppressive individuals (Elliot et al., 1991; Fefer et al., 1998; James et al., 2000; Mofredj et al., 2000; Fihman et al., 2006). *L. garvieae* was reported as an important zoonotic agent of in cheese, milk, raw product and vegetables (Foschino et al., 2006; Kawanishi et al., 2007; Fernandez et al., 2010). After the first isolation of L. garvieae from Europe, the agent emerged different country from America, Italy, Australia, South Africa, and Taiwan to Turkey (Ghittino and Prearo, 1992; Carson et al., 1993; Diler et al., 2002; Duman et al. 2017b).
In our country, *L. garvieae* was first isolated from Aegean region by Diler et al. (2002). Infection spread to other regions of Turkey and now it causes high economic losses because of *Lactococcosis* has found in almost each region of Turkey (Kubilay et al., 2005; Savaş and Ture, 2007; Ozer et al., 2008; Timur et al., 2011; Altun et al., 2013b).Fernandez et al., (2010) studied on *L. garvieae* which isolated from raw milk cheese identification by biochemically methods and reported that while there were a little differences as biochemically, isolates have high homogeneity. Vendrell et al., (2006) reported a detailed review on *L. garvieae*, β–Glucuronidase, Ribose and Sucrose were the most detected heterogenesis in biochemical test, *L. garvieae* is successfully identified by rapid test kits like as API Rapid 32 Strept and VITEK automatize systems (Russo et al., 2012; Altun et al., 2013a).

Material and Methods

Bacteria Isolates
In the present study, 30 isolates of each *Y. ruckeri* and *L. garvieae* were used which represent from different regions of Turkey between 2013 and 2017. Molecularly characterized isolates were identified in our previous papers and isolate information were given in Duman et al., 2017a and Duman et al., 2017b.

Phenotypic isolation
Previously identified isolates by molecular methods, were characterized according to gram staining, motility, oxidase, catalase, glucose fermentation in aerobic and anaerobic media (O/F), growth on MacConkey medium (MacConkey), hemolysis on blood agar (added 5% sheep blood, BA), Tryptic Soy Agar (TSA), Brain Heart Infusion Agar (BHIA) and Nutrient Agar (NA) (for *Y. ruckeri* isolates). The API RAPID ID 32E test strips for *Y. ruckeri* and API ID 32 STREPT for *L. garvieae* were used in this study (BioMerieux, France) according to the manufacturer instruction with some modification. We used 30°C and 5-7 hour for API RAPID ID 32E test strips and API ID 32 STREPT differently from manufacturer instruction (Abdel-Latif et al., 2014; Verner-Jeffreys et al., 2009; Ravelo et al., 2001; Altun et al., 2004; Evans et al., 2006; Aguado-Urda et al., 2014; Chen et al., 2001; Austin and Austin, 2016). All test strips were visually observed and test results were provided in apiweb database.

Results

Phenotypical characteristic of *Y. ruckeri*
All isolates were determined as gram negative, bacilli, motile, negative for oxidase, O/F fermentative, growth on MacConkey agar and non-hemolytic. All biochemical results were presented in table 1 and figure 1. Six isolates were found biochemically similar and five isolates have only differences on sorbitol test. Y199, Y95, Y7, Y56 and NCTC 12269 were determined as urease positive that characteristic is the atypical feature for *Y. ruckeri* (Table 1, figure 2).

Phenotypic results characteristic of L. garvieae
All isolates were determined as gram positive, coccus like as streptococci, non-motile, negative for oxidase and catalase, O/F fermentative and α-hemolytic. All biochemical results were presented in table 2. Six isolates were found biochemically similar and five isolates have only differences on sorbitol test. The isolates of L56, L114, L70, L51, L5, L89, L107, L109, L123, L133 and ATCC 49156 have similar biochemical features (Table 2, figure 2).

