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Comparison of molecular and biochemical heterogeneity of *Yersinia ruckeri* strains isolated from Turkey and the USA

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ABSTRACT

Forty isolates of *Yersinia ruckeri* from Italy (4), Turkey (24), the USA (10), and two type strains were compared by using biochemical test, pulsed-field gel electrophoresis (PFGE), and lipopolysaccharide (LPS) and outer membrane protein (OMP) profiles. Two biotype 1 and biotype 2 *Y. ruckeri* were determined based on motility and phospholipase activity. Twenty-two different pulse types were observed by cutting the DNA with *Spe*I restriction enzyme and running on PFGE. Four major clusters were generated using the UPGMA technique: cluster A contained Italian, Turkish and USA strains; cluster B included all the USA strains; and cluster C and cluster D contained all the Turkish strains. Based on the OMP profile, *Y. ruckeri* strains were divided into 4 clusters with 30 OMP types. A total of 30 different LPS types were observed. All the Italian, the USA, and most of the Turkish isolates were grouped together to form cluster A that consisted of 26 LPS types. It seems that all the four typing methods are highly discriminatory to distinguish even closely related strains. The overall similarities among the strains were 32.4 ± 6.1%, 58.7 ± 11.1%, and 79.5 ± 3.9% in LPS, PFGE, and OMP profiles, respectively. The PFGE, biochemical, OMP, and LPS profiles of none of the strains were found to be similar. Hence, each typing method showed its own discriminatory characteristics to distinguish between the individual strains. It is apparent that there is sufficient genetic diversity to justify using PFGE for analysis of horizontal transmission of *Y. ruckeri* in trout rearing facilities. It is possible that diverse environmental conditions resulted in a relatively high degree of genetic diversity in *Y. ruckeri*.

Statement of relevance

We believe that this manuscript entitled "Comparison of molecular and biochemical heterogeneity of *Yersinia ruckeri* strains isolated from Turkey and the USA" is relevant to aquaculture because *Y. ruckeri* is one of the most common bacterial fish diseases in salmonid culture. In the present study, *Y. ruckeri* strains were compared by using biochemical test, pulsed-field gel electrophoresis (PFGE), and lipopolysaccharide (LPS) and outer membrane protein (OMP) profiles to see which typing method has the most discriminatory power to distinguish strains in order to study epizootiology of yersiniosis.

1. Introduction

Yersinia ruckeri causes enteric redmouth disease (ERM) or yersiniosis, which is sometimes a problem in salmonid aquaculture. This bacterial species most commonly infects salmonids but has also been isolated from several non-salmonid fishes, earthworms, birds, and mammals (Willumsen, 1989; Horne & Barnes 1999), including one isolation from human bile (Farmer et al., 1985). It was first isolated in Idaho, USA, from rainbow trout *Oncorhynchus mykiss* (Ross et al., 1966) and has subsequently been found in most areas where salmonids are cultured including Turkey (Kayis et al., 2009; Ozturk and Altinok, 2014).

The phenotypic characteristics of *Y. ruckeri* have been widely studied to characterize the isolates. *Y. ruckeri* strains can be divided into two biotypes: biotype 1, generally motile and has phospholipase activity and isolates belonging to biotype 2 are nonmotile and do not have phospholipase activity (Davies and Frerichs, 1989). Traditionally, the diagnosis of this disease is carried out by agar cultivation and determining the phenotypic and serological properties of the pathogen (Pazos et al., 1996). Furthermore, some of the phenotypically similar bacteria such as *Cytophaga* and *Flavobacterium* could not be differentiated from each other by conventional diagnostic methods (Shewan and Mcmeekin, 1983). With the help of DNA-based approaches in recent decades, many genotypic methods have been used effectively in taxonomic and identification studies of a range of bacterial genera (Falcao et al., 2006; Kingston et al., 2009). Pulsed-field gel electrophoresis (PFGE) has only been used in epidemiological studies. Pulsed-field gel electrophoresis can be used to compare genotypic characteristics within the same species (Johnson et al., 1995). This method has high sensitivity, reproducibility, and discrimination ability compared with other molecular methods (Meays et al., 2004). In previous studies, biotype 2 *Y. ruckeri*

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isolated from Finland and UK was characterized by using PFGE (Strom-Bestor et al., 2010; Wheeler et al., 2009). As a result, little is known about the ability of different molecular typing methods to discriminate between different *Y. ruckeri* strains isolated from different countries. The objectives of this study were to describe the phenotypic and genetic characterization of *Y. ruckeri* isolates from rainbow trout with yersiniosis in Turkey between 1994 and 2010, and to compare Turkish strains with the strains of *Y. ruckeri* isolated from different cases of yersiniosis in the USA and Italy.

The cell envelope of Gram-negative bacteria is composed of the inner membrane and the outer membrane (OM). The OM consists of lipopolysaccharide (LPS), phospholipids, outer membrane proteins, and lipoproteins. Outer membrane proteins comprise almost 50% of the bacterial membranes of Gram-negative bacteria (Koebnik et al., 2000). Lipopolysaccharide is the major component of the outer membrane of Gram-negative bacteria, contributing greatly to the structural integrity of the bacteria, and protecting the membrane from certain kinds of chemical attack (Koebnik et al., 2000). The cell membrane is of crucial importance to Gram-negative bacteria, which upon mutation or removal leads to fatal consequences. The components of the bacterial capsules, LPS, and outer-membrane proteins are responsible for the complement resistance of bacteria (Rautemaa and Meri, 1999). The outer membrane proteins (OMPs) of Gram negative bacteria play an important role as virulence factors (Zierler and Galan, 1995) and are associated with pathogenicity and protective antigenicity (Lutwyche et al., 1995). These proteins have been used as epidemiological markers for animal pathogens (Barenkamp et al., 1981). Since LPS and outer membrane proteins (OMPs) have immunogenic properties (Hirst and Ellis, 1994; Kozinska and Guz, 2004), it is important to determine the virulent and avirulent strains based on OMP and LPS profiles. In the present study, we aimed to determine the LPS and outer membrane protein profiles of different strains and to compare them with PFGE and other biochemical tests.

