



## Fatty Acid Composition in *Yersinia ruckeri* Strains Isolated from Rainbow Trout Farms

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### ABSTRACT

In this study, a total of 33 *Yersinia ruckeri* isolates were obtained from rainbow trout farms in the five different region of Turkey. We determined to changes in fatty acids group of the strains. All the isolates were identified as *Y. ruckeri* based on colonial, cellular morphology and biochemical characters. All *Y. ruckeri* 8 major fatty acids, including 12:0 (Lauric acid), 14:0 (Myristic acid), 16:0 (Palmitic acid), 16:1n-7 (Palmitoleic acid), 17:1 (Heptadecanoic acid), 18:1n-9c (Oleic acid), 18:2n-6c (Linoleic acid), 18:3n-3c (Alfa-linoleic acid). Compared to isolates biochemical property, there was no difference between fatty acids and biochemical characteristics. The results of this study showed that the fatty acids composition of *Y. ruckeri* isolated strains from rainbow trout farms in Turkey is on a large scale homogenous.

### Research Article

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## Gökkuşığı Alabalığı Yetiştiricilik Tesislerinden İzole Edilen *Yersinia Ruckeri* Suşlarının Yağ Asit Kompozisyonu

### ÖZET

Bu çalışmada Türkiye'nin beş farklı bölgesindeki gökkuşığı alabalığı yetiştiricilik tesislerinden izole edilen 33 *Yersinia ruckeri* yağ asit miktarlarındaki değişimler incelenmiştir. Bütün suşların koloni ve hücre morfolojisi ile biyokimyasal özellikleri yönünden incelenmesi sonucu *Y. ruckeri* olduğu ortaya konulmuştur. Bütün *Y. ruckeri* suşlarında 12:0 (Laurik asit), 14:0 (Miristik asit), 16:0 (Palmitik asit), 16:1n-7 (Palmitoleik asit), 17:1 (Heptadekanoik asit), 18:1n-9c (Oleik asit), 18:2n-6c (Linoleik asit), 18:3n-3c (Alfa-linoleik asit) gibi 8 önemli yağ asidi tespit edilmiştir. İzolatların biyokimyasal özellikleri karşılaştırılmıştır. Yağ asidi ve biyokimyasal özellikler arasında farklılık bulunamamıştır. Bu çalışmada Türkiye'deki gökkuşığı alabalığı yetiştiricilik tesislerinden izole edilen *Y. ruckeri* suşlarının yağ asidi kompozisyonunun büyük oranda homojen olduğu görülmüştür.

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### INTRODUCTION

Enteric red mouth disease, known as ERM or Yersiniosis, was first reported in 1950's in the rainbow trout farms in the USA as a septicemic disease with high mortality rates in sub-acute and acute forms (Bullock and Anderson, 1984; Busch, 1982; Frerichs and Roberts, 1989; Lucangeli et al., 2000; Ross et al., 1966). ERM disease is widespread in many parts of the world (Bullock et al., 1977; Roberts, 1983; Lesel et al., 1983; Stevenson and Daly, 1982).

In Turkey, Yersiniosis caused by *Yersinia ruckeri* was identified in rainbow trouts in 1991. As the trout farms

increased in number, the disease became more widespread, starting to cause economic losses. This disease is currently frequently found in trout farms and has become the most important bacterial disease in Turkey (Çağırğan and Yürekli Türk, 1991; Kubilay, 1997; Kubilay and Diler, 1999; Timur and Timur, 1991).

A standardized and rapid identification system is offered by the API 20E system. Miniaturized multitest system was originally developed for the identification of Gram-negative enteric bacteria in clinical laboratories. During the last decade, this system has been increasingly used for the identification of marine

and fresh water fish pathogens (Tanrıkuş et al., 2004). Lipids are one of the most basic organic compounds in bacteria, as in other living things in the world. Lipids, which play an important role in the functions of cytoplasmic membranes, are affected by different environmental factors such as temperature, surface tension, osmotic pressure and pH. Fatty acids that make up lipids in defining and characterizing bacteria are accepted as one of the basic taxonomic criteria. It is considered that free fatty acid complexes, which are used in bacterial systematics, will be a good chemotaxonomic property in different growth environments in terms of microbial ecology (Karıptas, 1999).

The detection of the fatty acids of the bacteria using gas chromatography dates back to the 1960s. It has been suggested that mobile *Aeromonas* strains of clinical and non-clinical origin can be classified by liquid-gas chromatography according to their fatty acid metal ester profiles (Canonica and Pisono, 1985).

In this study, the analysis of the heterogeneity of *Y. ruckeri* isolated from fish in the trout farming plants in different regions of Turkey was carried out in order to reveal their fatty acid profiles. For this purpose, the biochemical properties of the strains used in the study,

in addition to the traditional biochemical tests, the relationship of epidemiological characters with the fatty acid composition were revealed.