Figure 1. API RAPID ID 32E test strips of Y. ruckeri isolates

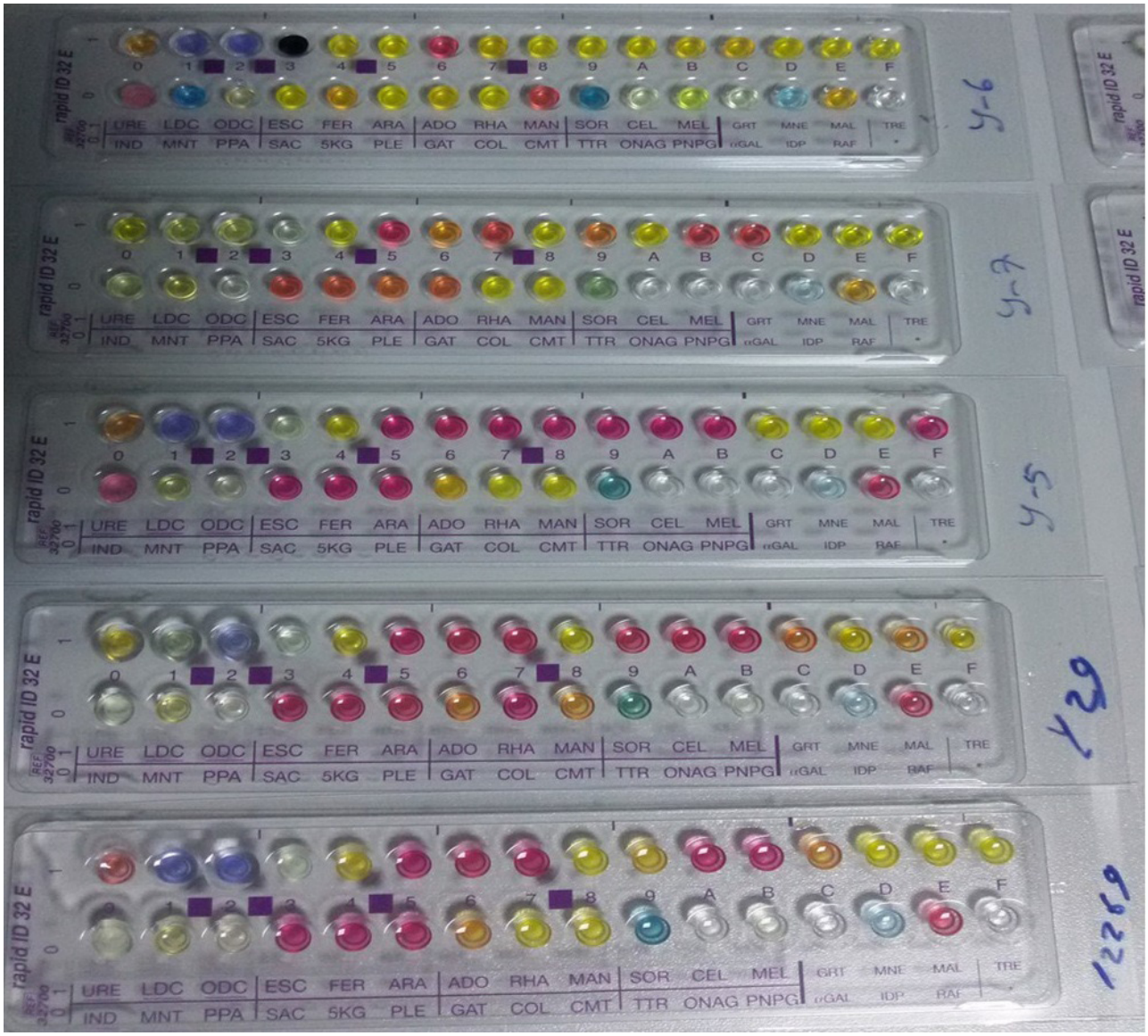

Figure 2. API RAPID ID 32STREPT test strips of L. garvieae isolates

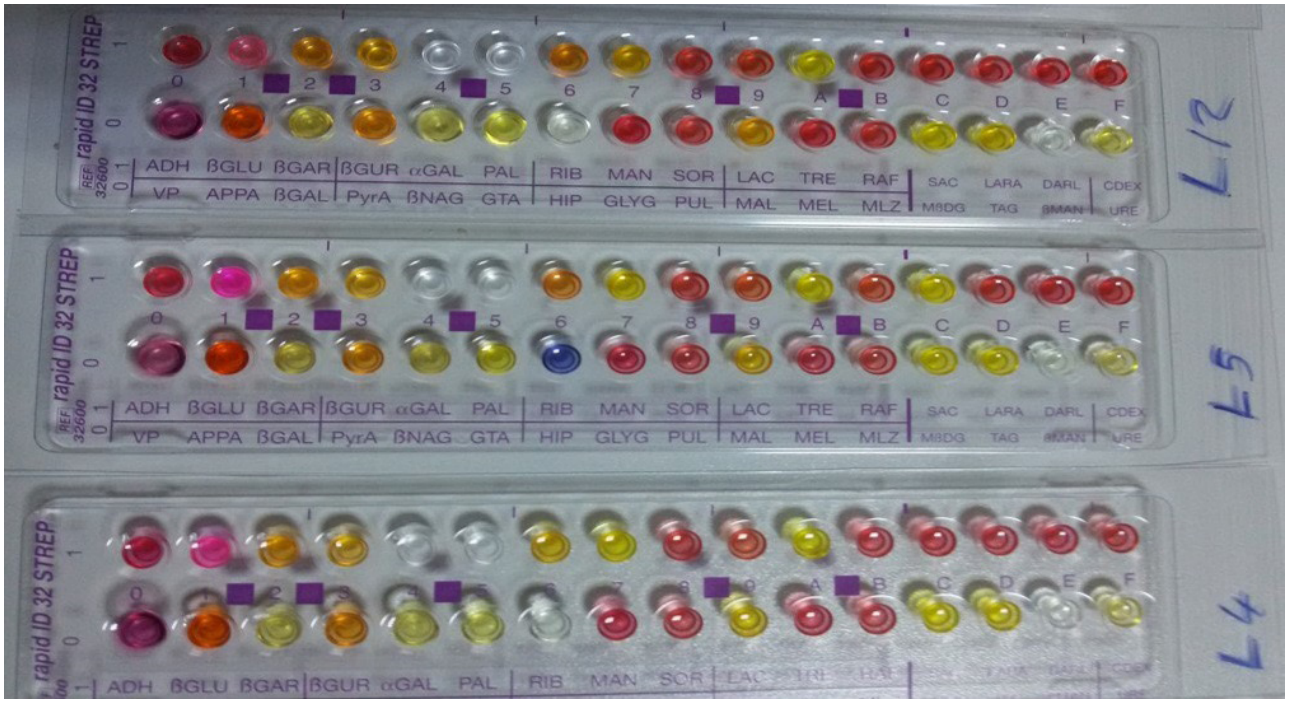

Table 1. Microbiological test and biochemically features of Y. ruckeri isolates determined by API RAPID ID 32E test

	Y58	Y106	Y107	Y147	Y2	Y39	Y262	Y263	NCTC 12268	NCTC 12270	Y237	Y248	Y253	NCTC 12266	NCTC 12267	NCTC 12269	Y56	Y36
Gram stain	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
O/F	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Growth on MacCon key	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Api Rapid 32E																		
URE	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—
LDC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ODC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESC	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
FER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ARA	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
ADO	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
RHA	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
MAN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SOR	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	—	—
CEL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
MEL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
IND	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
