Berl Münch Tierärztl Wochenschr 124, 10–18 (2011)
DOI 10.2376/0005-9366-124-10

ISSN 0005-9366

Korrespondenzadresse:
aciftci@omu.edu.tr

Eingegangen: 10.11.2010
Angenommen: 06.03.2011

Department of Diseases and Clinical Sciences, Faculty of Veterinary Medicine, University of Ondokuz Mayıs, Samsun, Turkey[1]
Department of Microbiology, Faculty of Veterinary Medicine, University of Ondokuz Mayıs, Samsun, Turkey[2]
Department of Biochemistry, Faculty of Veterinary Medicine, University of Ondokuz Mayıs, Samsun, Turkey[3]
Department of Microbiology, Faculty of Veterinary Medicine, University of Uludag, Bursa, Turkey[4]
Department of Aquaculture, Faculty of Fisheries, University of Rize, Rize, Turkey[5]
Department of Aquaculture, Faculty of Fisheries, University of Mersin, Mersin, Turkey[6]
Department of Microbiology, Faculty of Medicine, University of Ondokuz Mayıs, Samsun, Turkey[7]

Phenotypic and Molecular Characterization of *Yersinia ruckeri* Isolates from Rainbow Trout (*Oncorhynchus mykiss*, Walbaum, 1792) in Turkey

Phänotypische und molekulare Charakterisierung von Yersinia ruckeri Stämmen isoliert von Regenbogenforellen (Oncorhynchus mykiss, Walbaum, 1792) in der Türkei

Ertan Emek Onuk[1], Alper Ciftci[2], Arzu Findik[2], Gülay Ciftci[3], Soner Altun[4], Fikri Balta[5], Selmin Ozer[6], Ahmet Yılmaz Coban[7]

Summary

The aim of the study was the phenotypic and molecular characterization of *Yersinia* (*Y.*) *ruckeri* strains, the causative agent of Enteric Redmouth Disease (ERM), by antibiotyping, random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) and sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of whole cell proteins. For this aim, a total of 97 *Y. ruckeri* isolates were analyzed. The isolates were distinguished into ten antibiotypes and six phenotypes according to their resistance properties and whole cell protein profiles, respectively. Also, a glycoprotein band of approximately 25.5 kDa was observed in all *Y. ruckeri* strains tested. In all strains, six different RAPD types were observed. In conclusion, *Y. ruckeri* isolated from rainbow trout of fish farms in Turkey showed variation according to their phenotypic and genotypic characteristics, and the use of these three typing techniques in double and triple combinations could be more useful for discriminating the strains.

Keywords: Antibiotic resistance, Glycoprotein, RAPD-PCR, SDS-PAGE, *Yersinia ruckeri*

Zusammenfassung

Ziel der Studie war die phänotypische und molekulare Charakterisierung von *Yersinia*-(*Y.*)-*ruckeri*-Stämmen, dem Erreger der Rotmaulseuche bei der Regenbogenforelle, durch Antibiotyping, Random Amplified Polymorphic DNA-Polymerase-Kettenreaktion (RAPD-PCR) und Natriumdodecylsulfat-Polyacrylamidgelelektrophorese (SDS-PAGE) Muster der Ganzzell-Proteine. Für dieses Ziel wurden insgesamt 97 *Y.-ruckeri*-Isolate analysiert. Die Isolate wurden nach ihrer Antibiotika-Resistenz und ihren Ganzzell-Protein-Profilen in zehn Antibiotypen und sechs Phänotypen unterschieden. Auch wurde eine ungefähr 25,5 kDa grosse Glykoprotein Bande bei allen getesteten *Y.-ruckeri*-Stämmen beobachtet. Durch Genotypisierung der Stämme mit RAPD-PCR wurden sechs verschiedene RAPD Typen unterschieden. Die von Regenbogenforellen aus Fischfarmen in der Türkei isolierten *Y.-ruckeri*-Stämme zeigten Variationen im Hinblick auf ihre phänotypischen und genotypischen Merkmale. Die Verwendung dieser drei Typisierung-Techniken in Doppel- und Dreifach-Kombinationen könnte nützlich für die Unterscheidung der Stämme sein.

Schlüsselwörter: Antibiotika-Resistenz, Glykoprotein, RAPD-PCR, SDS-PAGE, *Yersinia ruckeri*

Introduction

Enteric Redmouth Disease (ERM) is known as a septicemic disease primarily in salmonids and also non-salmonid fishes. *Yersinia* (*Y.*) *ruckeri* is known as the causative agent of the disease. ERM leads to significant economical losses with respect to its direct and indirect effects such as fish mortality and export restrictions of broodstock and eggs in salmonid aquaculture worldwide (LeJeune and Rurangirwa, 2000). This disease is characterized by the presence of congestive or hemorrhagic zones in various tissues and organs, especially around the mouth and in the intestines and exophtalmos is also frequently seen (Coquet et al., 2002; Gibello et al., 1999). *Y. ruckeri* had been characterized biochemically and serologically in several studies (Davies and Frerichs, 1989; Pyle and Schill, 1987). Polymerase Chain Reaction (PCR), a rapid and simple diagnostic test specific for *Y. ruckeri*, is also widely used as an alternative to traditional identification methods (Gibello et al., 1999; LeJeune and Rurangirwa, 2000).

Various biochemical and molecular methods have been described for typing bacterial strains. Apart from the other phenotypical typing methods (biotyping, serotyping, phage typing), one of the most widely used phenotypical typing method is antibiotyping based on antibiotic susceptibility (Kapperud et al., 1999). Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) is considered as one of the most cost effective genotyping method for the investigation of large numbers of isolates (Tan et al., 2009). Furthermore, it has allowed detailed genotype screening without any previous knowledge of specific DNA sequence data (Argenton et al., 1996). Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) becomes increasingly important for differentiating and classifying microorganisms. This method has been reported to be used both singly and in combination with biochemical and phenotypic characteristics for identifying and classifying bacteria (Plikaytis et al., 1986). SDS-PAGE patterns can be obtained in very short times, are reproducible and do not require any sophisticated and expensive reagents or equipment as compared with molecular biology kits (Massa et al., 2007).

Glycoproteins are the proteins that consist of oligosaccharide chains covalently bound to the polypeptide skeleton. The presences of glycoproteins have recently been demonstrated in many of the bacteria. The glycoproteins are present in different forms such as membrane-associated glycoproteins, surface-associated glycoproteins and crystalline surface layers (S-layers), secreted glycoproteins and exoenzymes (Messner, 1997). After the first description of the presence of glycoproteins in Halobacteria, bacterial glycoproteins, especially S-layer glycoproteins have been continuously investigated (Upreti et al., 2003). Although many glycoproteins have been discovered in recent years, it has not been found any knowledge about glycoproteins of *Y. ruckeri*.