## MATERIAL and METHODS

### Bacteria

*Y. ruckeri* (33) isolates used in the study were isolated from classical fish farms in different regions of Turkey between 2005 and 2014 by classical methods. All strains were cultured at 25°C using Tryptic Soy Agar (TSA). Isolates that were revealed to be *Y. ruckeri* by biochemical tests were confirmed using the Polymerase Chain Reaction (PCR) method reported by Şeker et al. (2012). Sowing was performed in Shotts-Waltman Medium (SWM) and Ribose Ornithine Deoxycholate (ROD) selective fattening sites.

Isolates, which were previously isolated and added to glycerin in Tryptic Strain Broth (TSB), kept at -20°C, were freshly cultured in Tryptic Strain Agar (TSA) for sterility control before the study started, and it was checked whether the strains were pure. DSM18506 was used as the reference strain. Table 1 shows the geographical distribution of the isolates used in the study.

Table 1. The gathering places of *Y. ruckeri* used in the study

#### *Çizelge 1. Çalışmada kullanılan Y. ruckeri'nin toplanma yerleri*

Isolate no	Collection date	Region-city	Organ-isolated location
1-6	2005, 2014	Malatya	Kidney-lesion
7	2011	Aydın	Kidney
8	2012	Rize	?????
9-10	2008, 2012	Kayseri	Kidney
11-23	2005-2014	Elazığ	Kidney, spleen-lesion
24,25	2009	Mersin	?????
26-30	?????	Isparta	?????
31, 32	2011	Artvin	Liver
33	2010	Muğla	Liver

### Fatty acid extraction and analysis

Bacteria were incubated in 100ml TSB placed in 500ml flasks at 25 °C for 48 hours and collected by centrifugation at 2500 rpm for 10 minutes. The bacteria were washed twice with 0.85% NaCl and kept at room temperature for 2 hours. For hydrolysis of approximately 55 mg bacteria biomass, NaOH and 1 ml of methanol mixture were added and incubated for 30 minutes in a water bath at 95°C. Then, 2 ml of 6 N HCl prepared in methanol was added to methylation of free fatty acids and kept for 10 minutes in a water bath at 80°C. The aqueous phase for fatty acid extraction was collected and 1.15 ml of the hexane/methyl tert-butyl ether (1:1) mixture was added to each tube and inverted for 10 minutes. After the supernatant was removed, 3ml of 1.2% NaOH was added and shaken for 5 minutes. The supernatant organic phase was taken to the chromatography bottles (viallere) for fatty acid

determination.

Fatty acid analysis was carried out by using Ultra 2-HP (25m x 0,22 mm x 0,33mm) column and hydrogen gas in gas chromatography-mass spectrophotometer (GC-MS).

## RESULTS and DISCUSSION

It was determined that 33 *Y. ruckeri* isolates used in the study showed a homogeneous structure in terms of biochemical tests.

Results revealed that *Y. ruckeri* forms a ground glass-like zone due to the precipitation of calcium salts and hydrolysis of Tween 80 by forming green colored colonies in Shotts-Waltman medium (SWM) selective medium. *Y. ruckeri* was found to give yellow colonies in Ribose Ornithine Deoxycholate (ROD) medium as a result of fermentation of ribose and precipitation of sodium desoxycholate salts. The positive result of

isolate 7 in terms of Sorbitol test is that this strain has serotype 2 characteristics. This strain was immobile and found that it did not hydrolyze the tween 80.

It was determined that *Y. ruckeri* bacteria isolated from different regions of Turkey did not have a significant differences in fatty acid composition and

had a homogeneous structure. The composition of bacterial isolates to fatty acid is shown in Table 2. 8. Important fatty acids such as 12:0 (Lauric acid), 14:0 (Myristic acid), 16:0 (Palmitic acid), 16:1n-7 (Palmitoleic acid), 17:1 (Heptadecanoic acid), 18:1n-9c (Oleic acid), 18:2n-6c (Linoleic acid), 18:3n-3c (Alfa-linoleic acid) were detected in bacterial strains.

Table 2. Fatty acid levels of *Y. ruckeri* isolates.

*Çizelge 2. Y. ruckeri* izolatlarının yağ asit düzeyleri.

Fatty acids (%)								
Isolates	12:0	14:0	16:0	16:1n-7	17:1	18:1n-9c	18:2n-6c	18:3n-3c
1-6	4.98	1.32	22.00	0.34	0.55	1.38	10.05	2.0
7	4.99	0.97	24.95	1.02	1.09	1.41	12.45	2.59
8	5.3	1.39	19.90	0.29	0.55	1.37	11.00	2.33
9-10	5.09	1.30	19.95	0.30	0.54	1.38	10.95	2.53
11-23	4.98	1.33	21.00	0.30	0.51	1.37	10.95	2.51
24,25	5.06	1.40	21.53	0.32	0.51	1.36	11.00	2.71
26-30	5.12	1.42	19.89	0.32	0.55	1.36	11.02	2.65
31, 32	5.26	1.29	19.95	0.31	0.53	1.36	10.98	2.66
33	4.99	1.29	19.99	0.31	0.53	1.37	9.42	2.68
DSM18506	5.21	1.41	21.04	0.32	0.54	1.36	10.55	2.69