MNT	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PPA	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SAC	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5KG	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PLE	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
GAT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
COL	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
CMT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TTR	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
ONAG	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PNPG	+	+	+	+	+	+	—	—	+	+	+	+	+	+	—	—	—	+
GRT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MNE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
αGAL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
IDP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RAF	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
TRE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

LDC: L-lysine, ODC: L-ornithine, ESC: Aesculin ferric citrate, FER: D-glucose, ARA: L-arabinose, ADO: Adonitole, RHA: L-rhamnose, MAN: D-mannitol, SOR: D-sorbitol, CEL: D-cellobiyose, MEL: D-Melibiose, GRT: Sodium glucoronate, MNE: D-mannose, MAL: D-maltose, TRE: D-trehalose, IND: L-tryptophan, MNT: Sodium malonate, PPA: 4-nitrofenilalanin, SAC: D-saccharose (sucrose), 5KG: Potassium 5-ketogluconat, PLE: Palatinose, GAT: Galacturonic acid, COL: Colistin, CMT: Koumarate, TTR: Potassium tetrahionat, ONAG: 2-nitrofenil-N-asetil-βD-glukosaminidase, PNPG: 4-nitrofenil- βD-galaktopiranosidase, αGAL: 4-nitrofeil-αD-galaktopiranosid, IDP: 5-bromo-4-kloro-3-indolil-disodyum phosphate, RAF: D-raffinose, *: control for contamination

