Distribution and Virulence Gene Comparison of Aeromonas Strains Isolated from Diseased Fish and Water Environment

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Abstract

A total of 71 Aeromonas strains were isolated in the south of Jiangsu Province China in order to analyze the difference of Aeromonas spp. distribution between diseased fish and water environment. The sequence of 16S rDNA and gyrB demonstrated that the 71 Aeromonas isolates could be divided into 4 species, including A. veronii (55), A. hydrophila (11), A. salmonicida (3) and A. media (2). A. veronii was the most common species isolated from fish and water environment. All Aeromonas isolates were screened for three putative virulence genes, aer, hly and alt. hly was the most common gene among three virulence genes.

Keywords: Aeromonas, diseased fish, gyrB, 16S rDNA, virulence gene, water environment

Species of Aeromonas are common inhabitants of aquatic environments and have been described in connection with fish and human diseases (Saavedra et al., 2004; Figueras, 2005). At present the genus comprises 19 species: A. hydrophila, A. bestiarum, A. salmonicida, A. caviae (synonym A. punctata), A. media, A. eucnephophila, A. sobria, A. veronii (synonyms are A. ichthiosmia and A. culicicola), A. jandaei, A. schubertii, A. trota (synonym A. enteropelogenes), A. allosaccharophila, A. encheleia, A. popoffii, A. simiae, A. mouluscorum, A. bivalvium, A. aquariorum, and A. tecta, as well as two DNA homology groups without a species name, Aeromonas sp. HG11 (proposed to be the synonym of A. encheleia) and HG13 (Enteric group 501) (Demarta et al., 2008; Harf-Monteil et al., 2004; Martin-Carnahan and Joseph, 2005; Martinez-Murcia et al., 2008; Minana-Galbis et al., 2004; Minana-Galbis et al., 2007; Pidiyar et al., 2002; Saavedra et al., 2006).

Direct sequencing of the 16S rDNA gene is generally accepted as a stable and specific marker for bacterial identification (Marchandin et al., 2003). Although 16S rDNA gene sequencing has contributed notably to the elucidation of the phylogenetic interrelationships between Aeromonas species, the resolution of this molecular ‘clock’ has now been superseded by those of some protein-encoding housekeeping genes such as gyrB and rpoD (Yanez et al., 2003; Soler et al., 2004; Martin-Carnahan and Joseph, 2005; Saavedra et al., 2006). The gyrB gene that encodes the B subunit protein of DNA gyrase (topoisomerase type II) is a single copy gene and is essential for DNA replication. This gene has been extensively used for studying phylogenetic relationship with various bacterial genera and its comparison with DNA-DNA hybridization results (Kasai et al., 2000).

Aeromonas are native of aquatic environments, and are frequently found in foods, including meat, fish, vegetables, fresh and sea water (Gastro-Escarpulli et al., 2003). Species, such as A. hydrophila, A. bestiarum, A. veronii biovar sobria and A. sobria, have been associated with infections in kinds of fish species (Kozinska, 2007). Some strains of motile and non-motile Aeromonads are involved in different fish diseases, such as septicemia, ulcerative disease, and furunculosis (Aberoum and Joooyandeh, 2010; Cristi et al., 2007). The mechanism of pathogenesis is complex and unclear (Janda and Abbott, 2010; Parker and Shaw, 2011). All genes that encode for virulence associated factors that allow the pathogen to establish infection in the host are defined as virulence genes. Virulence of aeromonads is considered to be multifactorial including cytotoxic heat-labile (alt), and cytotoxic heat-stable enterotoxins (ast), cytotoxic heat-labile enterotoxin (act), aerolysin (aer), flagella A and flagella B (fla),...
lipase (lip), elastase (ela), serine protease (ser), ADP-ribosyltransferase toxin (aexT), and DNases (exu) (Sha et al., 2002; Chacon et al., 2003; Sen and Rodgers, 2004; Nam and Joh, 2007; Vilches et al., 2008; Nawaz et al., 2010). It is not clear whether there is a different virulent subset of Aeromonas species isolated from the diseased fish and the environment.

At the present study, in order to know the distribution difference of the Aeromonas between diseased fish and water environment, samples were collected from the diseased fish and pond water in the south of Jiangsu Province, China. All strains were sequenced with 16S rDNA and gyrB gene to identify the species. Meanwhile three virulence genes, aer, hly and alt, were screened to compare the difference between host and environment.

Presumptive Aeromonas strains were isolated from host (diseased fish) and water environment in the south of Jiangsu Province China. The diseased fish showed heavy dark, hemorrhaging in the eye and mouth, in the vicinity of the opercula, around the vent and the base of the fins, and on the surface of the body. Some of them even showed abdominal swelling with lots of ascetic fluid. Bacteria isolated from niches of diseased fish, such as fish body, gill, liver, intestine, vent, ascetic fluid etc., grew on plates of Rimler-Shotts Medium. Pond water were coated on plates of Rimler-Shotts Medium too. The isolates were presumptively identified as Aeromonas species by Gram-staining, cytochrome oxidase, catalase and oxidative/fermentative acid production from glucose (Hugh and Leifson, 1953). The putative Aeromonas strains (n = 71, 43 from diseased fish and 28 from water) were used in further analysis. DNA was extracted by using the UNIQ-10 Column Bacterial Genomic DNA Isolation Kit (Sangon Biotech (Shanghai) Co., Ltd, China). Strains were identified by the sequence of 16S rDNA and gyrB. Primers 16S rDNA and gyrB were in table I. Results were compared in a BLAST homology search with Aeromonas gene sequences deposited in the GenBank database.

