Is sea lamprey a potential source of infection with *Aeromonas salmonicida* for wild and farmed fish?

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Abstract
The present work reports the isolation of a typical pigment-producing strain of *Aeromonas salmonicida* ssp. *salmonicida* from sea lamprey (*Petromyzon marinus*). The organism was isolated from kidney or blood of both moribund and apparently healthy fish. The bacterial isolates proved to be virulent for rainbow trout with LD$_{50}$ ranging from $10^{2}$ to $10^{6}$. Therefore, sea lamprey can be a potential source of infection with *Aeromonas salmonicida* for wild and farmed fish.

Introduction
Furunculosis caused by *Aeromonas salmonicida* subspecies *salmonicida* is one of the most devastating bacterial diseases affecting cultured and wild salmonid fish throughout the world (Austin & Austin, 1999). The disease has also been described in several non-salmonids fish. In Spain, *A. salmonicida* ssp. *salmonicida* have been isolated as the causative agent of mortalities on valuable marine fish species such as turbot (*Scophthalmus maximus*, L.) and sea bream (*Sparus aurata*) (Toranzo et al, 1991; Toranzo and Barja, 1992; Real et al, 1994). The main external signs of furunculosis in affected fish are haemorrhagic zones at the bases of the pectoral fins and mouth and ulcerative lesions on the dorsal and ventral surface. Internally, haemorrhaging occurs in viscera, heart and abdominal wall (Austin & Austin, 1999; Toranzo and Barja, 1992; Real et al, 1994).

First described in Europe, *A. salmonicida* has now a worldwide distribution, including Australia and mainland Asia (Austin & Austin, 1999). Several studies have examined the role of water, sediment and detritus, as potential source of infection with *A. salmonicida*. However, factors that determine the dissemination of the pathogen as well as the precise route of transmission are still unclear (Rose et al, 1990; Toranzo et al, 1991; Austin & Austin, 1999). The role of fish as a source of infection with *A. salmonicida* has also been examined. Although, it is commonly accepted that fish may contribute to disseminate the disease by lateral and vertical transmission or by becoming carriers of *A. salmonicida*, the route of infection is not completely clear (Austin & Austin, 1999).

This work reports on the isolation and characterization of *A. salmonicida* subspecies
salmonicida from sea lampreys (Petromyzon marinus) captured in a river of Galicia (North-West of Spain). In order to investigate the possible implications for salmonid farming, the virulence of the isolated bacteria was evaluated for rainbow trout (Oncorhynchus mykiss).

Material and Methods

Fish
Moribund (2 fish) and apparently healthy sea lamprey (25 fish) were captured in a river of Galicia (North-West Spain) (Table 1). Sampled fish did not show external or internal clinical signs of disease.

Isolation and identification of bacteria
Samples from blood and kidney were cultured on Tryptic Soy Agar supplemented with 1% of NaCl (TSA-1, Difco) and incubated at 22°C for 24-48h. Pure cultures of the isolates obtained on TSA-1 agar plates were identified using standard morphological, physiological and biochemical test and API 20E system as previously described (Toranzo et al, 1991; Santos et al, 1993; El Morabit, 2002). Sensitivity to antimicrobial compounds was determined by the disc diffusion method (Barry and Thornsberry, 1991) using the following chemotherapeutic agents (microgram disc⁻¹) supplied by Oxoid: ampicillin (10), oxytetracycline (30), oxolinic acid (2), streptomycin (10), chloramphenicol (30), nitrofurantoin (300), enrofloxacin (2), trimetoprim-sulfametoxazol (23,7-1,2) and the vibriostatic agent O/129-Pteridine (150). Biochemical profiles were compared to those obtained using the reference strains ATCC14174 from the