The objective of this study was to characterize *Y. ruckeri* strains isolated from rainbow trout of different fish farms in Turkey by phenotypical and genotypical typing methods. For this aim, antibiotyping and SDS-PAGE typing of whole cell proteins as phenotypic methods and RAPD-PCR as a genotypic method were performed. Furthermore the strains were examined for the presence of glycoproteins.

Material and Methods

Bacterial isolates

In this study a total of 97 *Y. ruckeri* strains isolated from rainbow trout from different fish farms and different outbreaks in Turkey were characterized. These strains were isolated from various sources such as liver, kidney, spleen and stomach between 1997 and 2008 from four different geographical areas (Aegean, Black sea, Central Anatolia and Mediterranean) (Tab. 1). Samples from different origins were inoculated onto Tryptic Soy Agar and incubated at 22–25˚C for 24–48 h. The reference strain Y.ruckeri (ATCC 29473) was used in phenotypic and genotypic identification as a control strain. For the phenotypic confirmation of identification of *Y. ruckeri*, several characteristics such as negative Gram stain, negative catalase and urease, alkaline slant, acid butts, and lack of H2S, fermentation of glucose and non-fermentation of sucrose and lactose, non-utilization of rhamnose and arabinose and lack of indole production were examined (Holt et al., 1994). In addition to these characteristics, negative acetoin production (VP test), sorbitol fermentation, gelatin hydrolysis and positive Tween 80 hydrolysis were also examined in these strains (Austin and Austin, 2007). Also, PCR was performed to confirm the isolates as *Y. ruckeri*. For this aim, chromosomal DNA was extracted from cultures using a commercial kit (PureLink™ Genomic DNA Kits, Invitrogen Canada Inc., Canada). The designation of forward (5′-GCGAGGAGGAAGGGTTAAGTG-3′) and reverse (5′-GAAGGCACCAAGGCATCTCTG-3′) oligonucleotide primers and reaction conditions were performed according to the procedure reported by Gibello et al. (1999).

Antibiotic susceptibility testing and antibiotyping

The antibiotic susceptibility patterns of *Y. ruckeri* isolates were determined using the Kirby-Bauer Disc Diffusion Method according to the National Committee for Clinical Laboratory Standards (NCCLS) (2003). The used antibiotic discs were amoxicillin (10µg), ampicillin (10µg), enrofloxacin (5µg), erythromycin (15µg), neomycin (10µg), oxitetracycline (30µg) and trimethoprim (5µg). The results were recorded as susceptible, intermediate susceptible or resistant. The schematic diagram including these results have been drawn and the similarities of patterns were determined based on the Dice similarity coefficient using CHEF-DR® III, Quantity One® Software (Bio-Rad Laboratories, USA).

Genotyping by ERIC2 RAPD-PCR

To determine the RAPD patterns of *Y. ruckeri* isolates, ERIC2 Primer (5′- AAGTAAGTGACTGGGGTGAGCG-3′) specific for "Enterobacterial Repetitive Intergenic Concensus (ERIC)" was used. Amplification was performed by modifying the method reported by Versalovic et al. (1991). The RAPD reaction mix (25µl) included 2,5 mM of MgCl2, 200 µM of each dNTP, 2.5 U Taq DNA polimeraz, 25 pmol of ERIC2 primer and 5 µl of DNA extract. The amplification program was begun with initial denaturation at 95˚C for 1 min. Following initial denaturation, denaturation at 94˚C for 1 min, annealing at 36˚C for 1 min, extention at 72˚C for 3 min were repeated 35 times. A final extention at 72˚C for 5 min was included at the end of amplification cycles. The amplification products were detected by electrophoresis in 1.5% agarose gel and DNA was visualized by stain-

ing with ethidium bromide. The similarities and numbers of the bands between RAPD patterns were determined based on the Dice similarity coefficient. To create a dendogram that graphed genetic relatedness between *Y. ruckeri* isolates with the cut-off value of 75%, "Unweighted Pair Group Method with Arithmetic Averages (UPGMA)" was employed using CHEF-DR® III, Quantity One® Software (Bio-Rad Laboratories, USA).

Determination of protein profiles by SDS-PAGE

SDS-PAGE of whole cell proteins was conducted in 8% (w/v) polyacrylamide gel according to Laemmli (1970). To prepare whole cell lysate, the bacteria were propagated in Tryptic Soy Broth at 22–25˚C for 24 h. Then, the bacterial cells were pelleted at 800 g for 10 min and washed three times with phosphate buffer saline (pH 7.2). The pellet was suspensed with lysis buffer (125mM Tris-HCl, 0.1% SDS, pH 6.8) and boiled for 5 min. The suspension was centrifuged at 1800 g for 30 min and the supernatant was collected. Protein concentration was measured and equalized by Modified Lowry Protein Assay using a spectrophotometer at the wave length of 750 nm. Optimization of protein concentration for SDS-PAGE was done through a series of experimental SDS-PAGE. Each sample and the molecular weight markers (205-6.5 kDa) were loaded into the gel and electrophoresis was performed at a constant current of 20 mA. After electrophoresis, the gel was stained by Blue Silver staining solution. The SDS-PAGE results were evaluated for variation of band densities and similarities and numbers of the bands between patterns. The dendogram (UPGMA) were drawn based on the Dice similarity coefficient including the genetic relatedness between *Y. ruckeri* isolates with the cut-off value of 75% using CHEF-DR® III, Quantity One® Software (Bio-Rad Laboratories, USA).

Determination of glycoprotein profiles

To determine the glycoprotein patterns of *Y. ruckeri* isolates, a DIG Glycan Detection Kit (Roche Molecular Biochemicals, Germany) which detects the sugars in glycoconjugates by an enzyme immunoassay was used. According to this protocol, SDS-PAGE was performed in all strains in first step as described above. Prestained SDS-PAGE marker (6.5–120 kDa) was used as the membrane marker. Then, the gels were kept in the transfer tampon (48 mM Tris-HCl, 39 mM glycine, 0.037 % w/v SDS, and 20 % v/v methanol at pH 8.3) for 10 min. The membrane transfers of the proteins from the gel onto the Polyvinylidene Fluoride (PVDF) membrane (Hypond-P) were performed by keeping at 100V for 1 h with using membrane and 3 filtration papers by using semi-dry blot device (ECL-Semi-dry transfer device).

