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Re-identification of *Aeromonas* isolates from rainbow trout and incidence of class 1 integron and β-lactamase genes

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ABSTRACT

Forty-eight *Aeromonas* isolates from rainbow trout previously identified by the 16S rDNA-RFLP technique were re-identified using 2 housekeeping genes (*gyrB* and *rpoD*). After sequencing the prevalences of the species were *A. veronii* (29.2%), *A. bestiarum* (20.8%), *A. hydrophila* (16.7%), *A. sobria* (10.4%), *A. media* (8.3%), *A. popoffii* (6.2%), *A. allosaccharophila* (2.1%), *A. caviae* (2.1%), *A. salmonicida* (2.1%) and one isolate (2.1%) belongs to a candidate new species "*Aeromonas lusitana*". Coincident identification results to the 16S rDNA-RFLP technique were only obtained for 68.8% of the isolates. PCR amplification of the enterobacterial repetitive intergenic consensus (ERIC-PCR) indicated that the 48 isolates belonged to 33 different ERIC genotypes. Several genotypes were isolated from different farms and organs in the same fish, indicating a systemic dissemination of the bacteria. The presence of genes (bla_{IMP}, $bla_{CphA/IMIS}$, bla_{TEM}, bla_{SHV} and *intI1*) that encode extended-spectrum beta-lactamases (ESBLs), metallo-beta-lactamases (MBLs) and class 1 integrons were studied by PCR. Only 39.6% (19/48) of the strains showed the presence of one or more resistance genes. The gene $bla_{CphA/IMIS}$ was detected in 29.2% of the isolates, followed by the *intI1* (6.2%) and bla_{SHV} (4.2%) genes. The variable region of class 1 integrons of the 3 positive isolates was sequenced revealing the presence of the gene cassette *aadA1* (aminoglycoside transferase) that plays a role in streptomycin/spectinomycin resistance.

1. Introduction

The genus *Aeromonas* belongs to the class *Gammaproteobacteria*, order *Aeromonadales*, family *Aeromonadaceae* and includes species implicated in human and animal infections being contaminated water and food the major sources of infection (Janda and Abbott, 2010; Figueras and Beaz-Hidalgo, 2014). In aquaculture the species *Aeromonas salmonicida* is regarded as one of the main pathogenic fish species and is the causal agent of furunculosis, a hemorrhagic and ulcerative disease that affects trout, salmonids and other fish species (Beaz-Hidalgo and Figueras, 2013; Dallaire-Dufresne et al., 2014). However, studies using housekeeping genes have demonstrated that up to 10 *Aeromonas* species (*A. bestiarum*, *A. caviae*, *A. encheleia*, *A. eucrenophila*,

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A. hydrophila, *A. media*, *A. piscicola*, *A. salmonicida*, *A. sobria* and *A. tecta*) are isolated from healthy or diseased fish (Beaz-Hidalgo et al., 2010). Nowadays the use of housekeeping genes is the gold standard technique for the correct identification of *Aeromonas* species (Figueras et al., 2011).

An increased resistance to β-lactam antibiotics in the genus *Aeromonas* has been detected due to the presence of β-lactamases genes (Ndi and Barton, 2011; Carvalho et al., 2012; Chen et al., 2012). Extended-spectrum beta-lactamases (ESBLs), metallo-beta-lactamases (MBLs) and class 1 integrons include genes that encode antimicrobial resistance in the genus *Aeromonas* and have been identified in isolates recovered from aquaculture systems (Jacobs and Chenia, 2007; Kadlec et al., 2011). Integrons are able to capture several resistance gene cassettes from the environment and to incorporate them by using site-specific recombination and play a major role in the dissemination of antibiotic resistance genes (L'Abée-Lund and Sørum, 2001; Fluit and Schmitz, 2004). The class 1 integrons are the most commonly found in the genus *Aeromonas* (Pérez-Valdespino et al., 2009; Ndi and Barton, 2011; Sarria-Guzmán et al., 2014).

The aim of the current study was to re-identify 48 isolates recovered from rainbow trout using the *rpoD* and *gyrB* genes and to analyze their genotypes and the presence of β-lactamases genes and the class 1 integrons.