<table>
<thead>
<tr>
<th>Strains</th>
<th>Original host species</th>
<th>Isolation Source</th>
<th>LD₅₀ * for rainbow trout fingerlings (c.f.u./ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 14174</td>
<td>Diseased S. fontinalis</td>
<td>Unknown</td>
<td>10⁵₆</td>
</tr>
<tr>
<td>NF19.1</td>
<td>Diseased S. trutta, Spain</td>
<td>Kidney</td>
<td>10³</td>
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<td><strong>Lamprey strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L9R1</td>
<td>Healthy P. marinus, Spain</td>
<td>Kidney</td>
<td>10⁶</td>
</tr>
<tr>
<td>L3R</td>
<td>Healthy P. marinus, Spain</td>
<td>Kidney</td>
<td>ND</td>
</tr>
<tr>
<td>L9R2</td>
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<td>Kidney</td>
<td>10⁵₆</td>
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<td>Moribund P. marinus, Spain</td>
<td>Blood</td>
<td>10²</td>
</tr>
<tr>
<td>L10S1</td>
<td>Healthy P. marinus, Spain</td>
<td>Blood</td>
<td>10³₅</td>
</tr>
</tbody>
</table>

Table 1. Virulence for rainbow trout of A. salmonicida strains isolated from sea Lamprey.

* The degree of virulence expressed as the dose that causes 50% mortality of inoculated fish (LD₅₀), was determined by the method of Reed and Muench (1938).

ATCC: American Type Culture Collection.
American Type Culture Collection (ATCC, Rockville, MD, USA) and NCIMB2261 from the National Collection of Industrial and Marine Bacteria (NCIMB, Aberdeen, Scotland). The serological identity of the bacterial strain was determined using slide agglutination and Dot Blot assay as previously described (Santos et al., 1995). For the serological assays whole cell and “O” antigens and rabbit serum against the type strain ATCC 14174 were used.

Pathogenicity test
Virulence for fish of bacterial strains was evaluated in fingerling rainbow trout. Fish were intraperitoneally injected with bacterial doses ranging from $10^2$ to $10^8$ c.f.u./ml (5 fish per dose). Two reference strains (ATCC 14174 and NF19.1) from *Salvelinus fontinalis* and *Salmo trutta* respectively were used as positive controls in the trials. The degree of virulence (LD$_{50}$, the dose that causes 50% mortality) was determined by the method of Reed and Műnch (1938).

Results and Discussion
In July of 2000, moribund (2 fish) and apparently healthy (25 fish) sea lampreys captured in a river of Galicia (North-West Spain) were examined by conventional bacteriological methods for the presence of pathogenic microorganisms. Pigment-producing bacteria, presumptively identified as *A. salmonicida*, were isolated from the kidney (isolates L3R, L9R1 and L9R2) and blood (isolate L10S1) of four apparently healthy fish and from the blood of one moribund fish (isolate L3S1) (Table 1). Phenotypic tests demonstrated that all the isolates were non-motile Gram-negative rods, fermentative, oxidase and catalase positive and reduce nitrate. All the strains showed amylase, proteases and DNAse activities and produced acid from glucose, mannose, and galactose. All the isolates were negative for indol, Voges Proskauer, citrate and acid production from saccharose, inositol, sorbitol and lactose. Variable results among the isolates were observed in the following tests: acid production from arabinose, maltose and mannitol. The strains were also correctly identified as *A. salmonicida* using API 20E system. The slide agglutination and Dot Blot assays demonstrated that bacterial isolates from sea lamprey were antigenically homogeneous and belonged to *A. salmonicida* ssp. *salmonicida*.

The antimicrobial sensitivity analysis showed that all the strains displayed similar pattern being sensitive to most of the chemotherapeutic agent tested with the exception of oxytetracycline and the vibriostatic agent O/129-Pteridine. Other authors have previously described the isolation of oxytetracycline-resistant strains of *A. salmonicida* from diseased farmed-fish (Toranzo y col, 1991; Austin and Austin, 1999).

In order to investigate, whether or not sea lamprey can be a potential source of infection with *A. salmonicida* for wild and farmed fish, pathogenicity assays were carried out in fingerling rainbow trout. The four bacterial strains isolated from sea lamprey tested in the present study proved to be virulent for fish with a LD$_{50}$ (ranging from $10^2$ to $10^6$) similar to those obtained with the strains of *A. salmonicida* ssp. *salmonicida* ATCC 14174 and NF19.1 isolated from salmonids (table 1). *A. salmonicida* were re-isolated from internal organs of the inoculated rainbow trout. This study has shown that sea lamprey can act as
carriers of a strain of *A. salmonicida* which was pathogenic to rainbow trout fingerlings. Therefore, sea lampreys represent a risk of transmitting *A. salmonicida* to wild and farmed salmonid fish.

**References**


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