TABLE 1: *The distribution on the basis of geographical origin and profiles obtained by different typing methods of Yersinia ruckeri strains used in this study*

Isolate no	Antibio-type	SDS-PAGE Profile	RAPD-PCR genotype	Geographic Origin	Year of Isolatıon	Isolation Tissue
1	A1	SC4	RE1	Black sea	2007	Kidney
2	A1	SC4	RE1	Black sea	2007	Kidney
3	A1	SC4	RE1	Black sea	2007	Kidney
4	A1	SC4	RE1	Black sea	2007	Kidney
5	A1	SC4	RE1	Black sea	2007	Kidney
6	A1	SC4	RE1	Mediterranean sea	2005	Kidney
7	A1	SC4	RE1	Mediterranean sea	2005	Kidney
8	A1	SC4	RE1	Mediterranean sea	2005	Kidney
9	A1	SC4	RE1	Mediterranean sea	2005	Liver
10	A1	SC4	RE1	Central anatolia	1998	Kidney
11	A1	SC4	RE1	Central anatolia	1997	Liver
12	A1	SC4	RE1	Aegean	2006	Liver
13	A1	SC4	RE1	Aegean	2006	Liver
14	A1	SC4	RE2	Black sea	2007	Kidney
15	A1	SC4	RE2	Black sea	2007	Kidney
16	A1	SC4	RE2	Black sea	2005	Kidney
17	A1	SC4	RE2	Mediterranean sea	2005	Kidney
18	A1	SC4	RE2	Mediterranean sea	2005	Liver
19	A1	SC4	RE2	Central anatolia	1998	Kidney
20	A1	SC4	RD	Black sea	2004	Kidney
21	A1	SC4	RD	Black sea	2005	Kidney
22	A1	SC4	RD	Mediterranean sea	2005	Kidney
23	A1	SC4	RD	Aegean	2006	Liver
24	A2	SC4	RE2	Black sea	2004	Kidney
25	A2	SC4	RE2	Black sea	2004	Kidney
26	A2	SC4	RE2	Black sea	2001	Kidney
27	A2	SC4	RE2	Black sea	2005	Kidney
28	A2	SC4	RE2	Black sea	2007	Kidney
29	A2	SC4	RE2	Mediterranean sea	2005	Liver
30	A2	SC4	RE2	Central anatolia	2008	Kidney
31	A2	SC4	RE2	Aegean	2006	Liver
32	A2	SC4	RD	Black sea	2004	Kidney
33	A2	SC4	RD	Black sea	2005	Kidney
34	A2	SC4	RD	Black sea	2005	Kidney
35	A2	SC4	RD	Black sea	2007	Kidney
36	A2	SC4	RD	Mediterranean sea	2005	Kidney
37	A2	SC4	RD	Mediterranean sea	2005	Kidney
38	A2	SC4	RD	Mediterranean sea	2005	Liver
39	A2	SC4	RD	Central anatolia	2008	Kidney
40	A2	SC4	RD	Aegean	2006	Liver
41	A3	SC4	RE1	Black sea	2007	Spleen
42	A3	SC4	RE1	Black sea	2002	Kidney
43	A3	SC4	RE1	Black sea	2007	Spleen
44	A3	SC4	RE1	Black sea	2005	Kidney
45	A3	SC4	RE1	Mediterranean sea	2005	Kidney
46	A3	SC4	RE1	Mediterranean sea	2005	Kidney
47	A3	SC4	RE1	Mediterranean sea	2005	Kidney
48	A3	SC4	RE1	Aegean	2006	Liver
49	A3	SA	RE1	Black sea	2007	Kidney
50	A3	SA	RE1	Black sea	2007	Spleen
51	A3	SA	RE1	Central anatolia	2008	Kidney
52	A3	SC2	RE1	Black sea	2007	Spleen
53	A3	SC2	RE1	Black sea	2007	Kidney
54	A3	SC2	RE1	Black sea	2007	Liver
55	A4	SC4	RE2	Black sea	2005	Kidney
56	A4	SC4	RE2	Black sea	2005	Kidney
57	A4	SC4	RE2	Black sea	2007	Kidney
58	A4	SC4	RE2	Mediterranean sea	2005	Kidney
59	A4	SC4	RE2	Mediterranean sea	2005	Kidney
60	A4	SC4	RE2	Mediterranean sea	2005	Liver
61	A4	SC4	RE2	Aegean	2006	Liver
62	A4	SC4	RD	Black sea	2007	Kidney
63	A4	SC4	RD	Black sea	2007	Kidney
64	A4	SC4	RD	Mediterranean sea	2005	Kidney
65	A4	SC4	RD	Aegean	2006	Liver
66	A5	SC3	RC	Black sea	2007	Liver

Isolate no	Antibio-type	SDS-PAGE Profile	RAPD-PCR genotype	Geographic Origin	Year of Isolatıon	Isolation Tissue
67	A5	SC3	RC	Mediterranean sea	2005	Kidney
68	A5	SC3	RC	Aegean	2006	Liver
69	A5	SC3	RA	Black sea	2007	Kidney
70	A5	SC3	RA	Mediterranean sea	2005	Kidney
71	A5	SC3	RA	Mediterranean sea	2005	Kidney
72	A5	SC3	RA	Aegean	2006	Liver
73	A5	SC3	RB	Mediterranean sea	2005	Kidney
74	A5	SC3	RB	Aegean	2006	Liver
75	A6	SC3	RC	Black sea	2007	Spleen
76	A6	SC3	RC	Mediterranean sea	2005	Liver
77	A6	SC3	RA	Black sea	2007	Kidney
78	A6	SC3	RA	Mediterranean sea	2005	Liver
79	A6	SC1	RC	Mediterranean sea	2005	Liver
80	A6	SC1	RC	Black sea	2007	Liver
81	A6	SB	RB	Black sea	2007	Kidney
82	A6	SB	RB	Central anatolia	2008	Kidney
83	A7	SC4	RE1	Black sea	2005	Kidney
84	A7	SC4	RE1	Central anatolia	2008	Kidney
85	A7	SC4	RE1	Central anatolia	2008	Kidney
86	A7	SA	RE1	Central anatolia	2006	Liver
87	A7	SA	RE1	Central anatolia	2006	Liver
88	A8	SC3	RE2	Central anatolia	2008	Kidney
89	A8	SC3	RE2	Black sea	2004	Kidney
90	A8	SC3	RA	Mediterranean sea	2005	Liver
91	A8	SC3	RA	Mediterranean sea	2006	Liver
92	A9	SC4	RE1	Mediterranean sea	2006	Liver
93	A9	SC4	RE1	Mediterranean sea	2005	Stomach
94	A9	SC4	RE1	Central anatolia	2008	Kidney
95	A10	SC3	RB	Mediterranean sea	2005	Kidney
96	A10	SC3	RB	Central anatolia	2008	Kidney
97	A10	SC3	RB	Central anatolia	2008	Kidney

Then, a DIG Glycan Detection Kit (Roche Molecular Biochemicals, Germany) was used in PVDF according to the manufacturer's directions. The molecular weight(s) of the band(s) was determined using Kodak Molecular Imaging Software.