DSM18506: *Yersinia ruckeri* serotype O1 reference strain

Lipid compounds are abundant in the cell wall and cytoplasmic membrane of gram-negative microorganisms. Among the most common lipids are cyclopropane acid in *E.coli*, palmitic acid, cis-vaccenic acid, beta-hydroxy myristic acid in endotoxin of *E. coli*, beta-hydroxy acid in *P. aeruginosa*, cis-vaccenic acid in *Agrobacter tumefaciens*, palmitic acid in *S. marcescens* and 9, 10-methylen hexadecanvic acid, even and unsaturated fatty acids in microplasms, cholesterol and a small amount of caprylic acid and capric acid (Arda, 2000). In this study *Y. ruckeri* strains, 11 important fatty acids were detected such as 12:0 (Lauric acid), 14:0 (Miristik acid), 16:0 (Palmitik acid), 16:1n-7 (Palmitoleik acid), 17:1 (Heptadekanik acid), 18:1n-9c (Oleik acid), 18:2n-6c (Linoleik acid), 18:3n-3c (Alfa-linoleik acid).

Bacteria are the simplest and smallest microbial cells. Few of the bacteria with low oil production can synthesize the desired amount of oil (Denli and Tekin, 2000). Current information on the production of oil in bacteria is more related to pathogens. In the study conducted by Bunker (1963), *Mycobacterium* species stand out among the 5 microorganisms whose fat ratios vary between 7-21%. In a recent study (Beopoulos et al., 2009), it is stated that the total cell mass of *Rhodotorula glutinis* can store 72% fat. In the same article, although it is a very rare condition among bacteria, it is stated that *Rhodococcus opacus* bacteria can form a very high fat accumulation as 87%, but there is no information about the form of the oil. In this study, all of the samples are also pathogenic. Fat ratios obtained from samples range between 40.58% and 49.47% as with Malatya (1-6) 42.62%; Aydın (7) 49.47%; Rize (8) 42.13%; Kayseri (9-10) 42.04%; Elazığ

(11-23) 42.95%; Mersin (24-25) 43.89%; Isparta (26-30) 42.33%; Artvin (31-32) 42.34%; Muğla (33) 40.58%.

The examination of fatty acid profiles was performed on 33 *Yersinia ruckeri* species isolated from rainbow trout breeding facilities in 9 provinces in five different regions. As a result of gas chromatography analysis, fatty acid profile was determined as 12:0, 14:0, 16:0, 16:1n-7, 17:1, 18:1n-9c, 18:2n-6c, 18:3n-3c. When this results were compared to previous reports on FAME composition of *Y. ruckeri* isolates from the USA (Arias et al., 2007) and Fatty acid composition of *Yersinia ruckeri* isolates from aquaculture ponds in North West Germany (Huang et al., 2014), the fatty acids 12:0, 14:0 and 16:0 were found as main components in three studies. Jantzen and Lassen (1980) observed that strains from *Yersinia pseudotuberculosis*, *Yersinia enterocolitica* and *Yersinia pestis* all exhibited a similar fatty acid composition of 14:0, 16:1 and 16:0 and 16:0 was the most prominent fatty acid present in *Yersinia pestis* and *Yersinia pseudotuberculosis* (Tan et al., 2010). Canonica and Pisano (1988) reported in a study with *Aeromonas* species that they contain 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 16:1, 18:1, and 3-OH 14:0 fatty acids. Fatty acid contents of 10 Bacteria species of *Aeromonas* species isolated from 52 ice cream samples taken from ice cream in Kırşehir were examined and it was determined as 12:0, 14:0, 15:0, 15:0 3OH, 16:1, 16:0 ve 17:1, 17:0 3OH, 18:1 and 18:0 (Karıptaş and Yeniçeri, 2016). The fatty acid profile of *Y. ruckeri* obtained in this study with the fatty acid profiles determined as a result of studies with different species show (12:0, 14:0, 16:0 ve 17:1) similarities and (16:1n-7, 18:1n-9c, 18:2n-6c, 18:3n-3c) differences.



## CONCLUSION

Results of this study concluded that the fatty acid composition of *Y. ruckeri* strains isolated from rainbow trout farms in different geographic regions of Turkey revealed that *Y. ruckeri* strains sustain a very similar character in terms of fatty acid composition. It has been determined that serotype 1 strain is common in Turkey as in European countries. Since this research has been studied with *Y. ruckeri* strains isolated from businesses covering different geographic regions of Turkey, the results of the research obtained will contribute to future studies with this bacterium.

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## Researchers Contribution Rate Declaration Summary

The authors declare that they have contributed equally to the article.

## Conflicts of Interest Statement

None of the authors had any financial or personal relationships with other individuals or organizations that might inappropriately influence their work during the submission process.

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