2. Materials and methods

2.1. Bacterial strains

A total of 48 isolates from rainbow trout previously identified (Vega-Sánchez et al., 2014) using the 16S rDNA-RFLP technique (Borrell et al., 1997; Figueras et al., 2000) were included in the study. The sources and origins of isolates are shown in supplemental table S1.

Supplementary Table S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetmic.2014.06.012.

2.2. Aeromonas re-identification and typing

The DNA of the strains was extracted with the InstaGene™ Matrix (Bio-Rad Laboratories, Ltd., Missussauga, Ontario, Canada) according to the manufacturer's protocols. Re-identification of all isolates was performed by sequencing the *gyrB* and *rpoD* genes determining their similarity with the type strains and on the basis of their position in the phylogenetic tree constructed with the concatenated sequences of both genes as in previous studies (Soler et al., 2004; Martínez-Murcia et al., 2011). The nucleotide sequences were aligned by Clustal W software with the published reference sequences of all type strains described by Martínez-Murcia et al. (2011) including the recently described species *A. australiensis* and *A. dhakensis* (GenBank accession numbers for *gyrB*: FN691773; HQ442711 and *rpoD*: FN773335; HQ442800, respectively). Genetic distances were obtained by Kimura's two parameter model and phylogenetic trees were constructed using the Neighbor-Joining method implemented in MEGA 5.2 software (Tamura et al., 2011) as in previous studies (Martínez-Murcia et al., 2011). The enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) technique was used to genotype the isolates as described by Soler et al. (2003). Fingerprints with identical patterns were considered to be the same genotype or strain.

2.3. Detection of β-lactamase and class 1 integron genes

The β-lactamase genes bla_{TEM}, bla_{SHV}, bla_{IMP}, $bla_{CphA/IMIS}$ and class 1 integron gene (*intI*1) were amplified using primers and PCR conditions described elsewhere (Nüesch-Inderbinen et al., 1996; Navarro et al., 2001; Migliavacca et al., 2002; Henriques et al., 2006). Positive isolates for the *intI*1 gene were tested for the presence of inserted gene cassettes into the variable region of this integron as described earlier (Henriques et al., 2006). Briefly, the band obtained was extracted from the agarose gel and purified using Wizard® SV gel and PCR clean-up system (Promega, USA), according to the manufacturer's protocols and was submitted for sequencing.

2.4. Statistical analysis

To determine if there was any significant difference in the number of isolates recovered from different organs and in the number of isolates obtained of the different *Aeromonas* species, data were analyzed with ANOVA and Tukey's test using the software MegaStat version 10.1 of Excel 2007.

3. Results and discussion

3.1. Molecular identification

A total of 68.8% (33/48) of the strains showed coincident identification with those obtained in the previous study using the 16S rDNA-RFLP (Vega-Sánchez et al., 2014) and belonged to the species *A. bestiarum*, *A. hydrophila*, *A. media*, *A. popoffii*, *A. sobria* and *A. veronii* (Table 1). The 16S rDNA-RFLP identification method described by Borrell et al. (1997) and Figueras et al. (2000) generated species-specific profiles that enabled the identification of the 14 species that composed the genus in the year 2000. The addition of new species in the genus since 2000 limits the usefulness of this method, because the same RFLP pattern is obtained for the species *A. salmonicida*, *A. bestiarum* and *A. piscicola* as described by Beaz-Hidalgo et al. (2010). In the current study this occurred for 12.5% (6/48) of the strains that showed with the 16S rDNA-RFLP the common pattern for the species *A. salmonicida*, *A. bestiarum* and *A. piscicola*, and also 4.2% (2/48) of the isolates that showed the typical 16 rDNA-RFLP pattern described for the species *A. salmonicida*, although after sequencing they were identified as *A. bestiarum* ($n = 7$) and *A. salmonicida* ($n = 1$) (Table 1). Furthermore 8.3% (4/48) of the isolates showed atypical RFLP patterns and after sequencing they were shown to correspond to *A. allosaccharophila* ($n = 1$), *A. caviae* ($n = 1$) and *A. media* ($n = 2$) (Table 1 and supplemental Fig. S1) and 2.1% (1/48) showed the typical RFLP pattern described for the species *A. encheleia*, nevertheless after sequencing this isolate was identified as "*Aeromonas lusitana*" (Table 1 and Fig. 1). The remaining