Table 1 continuous

	Y14	Y241	Y226	Y44	Y73	Y121	Y75	Y89	Y199	Y95	Y11	Y255	Y7	Y10	Y79	Y92	Y93
Gram stain	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
O/F	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Growth on MacConkey	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Api Rapid ID 32E																	
URE	—	—	—	—	—	—	—	—	+	+	—	—	+	—	—	—	—
LDC	+	+	+	—	—	—	—	+	—	—	—	+	+	+	+	+	+
ODC	+	+	+	—	—	—	—	+	+	+	+	+	+	+	+	+	+
ESC	—	—	+	+	—	—	—	—	+	—	—	—	—	—	—	—	+
FER	+	+	+	+	+	+	+	+	—	—	—	—	+	+	+	+	+
ARA	—	—	—	—	—	—	—	—	—	—	+	+	+	+	—	+	+
ADO	—	—	—	—	—	+	+	—	—	+	—	—	—	—	—	—	—
RHA	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	+	+
MAN	+	+	+	+	+	+	+	—	—	+	+	+	+	+	+	+	+
SOR	—	+	—	—	—	+	+	—	—	—	—	—	—	—	—	+	+
CEL	—	—	—	+	—	+	+	—	—	—	—	—	—	—	+	+	+
MEL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
IND	—	—	—	+	—	—	—	+	+	—	—	—	—	—	—	+	+
MNT	—	—	—	—	—	—	—	—	—	—	+	+	+	+	—	—	+
PPA	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—
SAC	—	—	—	+	—	—	—	—	—	—	—	—	—	—	+	—	+
5KG	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
PLE	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+
GAT	+	+	+	—	+	—	—	+	—	—	—	+	—	—	—	+	+
COL	—	—	—	—	—	+	+	+	+	—	—	+	+	+	+	+	+
CMT	+	+	+	+	+	+	+	+	+	—	—	+	—	—	—	+	—
TTR	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—
ONAG	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PNPG	+	+	+	+	—	—	—	—	—	—	—	+	+	+	+	+	+
GRT	—	+	+	—	+	—	—	+	+	—	—	+	—	—	—	+	+
MNE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MAL	+	+	+	+	+	+	+	+	—	—	—	+	+	+	+	+	+
α GAL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
IDP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RAF	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
TRE	+	+	+	+	+	+	+	—	—	+	—	+	+	+	+	+	+
*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Table 2. Microbiological test and biochemically features of L. garvieae isolates determined by API RAPID ID 32STREPTtest

	ATCC 43921	ATCC 49156	ATCC 49157	L56	L114	L70	L51	L5	L89	L107	L109	L123	L133	L131	L64	L97	L31
Gram stain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Motility	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Oxidase	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Catalase	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
O/F	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
APİ 32 STREPT																	
ADH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
βGLU	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
βGAR	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
βGUR	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
αGAL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PAL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
RIB	—	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—
MAN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SOR	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
LAC	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
TRE	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RAF	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
VP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
APPA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
βGAL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PyrA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
β NAG	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
GTA	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
HIP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+
GLYG	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PUL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
MAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MEL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
MLZ	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SAC	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
LARA	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
DARL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
MBDG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TAG	—	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
β MAN	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CDEX	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
URE	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

O/F: Oxidation/Fermentation; ADH: L-arginine, βGLU: β-glucosidase; βGAR: β-Galactosidase; βGUR; β-glucuronidaseαGAL: α-galactosidase; PAL: alkaline phosphatase, RIB: D-ribose; MAN: Mannitol; SOR: Sorbitol; LAC: Lactose; TRE: D-trehalose; RAF: D-raffinose; VP: Voges Proskauer; APPA: L-alanyl-L-phenylalanyl-L-prolineß-naphthylamide, βGAL: β-galactosidase; PYRA: pyroglutamic acid-ß-naphthylamide, ß-NAG: N-Acetylß-Glucosaminidase, GTA: Glycyl-Tryptophan Arylamidase, HIP: Hydrolysis of HIPpurate, GLYG: Glycogen; PUL: Pullulan; MAL: D-maltose; MEL: D-Melibiose; MLZ: D-melezitose; SAC: D-saccharose; LARA: L-arabinose; DARL: D-arabitol; MBDG: methyl-ßD-glucopyranoside; TAG: D-tagatose; β MAN: β-mannosidase; CDEX: Dcyclodextrin; URE: Urease

Table 2. Continuous

	L116	L127	L136	L137	L114	L115	L132	L111	L129	L90	L14	L104	L138	L81	L140	L108
Gram stain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Motility	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Oxidase	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Catalase	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
O/F	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
APİ RAPID ID 32 STREPT																
ADH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+
βGLU	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
βGAR	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
βGUR	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
αGAL	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—
PAL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
RIB	—	—	—	—	+	+	+	+	+	+	+	+	—	+	+	—
MAN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SOR	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—
LAC	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—
TRE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RAF	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—
VP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
APPA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
βGAL	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—	—
PyrA	+	+	+	+	+	—	—	+	+	+	+	+	+	—	—	+
β NAG	—	—	—	—	—	—	+	+	+	+	+	+	+	—	—	—
GTA	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
HIP	+	+	+	+	+	+	+	+	+	—	—	+	—	—	+	+
GLYG	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PUL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—
MAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—
MEL	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—
MLZ	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—
SAC	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+	+
LARA	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—
DARL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—
MBDG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TAG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
β MAN	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—
CDEX	—	—	—	—	+	—	—	—	+	+	+	+	+	+	+	—
URE	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Discussion