2. Materials and methods

2.1. Bacterial isolates

Forty isolates of *Y. ruckeri* were studied (Table 1). Strains of *Y. ruckeri* were isolated from rainbow trout during epizootic outbreaks of yersiniosis during the period between 1994 and 2010, from different fish farms located in 7 different regions of Turkey (Turkey is divided into 7 geographic regions). *Y. ruckeri* isolated from Italy and the US was kindly provided by A. Manfrin (Istituto Zooprofilattico Sperimentale delle Venezie, Adria, Italy) and K. Hayden (Auburn University, Department of Fisheries and Allied Aquaculture, Auburn, AL), respectively. All the bacteria were subcultured on trypticase soy agar (TSA) to ensure purity of the culture.

2.2. Biochemical test

After purification, the following biochemical characteristics were used to identify *Y. ruckeri*: Gram staining, cytochrome oxidase, oxidation/fermentation, catalase, hemolysis on sheep blood agar,

Table 1
Yersinia ruckeri strains, sources, country, isolation year and pulse field type (PT), outer membrane type (OMT), lipopolysaccharide type (LPST) and biotype (BT). All Turkish strains were isolated from rainbow trout and from 7 different regions. All Turkish strains of *Y. ruckeri* were isolated from yersiniosis outbreaks.

Isolates (strains)	Country, city and regions	Year of isolation	Provided from	PT	OMT	LPST	BT
GA97126	USA	1997	K. Hayden	A1	O22	L4	2
GA99045	USA	1999	K. Hayden	A1	O21A	L4	1
GA9804	USA	1998	K. Hayden	A1	O24	L1	2
MSA3016	USA	2006	K. Hayden	A2	O23A	L8	1
ATCC29473	USA	1975	ATCC	B	O7	L9	1
MO688	USA	1997	K. Hayden	C	O24	L1	2
TMP089	Trabzon, Black Sea Region, Tr	2007	Laboratory collection	D	O8A	L18	2
TSE075	Trabzon, Black Sea Region, Tr	2007	Laboratory collection	E1	O8A	L5	2
RARDE071	Rize, Black Sea Region, Tr	2007	Laboratory collection	E1	O26	L29	1
TA0712	Trabzon, Black Sea Region, Tr	2007	Laboratory collection	E2	O9	L19	2
L14	Zonguldak, Black Sea Region, Tr	2006	Laboratory collection	E3	O8A	L5	1
TCO0710	Trabzon, Black Sea Region, Tr	2007	Laboratory collection	E3	O11	L6	1
691/97	Italy	1998	A. Manfrin	E3	O27	L15	2
NCIMB1315	USA	1965	NCIMB	E4	O5	L15	1
M22	Mugla, Aegean Region, Tr	1999	H. Cagirgan	F1	O25	L15	1
M45	Mugla, Aegean Region, Tr	2000	H. Cagirgan	F2	O2	L24	1
GA97016	USA	1997	K. Hayden	G	O12	L15	1
KM27	K. Maras, SE Anatolia Region, Tr	2002	Laboratory collection	H1	O10	L30	2
GA9707	USA	1997	K. Hayden	H2	O21A	L3	1
SAYr14	Sakarya, Marmara Region, Tr	2004	Laboratory collection	H3	O16A	L20	2
IYr2	Istanbul, Marmara Region, Tr	2008	N. Turk	H3	O4	L28	2
Yrnif	Izmir, Aegean Region, Tr	2000	N. Turk	H3	O17	L12	2
ANT10	Antalya, Mediterranean Region, Tr	2005	Laboratory collection	H3	O16A	L13	2
M72	Mugla, Aegean Region, Tr	2002	H. Cagirgan	H4	O16A	L20	1
M84	Mugla, Aegean Region, Tr	2005	H. Cagirgan	J	O19	L27	1
KA012	Kayseri, Central Anatolia Region, Tr	2008	Laboratory collection	K	O8A	L26	1
3019	Italy	2003	A. Manfrin	L1	O14	L6	1
ME128	Mersin, Mediterranean Region, Tr	2007	S. Ozer	L2	O3	L17	2
3020	Italy	2003	A. Manfrin	M	O13	L23	1
3018	Italy	2003	A. Manfrin	M	O8A	L11	1
ALG9488	USA	1994	K. Hayden	O1	O23A	L10	1
LV01	USA	2008	K. Hayden	O1	O16A	L2	1
ALG94883	USA	1994	K. Hayden	O2	O6	L10	2
ANT117	Antalya, Mediterranean Region, Tr	2003	Laboratory collection	P	O18	L22	2
TT072	Trabzon, Black Sea Region, Tr	2007	Laboratory collection	R	O30	L17	2
DEN12	Denizli, Aegean Region, Tr	2005	Laboratory collection	S	O1	L16	1
ME17	Mersin, Mediterranean, Tr	2007	S. Ozer	T	O28	L25	1
Yr118	Antalya, Mediterranean, Tr	2007	Laboratory collection	U	O20	L21A	2
EL14	Elazig, East Anatolia Region, Tr	2001	E. Seker	V	O29	L14	1
ISYr1	Isparta, Mediterranean Region, Tr	2006	Laboratory collection	Y	O8A	L7	2