Determination of the reproducibility, discriminatory power and confidence intervals of typing methods

To determine the reproducibility of SDS-PAGE typing, analysis were repeated in triplicate. To determine the reproducibility of RAPD-PCR typing by interassay analysis, all isolates were tested for 5 consecutive days. A numerical index of discrimination was used for evaluating the discriminatory power. To calculate the discriminatory indices (DI) and the confidence intervals (CI) of each typing methods and their different combinations, the formula described previously was used (Grundmann et al., 2001; Hunter and Gaston, 1988). The formula for calculating DI is as follows:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} n_j(n_j - 1)$$

In the formula, S is the number of types, nj is the number of population members falling into the jth type, and N is the size of the population.

Results

All isolates were identified biochemically as *Y. ruckeri* (Tab. 1). All the isolates gave bands of 575 bp by PCR and all of them were confirmed also genotypically as *Y. ruckeri*. Identical profiles between 97 *Y. ruckeri* isolates and reference *Y. ruckeri* strain were observed in biochemical reactions examined.

Antibiotic susceptibility testing and antibiotyping

Yersinia ruckeri isolates were grouped into 10 different antibiotypes (A1–A10) depending upon their susceptibilities to 7 different antimicrobials (Tab. 2). In all groups except A1, A7 and A9, the resistance against at least one antibiotic was determined. Fiftytwo (53.6%) and fortytwo (43.3%) isolates were resistant to erythromycin and neomycin, respectively. Most of the isolates (75.3%) were susceptible to amoxicillin, ampicillin, enrofloxacin, oxytetracycline and trimethoprim. The resistance to trimethoprim was found only in 7 isolates (7.2%) in two groups (A8 and A10). The distribution of the antibiotypes by geographical origin is shown in Table 1.

Genotyping by ERIC2 RAPD-PCR

All *Y. ruckeri* isolates examined in this study generated bands with ERIC2 primer. There was a genetic diversity between these strains. *Y. ruckeri* isolates were grouped into 5 unique types (RA-RD) and 1 cluster (RE) including 2 subtypes (RE1 and RE2) based on their RAPD band patterns. The RA, RB, RC, RD and RE genotypes included 8.2%, 7.2%, 7.2%, 17.5% and 59.8% of isolates, respectively. Most of the isolates (36.1%) were assigned to RE1 subtype. The second predominant group, RE2, included 23 (23.7%) isolates. The similarity between these two groups was found as 83%. The agarose gel electrophoresis patterns and phylogenetic tree of *Y. ruckeri* isolates was shown in Figure 1.

The protein profiles by SDS-PAGE

All 97 *Y. ruckeri* strains were typed according to their SDS-PAGE band profiles of whole cell proteins. Strains were clustered by the unweighted pair-group with arithmetic averages (UPGMA) method at 75% similarity level. Strains were divided into a large cluster (SC) and 2 unique types (SA and SB). The unique types SA and SB contained 5 (5.2%) and 2 (2.1%) strains, respectively. The SC cluster included 4 subtypes named as SC1, SC2, SC3 ve SC4. Two of these subtypes, SC3 and SC4, showed 90% similarity and they included 20 (20.6%) and 65 (67.0%) of all strains, respectively. The degree of relation-

TABLE 2: *The antibiotic resistance profiles of Y. ruckeri isolates*

	AMO	AMP	ENR	ERT	NEO	OXY	TRM	n (%)
A1	S	S	S	I	I	S	S	23 (23.71)
A2	S	S	S	R	R	S	S	17 (17.52)
A3	S	S	S	I	R	S	S	14 (14.43)
A4	S	S	S	R	I	S	S	11 (11.34)
A5	R	R	S	R	I	S	S	9 (9.27)
A6	R	R	S	R	R	S	S	8 (8.24)
A7	S	S	S	S	I	S	S	5 (5.15)
A8	R	R	S	R	I	S	R	4 (4.12)
A9	S	S	S	S	S	S	S	3 (3.09)
A10	R	R	S	R	R	S	R	3 (3.09)

A1–A10: antibiotypes, S: susceptible, I:intermediate resistant; R:resistant; AMO: amoxicillin, AMP:ampicillin, ENR: enrofloxacin, ERT: erythromycin, NEO: neomycin, OXY: oxytetracycline, TRM: trimethoprim.

ship of the whole cell protein profiles of SC1 (2 strains, 2.1%) and SC2 (3 strains, 3.1%) subtypes belonging to the same large cluster was 83%. The relatedness of the remaining strains which were included in two unique types, SA and SB were 69% and 72%, respectively.

In this study, the major bands observed after SDS-PAGE analysis of the whole cell proteins of *Y. ruckeri* isolates were between 36 and 116 kDa. Approximately 41, 47, 53, 56, 66, 86, 98, 114 and 153kDa of bands were detected as major bands in the groups. SA included 3 major bands of 47, 66 and 86 kDa. SB included 5 major bands of 41, 47, 53, 66 and 114 kDa. SC1 included 4 major bands of 41, 53, 66 and 114 kDa. SC2 included 2 major bands of 47 and 66 kDa. SC3 included 6 major bands of 41, 53, 56, 66, 98 and 153 kDa. SC4 included 6 major bands of 41, 53, 56, 66, 98 and 114 kDa. The SDS-PAGE patterns and phylophenotic tree of each type are illustrated in Figure 2.

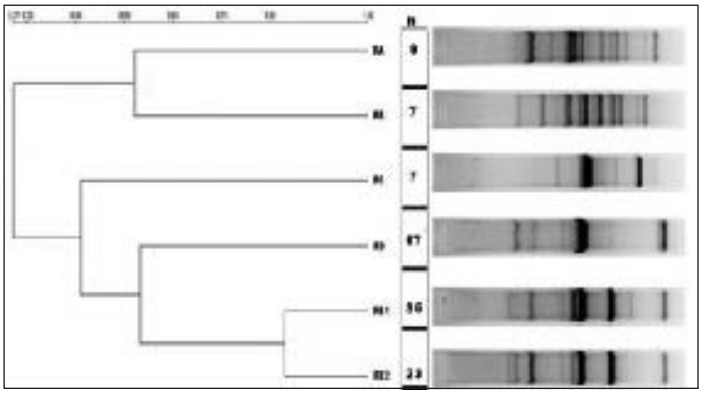

FIGURE 1: *The phylogenetic tree of genotypes of Y. ruckeri isolates with RAPD-PCR pattern similarities.*