The determination of biochemical differences of *Y. ruckeri* and L. garvieae isolates that were recovered from different month, fish weight and regions of Turkey were aimed in the present study. The rapid identification kits are generally used in private sector for bacterial identification within one day after isolation and also many researcher used to this kits in basic microbiologic test. According to our results, *Y. ruckeri* and *L. garvieae* isolates could be identified by using biochemical tests and rapid test strips by taking into consideration of biochemically differences.

Y. ruckeri was easily isolated from disease outbreaks and carrier fish with cultured on TSA, BHIA, BA and nutrient agar (NA) aerobically and anaerobically (Austin and Austin,, 2016).Determination of biochemical features especially shoul be done by culturing of isolates on 22-25°C otherwise features could be change on different conditions like as *Y. ruckeri* was reported as non-motile on 30°C reported in previous studies (Rintamaki et al., 1986). While *Y. ruckeri* could be identified on basic microbiologic test, they are generally time consuming technique; therefore rapid test kits were evaluated. In the used test kits, API 20E is the most common used kits that identified *Y. ruckeri* easily (Onuk et al., 2011; Tinsley et al., 2011; Altun et al., 2013a; Austin and Austin, 2016). Even though most isolate has similar features on microbiologic test, gelatin, differences on tween hydrolysis and VP reaction were demonstrated in previous research (Onuk et al., 2011; Bastardo et al., 2012; Altun et al., 2013a; Austin and Austin, 2016). We determined as half of *Y. ruckeri* isolates biochemically similar character and *Y. ruckeri* could be identified by using microbiologic test and rapid test kit which demonstrated in our study. We used API RAPID ID 32E test strips differently other studies and the recovered isolates could be successfully identified into 5-7 hours due to some differences on enzyme test. Using of rapid test kit showed primary identification could be done within 5-7 hours. *Y. ruckeri* isolates were detected as urease negative before (Altun et al. 2013a; Akhlaghi and Sharifi Yazdi, 2008; Ewing et al., 1978) yet we observed Y56, Y199, Y95 and Y7 as far as NCTC 12269 (16.6%) positive on urease.

The sorbitol positive *Y. ruckeri* isolates were reported as similar as our results. Despite of, a number of isolates have differences in urease, sorbitol, colistin and 4-nitrofenil-βD-galaktopiranosidase, some isolates have differences all biochemical test exclude for 4-nitrofeil-αD-galaktopiranosid, 5-bromo-4-kloro-3-indolil-disodyum phosphate- and D-mannose in Table 1. These findings revealed that *Y. ruckeri* biochemical structure could show differences and when the isolate trying to identify using rapid test kit, all variations should be taking into consideration.

Other most important agent in aquaculture is *L. garvieae* especially water temperature above on 16°C (Altun et al., 2013b; Duman et al., 2017b). Lactococcosis has characteristic symptoms like as hyperpigmentation and bilateral exophthalmia as it is easily diagnosed visually in pond (Austin and Austin, 2016). The agent could be easily recovered from both diseased fish and also carrier fish culturing on TSA, BHIA and BA (with hemolysis on added 5% sheep blood), as gram positive streptococcus, non-motile, negative for oxidase and catalase, fermentative for O/F and growth capability on 45°C (Austin and Austin, 2016). The mentioned biochemical parameters could be combined with bacteria colony type and symptoms of fish ever disease diagnosed by basic biochemical methods and symptoms of fish. In this respect, we observed bacteria colony type differentiation on blood agar for three days on 22°C incubation and results showed us the bacteria need three days because of colony type differentiation. The colonies were like as pinpoint type in first day and nearly second day on 22°C, in addition purity of bacteria on agar plate should be done after three days. *L. garvieae i*solates that recovered from different source as well as human, milk, and other terrestrial animal, have similar biochemical features while there are some test were different ß-galactosidase, N-acetyl-ß-glucosaminidase and hippuric acid (Cheng and Chen, 1998). The homogenous structure of this agent enable to easily identification with rapid biochemical test like as API Rapid ID 32 STREPT (Biomerieux, France) which identify *L. garvieae* 99.9% similarity index within 5-7 hour (Vela et al., 1999; Ravelo et al., 2001).Vela et al., (2000) observed that *L. garvieae* recovered from rainbow trout, human, beef and water buffalo has determined 13 biotypes based on sucrose, tagatose, mannitol, acid production from cyclodextrin, proglutamic acid, arilamidase and N-acetyl-ß-glukosaminidase.