Table I

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primers</th>
<th>Tm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rDNA</td>
<td>F: 5’-CAC GGA TCC AGA GTT TGA T(C/T) (A/C) TGG CTC AG-3’&lt;br&gt;R: 5’-GTG AAG CTTACG G (C/T)T ACC TTG TTA CGA CTT-3’</td>
<td>52</td>
</tr>
<tr>
<td>gyrB</td>
<td>F: 5’-TCC GGC GTG CTG CAC GCC GT-3’&lt;br&gt;R: 5’-TTG TCC GGG TTG TAC TCG TC-3’</td>
<td>59</td>
</tr>
<tr>
<td>aer</td>
<td>F: 5’-GCTGAACCCCATCTATCCTG-3’&lt;br&gt;R: 5’-TTTCTCCGGTAAACGGATTG-3’</td>
<td>50</td>
</tr>
<tr>
<td>hly</td>
<td>F: 5’-GGCCGTTGCGCCGAGATACGGG-3’&lt;br&gt;R: 5’-GGCGGGCCCGGAGACCGG-3’</td>
<td>65</td>
</tr>
<tr>
<td>Alt</td>
<td>F: 5’-TGACCCAGTCCTGGCACGGC-3’&lt;br&gt;R: 5’-GGTGATCGATCACCACCAGC-3’</td>
<td>60</td>
</tr>
</tbody>
</table>

Table II

| Aeromonas isolates obtained from diseased fish and water environment (%) |
|-------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Diseased fish    | Crucian Carp | Black Carp | Grass Carp | Subtotal | Water Environment | Bluntnose Black Bream | Total |
| Aeromonas veronii | 7              | 8            | 2           | 13         | 30              | 25              | 55             |
| Aeromonas hydrophila | 1            | /            | 5           | 4         | 10              | 1               | 11             |
| Aeromonas salmonicida | /           | /            | 3           | 0         | 3               | /               | 3              |
| Aeromonas media   | /              | /            | /           | /         | /               | 2               | 2              |
| Subtotal          | 8              | 8            | 10          | 17        | 43              | 28              | 71             |
could found that there was no difference of A. veronii between diseased fish and water environment, 69.77% and 89.29% respectively, but A. hydrophila was significantly different ($P<0.05$). A. hydrophila isolates were significantly more frequent from diseased fish than from water. A similar result was found by Nielsen et al. (2001).

All Aeromonas isolates were screened for three putative virulence genes, aer, hly and alt. The primers are shown in table I. Of 71 strains analyzed, 50 (70.43%) were positive for at least one of the virulence genes examined. Three virulence genes were present in 4/71 (5.63%) of isolates, among which 3 strains were isolated from diseased fish and the other was from water environment. While 17 (23.94%) isolates contained two virulence genes, among which 10 were isolated from diseased fish and 7 from water environment. In 17 (23.94%) isolates contained two virulence genes, among which 10 were isolated from diseased fish including 5 A. veronii, 4 A. hydrophila and 1 A. salmonicida, and 7 were isolated from water environment including 6 A. veronii and 4 A. hydrophila. The number of Aeromonas isolates containing one virulent gene was the highest. 31 (43.66%) isolates, and included 18 from diseased fish and 13 from water environment.

The positive rate of virulence gene of A. veronii and A. hydrophila was compared between isolates from diseased fish and water environment (table III). In A. veronii, hly was present in nearly half of the strains from diseased fish while alt was present in half of the isolates from water environment. In A. hydrophila, hly was positive in most of the isolates not only diseased fish but also water environment. The positive rate hly was highest among three virulence genes. Several recent studies reported the involved virulence factors in fish infections (Boyd et al., 2008; Dacanay et al., 2010; Li et al., 2011). Janda and Abbott (2010) found that only a small subset of strains containing genes for potential virulence factors seems to cause infection. It is not clear whether there is a virulent subset of Aeromonas species prevalent in clinical isolates with the ability to cause freshwater fish infections.

Our results showed that the species distribution in Aeromonas isolates from diseased fish and water environment in Jiangsu Province of China was similar without significant differences. They indicated that Aeromonas species in aquatic environments are varied and have considerable virulence potential.

### Table III

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolated from</th>
<th>aer</th>
<th>hly</th>
<th>alt</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. veronii</td>
<td>Diseased fish</td>
<td>23 (7/30)</td>
<td>41 (12/30)</td>
<td>33 (10/30)</td>
</tr>
<tr>
<td></td>
<td>Pond water</td>
<td>24 (6/25)</td>
<td>28 (7/25)</td>
<td>50 (15/30)</td>
</tr>
<tr>
<td>A. hydrophila</td>
<td>Diseased fish</td>
<td>20 (2/10)</td>
<td>80 (8/10)</td>
<td>20 (2/10)</td>
</tr>
<tr>
<td></td>
<td>Pond water</td>
<td>100 (1/1)</td>
<td>100 (1/1)</td>
<td>0 (0/1)</td>
</tr>
</tbody>
</table>

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### Literature