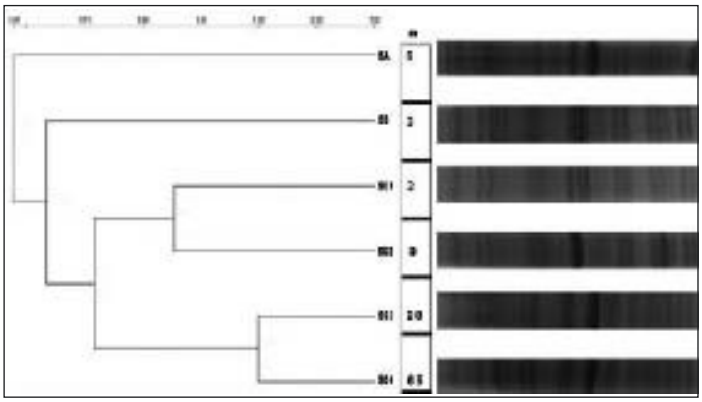

FIGURE 2: *The representation of dendogram and whole-cell protein profiles of Y. ruckeri isolates with SDS-PAGE pattern similarities.*

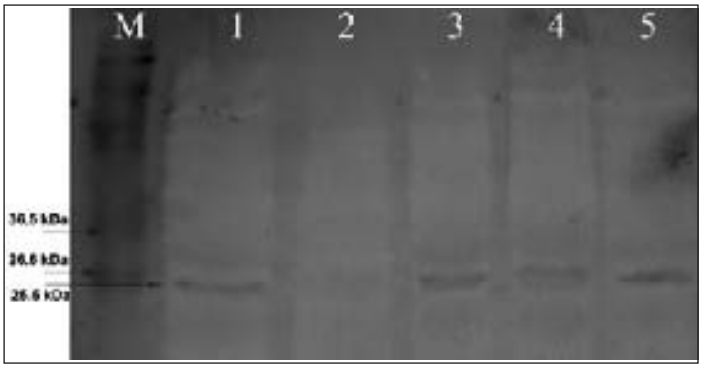

FIGURE 3: *The glycoprotein profile of Y. ruckeri isolates on PVDF membrane.*

Glycoprotein detection

The glycoprotein detection performed by an enzyme immunoassay using a commercial kit revealed a single 25.5 kDa major band common in all isolates (Fig. 3.).

The reproducibility, discriminatory power and confidence intervals of typing methods

The reproducibility of RAPD-PCR was found as 100%. Also, it was shown that the differences in SDS-PAGE band patterns were highly reproducible. The DI and CI for each typing methods and their combinations are summarized in Table 3.

Discussion

Several typing schemes have been created based on phenotypic bacterial characteristics. Strains of *Y. ruckeri* could be characterized and divided into two biotypes based on their sorbitol fermentation capability (Tobback et al., 2007). Also, 5 different serovar (O1, O2, O5, O6 and O7) have been distinguished based on their heat stable O-antigens (Davies, 1990). Apart from these typing techniques, antibiotic resistance properties dependent on antibiotyping is also classified as a phenotypical bacterial typing method which has been used in epidemiological studies. Antimicrobial resistance patterns are valuable as a guide to empirical therapy, as a typing method, and as an indicator of dissemination of antimicrobial resistance determinants (Alcoba-Flórez et al., 2005). The determination of antibiotic resistance patterns might provide important information towards specific control measures. De Grandis and Stevenson (1985) have determined antibiotic susceptibility patterns of fifty *Y. ruckeri* strains and reported that all strains shared a uniform pattern of susceptibility to many of the antibiotics tested. Almost the same antibiotic susceptibility patterns were found in all 97 *Y. ruckeri* strains tested in this study to enrofloxacin, oxytetracycline and trimethoprim. They have reported that erythromycin showed poor activity against all *Y. ruckeri*, whereas tetracycline, oxytetracycline and trimethoprim were included in the group containing the most active antibiotics against these strains. The strains in this study were variable for erythromycin susceptibility and mostly found to show intermediate and resistance patterns. Although *Y. ruckeri* is sensitive to many antibiotics, acquired resistance of *Y. ruckeri* strains to various antimicrobial agents, especially oxytetracycline and sulphonamides has been reported (De Grandis and Stevenson, 1985). Kırkan et al. (2006) have found 30% and 40% resistance to tetracyclin of 10 *Y. ruckeri* isolates tested by Agar Dilution and E-test, respectively. They have also determined that *Y. ruckeri* strains showed increased resistances to trimethoprim-sulphamethaxazole. However, in this study all strains were found susceptible to oxytetracycline and almost all strains except seven strains were susceptible to trimethoprim. These different results concerning tetracylin and trimethoprim-sulphamethaxazole may be due to different methods used. Kırkan et al. (2000) have reported that all *Y. ruckeri* strains (8 isolates) in their study pre-

TABLE 3: *Discriminatory indices (with Confidence intervals) of the typing methods used in this study*

Typing methods	Number of types	Discriminatory indices with Confidence intervals (%)
AT	10	0.86 (82–89%)
SDS-PAGE	6	0.55 (40–69%)
RAPD-PCR	6	0.77 (70–83%)
AT+SDS-PAGE	15	0.88 (82–93%)
AT+ RAPD-PCR	19	0.93 (89–96%)
SDS-PAGE+ RAPD-PCR	11	0.83 (76–89%)
AT+SDS-PAGE+ RAPD-PCR	23	0.94 (91–97%)

AT: antibiotyping; SDS-PAGE: sodium dodecylsulphate polyacrylamide gel electrophoresis; RAPD-PCR: randomly amplified polymorphic DNA-polymerase chain reaction.

sented resistance to erythromycin and ampicillin. In this study only 24 (24.7%) of our isolates showed ampicillin resistance, whereas most of strains (53.6%) were found to be resistant to erythromycin. Antibiotic resistance may occur due to exposure to inadequate, variable dosage, incompleteness of treatment of recommended course, and repeated short-term antibiotic treatment. Enrofloxacin, oxytetracyclin and trimethoprim are commonly used for treatment of bacterial fish diseases in Turkey. It is considered that in this study, the general lack of resistance to enrofloxacin and oxitetracyclin and 7.2% of resistance to trimethoprim observed among *Y. ruckeri* isolates may be due to the absence of misuse of these antibiotics in rainbow trout of fish farms. According to antibiotyping results (Tab. 1) the most prevalent antibiotype among our isolates was A1 (23.71%) and the closest follower was A2 (17.52%). Although A1 and A2 types were distributed among four different regions, these were more frequently observed in Black sea and Meditterrenaen than in the other regions. The antibiotypes that show resistance against at least four antibiotics were A6, A8 and A10. These antibiotypes were not observed in Aegean and were also generally less observed among the other regions. There was not so many antibiotyping studies on *Y. ruckeri* isolates from rainbow trout in Turkey and this study may be useful to choose correct antibiotic for treatment of ERM observed in different regions of Turkey.Several biochemical tests are used to identify *Y. ruckeri* isolates, however it has been reported that there is an uniformity in biochemical reactions in *Y. ruckeri* strains (Joh et al., 2010). Some biochemical characteristics such as acetoin production (VP test), gelatin hydrolysis, sorbitol fermentation and Tween 80 hydrolysis have been reported to be variable among *Y. ruckeri* strains (Furones et al., 1993). In a study, Altun et al. (2010) have reported that all *Y. ruckeri* isolates from different geographical regions of Turkey were homogenous for biochemical parameters examined by conventional tests. Similarly in this study, no differences were found among all isolates for biochemical reactions examined by classical biochemical tests.