We observed most of *L. garvieae* isolate have similar biochemical characteristic in API RAPID ID 32 STREP test kit and the test kit successfully identified almost all isolate as *L. garvieae* while some isolates have different biochemical parameter for ex. Ribose, saccharose, hippurate and N-ace-

tyl-ß-glukosaminidase similar as previous report by Altun et al., (2013b).

Consequently, some of *Y. ruckeri* isolate has different biochemical features comparing of reference strain and typical characteristic, there are a little report of urease positive *Y. ruckeri* while we observed 16.6% isolates as positive for urease. And also *Y. ruckeri* isolates could be identified within 5-7 hours by API RAPID ID 32E that is not commonly used kit for identification of *Y. ruckeri*. Comparing to *Y. ruckeri* isolates, *L. garvieae* has more homogenous biochemical structure and identified successfully with minor variations. The molecular identification should be done for all bacteria species while they could identify by rapid test kit correctly for supporting to certain identification.

References

Abdel-Latif HM, Khalil RH, Saad TT, El-Bably RY. Identification and molecular characterization of Yersinia ruckeri isolated from mass mortalities of cultured Nile tilapia at Kafr El-sheikh governorate. Global Journal of Fisheries and Aquaculture Researches 1(2), 01-17,2014.

Aguado-Urda M, Rodriguez-Bertos A, Ana I, Blanco MM, Acosta F, Cid R, Gibello A. Experimental Lactococcus garvieae infection in zebrafish and first evidence of its ability to invade non-phagocytic cells. Veterinary microbiology 171(1), 248-254,2014.

Akhlaghi M. & Sharifi YH. Detection and identification of virulent Yersinia ruckeri: the causative agent of enteric redmouth disease in rainbow trout (Oncorhynchus mykiss) cultured in Fars province, Iran,2008.

Altinok I, Capkin E, Boran H. Comparison of molecular and biochemical heterogeneity of Yersinia ruckeri strains isolated from Turkey and the USA. Aquaculture, 450, 80-88,2016.

Altun S, Buyukekiz A, Onuk EE, Duman M, and Ciftci A. Phenotypic, genotypic characterisation and antimicrobial susceptibility determination of Lactococcus garvieae strains. Kafkas Universitesi Veteriner Fakültesi Dergisi, 19 (3): 375-381. doi: 10.9775/kvfd.2012.7754, 2013b.

Altun S, Diler O, Adiloglu AK. Genotyping of Lactococcus garvieae strains from rainbow trout (Oncorhynchus mykiss) by 16S rDNA sequencing. Bulletın-European Asocıatıon Of Fısh Pathologısts 24(2), 119-125,2004.

Altun S, Kubilay A, and Diler O.Yersinia ruckeri suslarının fenotipik ve serolojik özelliklerinin incelenmesi. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 16(1): 223-229, 2010.

Altun S, Onuk EE, Ciftci A, Duman M, and Büyükekiz AG. Determination of Phenotypic, Serotypic and Genetic Diversity and Antibiotyping of Yersinia ruckeri Isolated from Rainbow Trout. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 19(2), pp.225-232,2013a.

Austin B, and Austin DA. Bacterial fish pathogens. 6. th edition. Springer International Publishing, Switzerland, pp: 21-82; 161-321; 323-396; 643-721,2016.

Bastardo A, Ravelo C, Romalde JL. Multilocus sequence typing reveals high genetic diversity and epidemic population structure for the fish pathogen Yersinia ruckeri. Environ Microbiol 14:1888–1897, 2012.

Carson J, Gudkovs N, Austin B. Characteristics of an Enterococcus-like bacterium from Australia and South Africa, pathogenic for rainbow trout (Oncorhynchus mykiss Walbaum). J. Fish Dis., 6: 381-388,1993.

Chen SC, Lin YD, Liaw LL, Wang PC.Lactococcus garvieae infection in the giant freshwater prawn Macrobrachium rosenbergii confirmed by polymerase chain reaction and 16S rDNA sequencing. Dis Aquat Org 45:45–52,2001.

Cheng W, Chen JC. Isolation and characterization of an Enterococcus-like bacterium causing muscle necrosis and mortality in Macrobrachium rosenbergii in Taiwan. Dis Aquat Org;34:93–101,1998.

Diler O, Altun S, Adiloglu A, Kubilay A, and Isikli BT. First occurrence of streptococcosis affecting farmed rainbow trout (Oncorhynchus mykiss) in Turkey. Bulletin of the European Association of Fish Pathologists 22 (1): 21, 2002.

Duman M, Buyukekiz AG, Saticioglu IB, Cengiz M, Sahinturk P, Altun S. Epidemiology, Genotypic Diversity,

and Antimicrobial Resistance of Lactococcus garvieae in Farmed Rainbow Trout (Oncorhynchus mykiss). Iranian Journal of Fisheries Science. In press,2017b.

Duman M, Altun S, Cengiz M, Saticioglu IB, Buyukekiz AG, Sahinturk P. Genotyping and antimicrobial resistance genes of Yersinia ruckeri isolates from rainbow trout farms. Diseases of Aquatic Organisms, Vol. 125: 31–44, 2017. DOI https://doi.org/10.3354/dao03132, 2017a.

Elliot JA, Collins MD, Pigott NE, Facklam RR. Differentiation of Lactococcus lactis and Lactococcus garvieae from humans by comparison of whole-cell protein patterns. J Clin Microbiol 20:2731–4,1991.

Evans JJ, Pasnik DJ, Klesius PH, Al-Ablani S. First report of Streptococcus agalactiae and Lactococcus garvieae from a wild bottlenose dolphin (Tursiops truncatus). Journal of wildlife diseases, 42(3), 561-569,2006.

Ewing WH, Ross AJ, Brenner DJ, Fanning GR.Yersinia ruckeri sp. nov., the red mouth (RM) bacterium. International Journal of Systemic Bacteriology, (28): 37-44,1978.

Fefer JJ, Ratzan KR, Sharp SE, Saiz E.Lactococcus garvieae endocarditis: report of a case and review of the literature. Diagn Microbiol Infect Dis 32:127–30,1998.

Fernandez E, Alegrıa A, Delgado S, Mayo B. Phenotypic, genetic and technological characterization of Lactococcus garvieae strains isolated from a raw milk cheese. Int Dairy J 20: 142–148,2010.

Fihman V, Raskine L, Barron Z, Kiffel C, Riahi J, Berc-ot B.Lactococcus garvieae endocarditis: identification by 16S rRNA and sodA sequence analysis. J Infect 52:e3–6,2006.

Foschino R, Picozzi C, Borghi M, Cerliani MC, Cresci E. Investigation on the microflora of Caprino Lombardo cheese from raw goat milk. Ital J Food Sci 18: 33–49,2006.

Ghittino P. and Prearo M. Report of Streptococcosis in rainbow trout (Oncorhynchus mykiss) in Italy: preliminary note. Boll. Soc. Ital. Pathol. Ittica 8: 4-9,1992.

James PR, Hardman S, Patterson D. Osteomyelitis and possible endocarditis secondary to Lactococcus garvieae: a first case report. Postgrad Med J 76:301–3,2000.

Kawanishi M, Yoshida T, Kijima M, Yagyu K, Nakai T, Okada S, Endo A, Murakami M, Suzuki S, Morita H. Characterization of Lactococcus garvieae isolated from radish and broccoli sprout that exhibited a KG phenotype, lack of virulence and absence of a capsule. Lett Appl Microbiol 44: 481–487,2007.

Keukeleire S, Bel A, Jansen Y, JanssensM, Wauters G, Piérard D.Yersinia ruckeri, an unusual microorganism isolated from a human wound infection. New microbes and new infections, 2(4), 134-135,2014.

Kubilay A, Altun S, Ulukoy G and Diler O.Lactococcus garvieae suşlarının antimikrobiyal duyarlılıklarının belirlenmesi. Eğirdir Su Ürünleri Fakültesi Dergisi, 1(1): 39-48, 2005.

Mofredj A, Baraka D, Cadranel JF, LeMaitre P, Kloeti G, Dumont JL.Lactococcus garvieae septicemia with liver abscess in an immunosuppressed patient. Am J Med 109:513–4,2000.

Onuk EE, Ciftci A, Findik A, Ciftci G, Altun S, Balta F, Ozer S, Coban AY. Phenotypic and molecular characterization of Yersinia ruckeri isolates from rainbow trout (Oncorhynchus mykiss, Walbaum, 1792) in Turkey. Berl Munch Tierarztl Wochenschr 124: 320–328,2011.