To classify and identify the microorganisms, SDS-PAGE of cell proteins is used increasingly, nowadays. Protein band patterns are usually compared visually but computerized comparisons of electrophoretic protein patterns can be a fast, easy and powerful tool for classification and identification of bacteria (Kersters and De Ley, 1975). SDS-PAGE patterns can be obtained in very short times, are reproducible and do not require any sophisticated and expensive reagents or equipment, as compared with molecular biology kits (Massa et al., 2007). Studies on the characterization of *Y. ruckeri* strains by analysis of their whole cell protein patterns were limited. Some researchers have typed *Y. ruckeri* isolates according to the outer-membran protein (OMP) profiles and different numbers of OMP types have been identified among *Y. ruckeri* isolates (Romalde et al., 1993; Sousa et al., 2001). Several studies on outer membran protein (OMP) analysis have been conducted (Coquet et al., 2002; Sousa et al., 2001). It has been reported that SDS-PAGE technique is chosen in preference to OMP profile analysis because it is more discriminatory than OMP typing and because of an advantage in that a single technical manipulation provides more markers than by the OMP typing techniques (Paterson et al., 1987). In a study, it has been reported that all the *Y. ruckeri* isolates examined showed very similar profiles and three different profiles could be distinguished shared by serotypes examined (Bastardo et al., 2009). They have detected the major bands of O1a and O1b serotypes approximately between 38 and 41 kDa and a range of 35 and 38 kDa of serotype O2b. In this study, the major bands after SDS-PAGE analysis of the whole cell proteins of *Y. ruckeri* isolates were observed between 36 and 116 kDa. Approximately 41, 47, 53, 56, 66, 86, 98, 114 and 153kDa of bands were detected as major bands in groups. The most of the isolates (92.8%) were assigned to a cluster. In this cluster the majority of the isolates assigned to SC3 and SC4 showed a similarity of 90%. Although it was observed some variability in whole cell protein profiles of *Y. ruckeri* isolates examined in this study, most of the isolates were found to have homogenous SDS-PAGE profiles. None of the isolates except from the isolates grouped in SC2 showed a clear regional specifity. SC2 type (included only three isolates) was observed only in Blacksea. However it should be remembered that most of the isolates originated from the Black sea. SDS-PAGE analysis of the whole cell proteins provided a reproducible and typeable method for characterization of *Y. ruckeri* strains. However, the discrimination power of SDS-PAGE typing for *Y. ruckeri* strains was low. Therefore SDS-PAGE was considered to be unsuitable as a stand-alone method for typing *Y. ruckeri* isolates. It should be used together with the other typing methods.

The presence of glycoprotein(s) in procaryotes has recently been accepted. Bacterial glycoproteins have been reported to be comprising a wide range of different cell envelope components as well as secreted glycoproteins and exoenzymes (Messner, 1997; Cicek and Kirkan, 2010). To the best of our knowledge, any report on the detection of glycoproteins of *Y. ruckeri* isolates has not been found in the literature. Several pathogens such as Neisseria, Mycobacteria and Streptococci synthesize glycoproteins some of which may play a critical role in pathogenesis. Similarly a glycoprotein of enterotoxigenic E. coli, TibA, has been characterized as an adhesion factor (Lindenthal and Elsinghorst, 2001). In this study, the approximately 25.5 kDa of glycoprotein was detected in all *Y. ruckeri* isolates. Because of having probability to use of the glycoproteins in development of diagnostic kits or vaccines, it might be important to characterize the antigenic potential of this glycoprotein and further studies are needed for this aim.

Phenotypical techniques used for typing various bacteria are time consuming, laborious and expensive (Frazin and Cabodi, 2003), furthermore the homogeneity in some phenotypic characteristics determined among *Y. ruckeri* isolates may bring about difficulties in epidemio-

logical studies. PCR-based genotyping methods such as PCR-RFLP, PCR-ribotyping, REP (Repetitive extragenic palindromic)-PCR, ERIC (Enterobacterial repetitive intergenic)-PCR, RAPD-PCR and DNA amplification fingerprinting have played an important role in bacterial typing schemes for the last two decades (Frazin and Cabodi, 2003). However these techniques have rarely been used to characterize the fish pathogens compared to both human and amimal pathogens (Lucangeli et al., 2000). Until now, plasmid profile analysis, ribotyping (Garcia et al., 1998; Sousa et al., 2001), Pulsed-field gel electrophoresis (PFGE) (Garcia et al., 1998; Lucangeli et al., 2000), PCR-based fingerprinting (Argenton et al., 1996; Coquet et al., 2002; Lucangeli et al., 2000) have been used successfully as alternatives to the phenotyping techniques for genotyping of *Y. ruckeri* strains. RAPD typing, also known as arbitrarily primed-polymerase chain reaction (AP-PCR), is one of the useful typing technique that uses single oligonucleotides of arbitrarily chosen sequence as primers for DNA synthesis and that generates strain-specific arrays of amplified DNA fragments (Ozer et al., 2008). It has been described as a rapid and simple method which has been successfully used for genetic fingerprinting and molecular typing for microorganisms such as *Lactobacillus*, *Salmonella*, *E. coli*, yeast, *Bacillus* (Gao et al., 1996; Zulkifli et al., 2009).

Enterobacterial repetitive intergenic consensus (ERIC2) sequence and M13 primers have succesfully been used as universal primers to type various species of bacteria (Tazumi et al., 2009). The position of ERIC elements in enterobacterial genomes varies between different species and has been used as a genetic marker to characterize isolates within a bacterial species (Versalovic et al., 1991). RAPD-PCR has been considered as an alternative to PFGE (Tazumi et al., 2009). RAPD-PCR has been reported to give an advantage in which molecular preliminary information of the species is not neccessary and clonal relatedness can be determined rapidly and cheap. The RAPD data could be helpful for taking some measures against micoorganisms in point. If necessary the RAPD data may confirm by PFGE and constitute a base for fine-tuning the sanitary or clinical measures (Bastardo et al., 2009; Renders et al., 1996). In this study, ERIC2 primer was used to perform RAPD-PCR analysis of *Y. ruckeri* strains. In previous studies, ERIC2 primer has been used to differentiate the subspecies of P. aeruginosa (Clarke et al., 2008; Renders et al., 1996). Some researchers, have used ERIC primers in Interspersed repetitive sequence (IRS)-PCR to characterize several bacterial pathogens (Rivera et al., 1995; Versalovic et al., 1991). Lucangeli et al. (2000) have also used ERIC2 primer for determining the patterns of IRS-PCR of both *Y. ruckeri* and Photobacterium damsela and they have found that this method was not capable of discriminating among *Y. ruckeri* serovar O1 strains. These researchers had reported that a single profile was shown for all *Y. ruckeri* isolates analysed by IRS-PCR. No report has been found on RAPD typing of *Y. ruckeri* strains using ERIC2 primer. In this study, *Y. ruckeri* isolates were grouped into 6 RAPD types at 75% similarity level. The great majority of *Y. ruckeri* strains were assigned to RE1 and RE2 and these two types showed 83% genetic similairty. Except from RE1 and RE2 which were assigned to the same cluster, the similarity between RAPD types varied from 27 to 53%. Only a small proportion of the strains (19.5%) were genetically less related. The majority of strains that caused outbreaks in Central Anatolia and Black Sea were found to assign to prevalent (dominant) types, RE1 and RE2, and these strains were closely related. The half of (15/30) and 54.54% (6/11) of the isolates grouped in RA, RB, RC and RD from Mediterranean and Aegean, respectively, showed more variability than Black sea and Central Anatolia isolates grouped in these types. RA and RC types were not observed in Central Anatolia. However, generally all RAPD types of *Y. ruckeri* strains were found to distribute among all regions. This may mean that transfer of fish populations between different farms has occured.

In this study, according to the discriminative powers of the typing methods, the most discriminative method for typing of *Y. ruckeri* isolates was antibiotyping (DI = 0.86) followed by RAPD (DI = 0.77) and SDS-PAGE (DI = 0.55) typing. However, the combination of these typing methods with each other increased the discriminatory capability. The most discriminative combination was ATB+SDS-PAGE+RAPD-PCR (DI = 0.94) which was slightly higher than ATB+RAPD-PCR (DI = 0.93). These results showed that antibiotyping was a valuable typing method, especially in combination with the other techniques applied in this study. However, due to the development of acquired antibiotic resistance, an identical strain may show different typing patterns. Although the DI of antibiotyping was high, this method alone should not be considered as an invaluable method for epidemiological studies. SDS-PAGE typing was much poorer for discrimination than the others and it was considered that this method should be used together with any of the typing techniques mentioned in this study.

In conclusion, *Y. ruckeri* strains isolated from rainbow trout in fish farms in different regions of Turkey showed a limited variation according to their whole cell SDS-PAGE profile and RAPD profiles. High variation among strains by antibiotypes may be due to the possibility of the emergence of antibiotic resistance. It was considered that the use of the typing techniques in double and triple combination with each others could be more useful for discriminating the strains. This is the first report of the typing of *Y. ruckeri* strains by their SDS-PAGE, antibiotic susceptibility and RAPD profiles in different regions of Turkey. Also, this is the first report about glycoprotein presence of *Y. ruckeri*. The determination of the antigenic potential of this glycoprotein was considered to be needed to reveal its availability for the development of diagnostic kits or vaccines. For this reason, further studies should be performed. The results obtained from this study can provide initial information about the variability of *Y. ruckeri* isolates in some regions of Turkey and can lead to taking measures to control infections caused by *Y. ruckeri* providing opinion on the vaccine development.

Conflict of interest

We have no protected, financial, occupational or other personal interests in a product, service and/or a company which cold influence the content or opinions presented in the manuscript.

References

Alcoba-Flórez J, Pérez-Roth E, González-Linares S, Méndez-Álvarez S (2005): Outbreak of Shigella sonnei in a rural hotel in La Gomera, Canary Islands, Spain. Int Microbiol 8: 133–136.

Altun S, Kubilay A, Diler, Ö (2010): Investigation of phenotypical and serological properties of *Yersinia ruckeri* strains. Kafkas Univ Vet Fak Derg 16 (suppl B): 223–229.

Argenton F, De Mas S, Malocco C, Dalla Valle L, Giorgetti G, Colombo L, (1996): Use of random DNA amplification to generate specific molecular probes for hybridization tests and PCR-based diagnosis of *Yersinia ruckeri*. Dis Aquat Org 24: 121–127.

Austin B, Austin DA (2007): Diagnosis. In: Bacterial Fish Pathogens Disease in Farmed and Wild Fish, 3rd (Revised) ed., Praxis, 215–235,

Bastardo A, Bohle H, Toranzo AE, Romalde JL (2009): Phenotypical, serological and molecular characterization of *Yersinia ruckeri* strains isolated from Atlantic salmon (Salmo salar) farmed in Chile. In: Diseases of Fish and Shelfish, 14th EAFP International Conference Praque, September 14–19, 319–320.

Cicek E, Kirkan S (2010): Bacterial glycoproteins. Journal of Bornova Vet Cont Res Inst 32: 51–58.

Clarke L, Moore JE, Millar BC, Crowe M, Xu J, Goldsmith CE, Murphy RG, Dooley JS, Rendall J, Elborn JS (2008): Molecular epidemiology of Pseudomonas aeruginosa in adult patients with cystic fibrosis in Northern Ireland. Brit J Biomed Sci 65: 18–21.

Coquet L, Cosette P, Quillet L, Petit F, Junter GA, Jouenne T (2002): Occurrence and phenotypic characterization of *Yersinia ruckeri* strains with biofilm-forming capacity in a rainbow trout farm. Appl Environ Microbiol 68: 470–475.

Davies RL, Frerichs GN (1989): Morphological and biochemical differences among isolates of *Yersinia ruckeri* obtained from wide geographical areas. J Fish Dis 12(4): 357–365.

Davies RL (1990): O-serotyping of *Yersinia ruckeri* with special emphasis on European isolates. Vet Microbiol 22: 299–307.

De Grandis SA, Stevenson RMW (1985): Antimicrobial susceptibility patterns and R plasmid-mediated resistance of the fish pathogen *Yersinia ruckeri*. Antimicrob Agent Chemother 27: 938–942.

Frazin L, Cabodi D (2003): Molecular typing of *Yersinia* strains by pulsed-field gel electrophoresis and RAPD-PCR. In: Advances in Experimental Medicine and Biology, Kluwer Academic/Plenum Publishers, New York, 349–352.

Furones MD, Rodgers, CJ, Munn CB (1993): *Yersinia ruckeri,* the causal agent of enteric redmouth disease (ERM) in fish. Ann Rev Fish Dis 3: 105–125.

Gao Z, Jackson KM, Leslie DE (1996): Pitfalls in the use of random amplified polymorphic DNA (RAPD) for fingerprinting of Gram negative organisms. Pathol 28: 173–177.

Garcia JA, Dominguez L, Larsen K, Pedersen K (1998): Ribotyping and plasmid profiling of *Yersinia ruckeri*. J Appl Microbiol 85(6): 949–955.