Ozer S, Bulduklu PS and Dönmez E. Mersin ilinde yetiştiriciliği yapılan gökkuşağı alabalıklarında (Oncorhynchus mykiss, Walbaum) streptokokkozis varlığı. Journal of Fisheries Sciences 2(3): 272-283,2008.

Ravelo C, Magarinos B, Romalde JL, Toranzo AE. Convencional versus miniaturizad systems for the phenotypic characterization of Lactococcus garvieae strains. Bull Eur Ass Fish Pathol 21:136–44,2001.

Rintamaki P, Valtonen ET, Frerichs GN. Occurrence of Yersinia ruckeri infection in farmed whitefish, Coregonus peled Gmelin and Coregonus muksun Pallas, and Atlantic salmon, Salmo salar L., in northern Finland. Journal of Fish Diseases, 9(2), pp.137-140,1986.

Russo G, Iannetta M, D'Abramo A, Mascellino MT, Pantosti A, Erario L, Vullo V. Lactococcus garvieae endocarditis in a patient with colonic diverticulosis: first case report in Italy and review of the literature. Microbiologica-Quarterly Journal of Microbiological Sciences, 35(4), 495,2012.

Savaş H. and Türe M. Bölgemizde doğal ve kültürü yapılan balıklarda görülen hastalıklar. SUMAE Yunus Araştırma Bülteni 7: 10-13,2007.

Seker E, Karahan M, Ispir U, Cetinkaya B, Saglam N. and Sarieyyupoglu M. Investigation of Yersinia ruckeri infection in rainbow trout (Oncorhynchus mykiss walbaum 1792) farms by polymerase chain reaction (PCR) and bacteriological culture. Kafkas Universitesi Veteriner Fakultesi Dergisi, 18 (6): 913- 916, 2012.

Sharifiyazdi H, Akhlaghi M, Tabatabaei M, and Mostafavi Zadeh SM. Isolation and Characterization of Lactococcus garvieae from Diseased Rainbow Trout (Oncorhynchus mykiss, Walbaum) Cultured in Iran. Iranian Journal of Veterinary Research 11, 342-350,2010.

Timur G, Yardimci RE, Urku C, and Canak O. Marmara Bölgesi kültür gökkuşağı alabalıklarında lactococcosisin bakteriyolojik ve histopatolojik metotlarla teşhisi. İstanbul Üniversitesi, Su Ürünleri Dergisi, 26 (1): 63-81, 2011.

Timur G, Timur M. An outbreak of enteric redmouth disease in farmed rainbow trout (O. mykiss) in Turkey. Bulletin of the European Association of Fish Pathologists (United Kingdom),1991.

Tinsley JW, Lyndon AR, Austin B. Antigenic and crossprotection studies of biotype 1 and biotype 2 isolates of Yersinia ruckeri in rainbow trout, Oncorhynchus mykiss (Walbaum). Journal of applied microbiology 111(1), pp.8-16,2011.

Vela AI, Vázquez J, Gibello A, Blanco MM, Moreno MA, Liébana P, Albendea C, Alcalá B, Mendez A, Domínguez L, and Fernández-Garayzábal JF. Phenotypic and Genetic Characterization of Lactococcus garvieae Isolated in Spain from Lactococcosis Outbreaks and Comparison with Isolates of Other Countries and Sources. Journal of Clinical Microbiology, 38(10), pp.3791-3795,2000.

Vela I, Liebana P, Albendea C, Gibello A, Blanco M, Moreno M, Cutúli M, García JA, Doméneche A, Domínguez L, and Vázquez J. September. Caracterización fenotípica y molecular de Lactococcus garvieae. In Proceedings of the XIII, 1999.

Vendrell, D., Balcázar, J. L., Ruiz-Zarzuela, I., De Blas, I., Gironés, O., & Múzquiz, J. L. (2006). Lactococcus garvieae in fish: a review. Comparative immunology, microbiology and infectious diseases, 29(4), 177-198.

Verner-Jeffreys DW, Welch TJ, Schwarz T, Pond MJ, Woodward MJ, Haig SJ, Rimmer GS, Roberts E, Morrison V, Baker-Austin C. High prevalence of multidrug-tolerant bacteria and associated antimicrobial resistance genes isolated from ornamental fish and their carriage water. PLoS One. doi:10.1371/journal.pone.0008388, 2009.

Wortberg F, Nardy E, Contzen M, Rau J. Identification of Yersinia ruckeri from diseased salmonid fish by fourier transform infrared spectroscopy. J Fish Dis 35: 1–10,2012.