Gibello A, Blanco MM, Moreno MA, Cutul MT, Domenech A, Dominguez L, Fernandez-Garayzabal JF (1999): Development of a PCR assay for detection of *Yersinia ruckeri* in tissues of inoculated and naturally infected trout. Appl Environ Microbiol 65: 346–350.

Grundmann H, Hori S, Taner G (2001): Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. J Clin Microbiol 39: 4190–4192.

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994): Facultatively anaerobic Gram negative rods. In: Bergey's Manual of Determinative Bacteriology, 9th ed., 175–289. Williams and Wilkins, Baltimore, MD

Hunter PR, Gaston MA (1988): Numerical index of the discriminatory ability of typing systems: An application of Simpson's index of diversity. J Clin Microbiol 26: 2465–2466.

Joh SJ, Kweon CH, Kim MJ, Kang MS, Jang H, Kwon JH (2010): Characterization of *Yersinia ruckeri* isolated from the farm-cultured eel Anguilla japonica in Korea. Korean J Vet Res 50: 29–35.

Kapperud G, Nesbakken T, Aleksic S, Mollaret HH (1990): Comparison of restriction endonuclease analysis and phenotypic typing methods for differentiation of *Yersinia enterocolitica* isolates. J Clin Microbiol 28: 1125–1131.

Kersters K, De Ley J (1975): Identification and grouping of bacteria by numerical analysis of their electrophoretic protein patterns. J Gen Microbiol 87: 333–342.

Kirkan S, Goksoy EO, Kaya O (2000): The isolation of *Yersinia ruckeri* from rainbow trouts in Aydin region. J Pendik Vet Microbiol 31: 27–30.

Kirkan S, Goksoy EO, Kaya O, Tekbiyik S (2006): In-vitro antimicrobial susceptibility of pathogenic bacteria in rainbow trout (*Oncorhynchus mykiss*, Walbaum). Turk J Vet Anim Sci 30: 337–341.

Laemmli K (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685.

LeJeune JT, Rurangirwa FR (2000): Polymerase chain reaction for definitive identification of *Yersinia ruckeri*. J Vet Diagn Invest 12: 558–561.

Lindenthal C, Elsinghorst EA (2001): Enterotoxigenic *Escherichia coli* tibA glycoprotein adheres to human ıntestine epithelial cells. Infect Immun 69: 52–57.

Lucangeli C, Morabito S, Caprioli A, Achene L, Busani L, Mazzolini E, Fabris A, Macri A (2000): Molecular fingerprinting of strains of *Yersinia ruckeri* serovar O1 and Photobacterium damsela subsp. piscicida isolated in Italy. Vet Microbiol 76: 273–281.

Massa R, Bantar C, Lopardo H, Vay C, Gutkind G (2007): Whole-cell protein profiles are useful for distinguishing enterococcal species recovered from clinical specimens. Braz J Microbiol 39: 199–203.

Messner P (1997): Bacterial glycoproteins. Glycoconjugate J 14: 3–11.

National Committee for Clinical Laboratory Standards (2003): Performance standards for antimicrobial susceptibility testing; fourteenth informational supplement: M2–A8 performance standards for antimicrobial disk susceptibility test; approved standard. 8th ed. National Committee for Clinical Laboratory Standards, Wayne, PA.

Ozer S, Bulduklu P, Dönmez E, Koyuncu E, Serin MS, Aslan G, Tezcan S, Aydin E, Emekdas G (2008): Phenotypic and genetic homogeneity of *Yersinia ruckeri* strains isolated from farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum), in Mersin Province, Turkey. Bull Eur Assoc Fish Pathol 28: 97–104.

Paterson AJ, Macsween KF, Pennington TH (1987): Haemophilus influenzae subtyping by SDS-PAGE of whole-cell polypeptides. Epidemiol Infect 99: 179–189.

Plikaytis BD, Carlone GM, Plikaytis BB (1986): Numerical analysis of normalized whole-cell protein profiles after Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis. J Gen Microbiol 132: 2653–2660.

Pyle SW, Schill WB (1987): Further characterization of biochemical and serological characteristics of *Yersinia ruckeri* from different geographic areas. Microbiol Lett 35: 87–93.

Renders N, Römling U, Verbrugh H, Van Belkum A (1996): Comparative typing of Pseudomonas aeruginosa by random amplification of polymorphic DNA or pulsed-field gel electrophoresis of DNAmacrorestriction fragments. J Clin Microbiol 34: 3190–3195.

Rivera IG, Chowdhury MAR, Huq A, Jacobs D, Martins MT, Colwell RR (1995): Enterobacterial repetitive intergenic consensus sequences and the PCR to generate fingerprints of genomic DNAs from Vibrio cholerae O1, O139, and non-O1 strains. Appl Environ Microbiol 61: 2898–2904.

Romalde JL, Magarınos B, Barja JL, Toranzo AE (1993): Antigenic and molecular characterization of *Yersinia ruckeri* proposal for a new intraspecies classification. Syst Appl Microbiol 16: 411–419.

Sousa JA, Magariæos B, Eiras JC, Toranzo AE, Romalde JL (2001): Molecular characterization of Portuguese strains of *Yersinia ruckeri* isolated from fish culture systems. J Fish Dis 24: 151–159.

Tan YF, Haresh KK, Chai LC, Son R (2009): Antibiotic susceptibility and genotyping by RAPD of Campylobacter jejuni isolated from retailed ready-to-eat sushi. Int J Food Res J 16: 31–38.

Tazumi A, Maeda Y, Buckley T, Millar BC, Goldsmit CE, Dooley JSG, Elborn JS, Matsuda M, Moore JE (2009): Molecular epidemiology of clinical isolates of *Pseudomas aeruginosa* isolated from horses in Ireland. Irish Vet J 62: 456–459.

Tobback E, Decostere A, Hermans K, Haesbrouck F, Chiers K (2007): *Yersinia ruckeri* infections in salmonid fish. J Fish Dis 30: 257–268.

Upreti RK, Kumar M, Shankar V (2003): Bacterial glycoproteins: functions, biosynthesis and applications. Proteomics 3: 363–379.

Versalovic J, Koeuth T, Lupski R (1991): Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nuc Acid Res 19: 6823–6831.

Zulkifli Y, Alitheen NB, Son R, Raha AR, Samuel L, Yeap SK, Nishibuchi M (2009): Random amplified polymorphic DNA-PCR and ERIC PCR analysis on Vibrio parahaemolyticus isolated from cockles in Padang, Indonesia. Int Food Res J 16: 141–150.

AUTORKOREKTUR

Address for correspondence:
Alper CIFTCI, DVM, PhD
University of Ondokuz Mayıs
Faculty of Veterinary Medicine
Department of Microbiology
55139, Kurupelit/Samsun
Turkey
aciftci@omu.edu.tr