Serotype and Siderophore Production of *Edwardsiella tarda* Isolated from Water at Eel Farms

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**ABSTRACT**—Appearance of *Edwardsiella tarda* were monitored in pond water culturing eel *Anguilla japonica* in Kagoshima prefecture. *E. tarda* were isolated at almost all sampling times. Serotype and siderophore production of the *E. tarda* isolates, which are involved with its pathogenesis, were determined. Out of 370 isolates, 194 isolates (52%) were classified into serotype A and 246 isolates (66%) produced siderophore. Ninety-one percent of the isolates that were classified into serotype A produced siderophore; 72% of those that produced siderophore were classified into serotype A. There was no cross linkage between serotype and siderophore production in *E. tarda*.

**Key words:** Edwardsiella tarda, serotype, siderophore, *Anguilla japonica*

Edwardsiellosis caused by *Edwardsiella tarda* is prevalent in not only cultured eel *Anguilla japonica* but also various freshwater and marine fishes in the world. *E. tarda* has been constantly isolated from water and sediment of eel culture ponds since the middle of 1970’s, when so-called greenhouse system was introduced into many eel farms all over Japan and thereby water in the greenhouse ponds was kept warm throughout the year. Many workers have been researching virulent factors of *E. tarda*. Park et al. reported that most strains of *E. tarda* classified into serotype A by O-agglutination test, showed high virulence against eel. Igarashi et al. demonstrated that siderophore for iron uptake was essential for the virulence of *E. tarda*. The present authors had an opportunity to take a preliminary survey of edwardsiellosis in eel in Kagoshima prefecture, where the greatest production of cultured eel in Japan has been achieved, and examined the relationship between serotype and siderophore production in *E. tarda* isolates from eel culture ponds.

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**Materials and Methods**

**Duration and frequency of examination**

Samples were collected once a month from March to July in 2003 and from June to December in 2004. As eel were selected based on their weight and transferred to the other ponds almost every month, the ponds to which the group of eel in the heaviest weight was transferred were traced until the end of the monitoring. No chemotherapeutic agents were administrated to eel in the examined ponds during the monitoring.

**Isolation of bacteria**

Serial 10-fold dilutions of pond water were made, aliquots of each dilution was then inoculated on Trypto Soy Agar (Nissui) for the total viable cells and on SS Agar (Nissui) containing 1% of mannitol for *E. tarda* just after collecting samples at the pond site. The plates were aerobically incubated at 30°C for 48 h. Colonies with black pigmentation on SS Agar were picked off for further characterization to identify to *E. tarda*. Cultures of *E. tarda* are Gram-negative, motile and fermentative rods that produce catalase, hydrogen sulfide, indole and lysine decarboxylase but not cytochrome oxidase.

**Serotype and siderophore production of *E. tarda***

Four types of rabbit antisera (A, B, C and D) were obtained from rabbits (Japan white strain) immunized against each types of *E. tarda* (A, B, C and D) according to Park et al. All isolates of *E. tarda* were classified into four serotypes and an unclassified group by agglutination test on glass slides. Modified CAS assay was also carried out to demonstrate the ability of siderophore production: Colonies with obvious orange halos were judged positive, while both colonies with small halos (within a radius of 3 mm) and orange pigmented colonies without halos were judged negative.

**Results and Discussion**

The number of total viable cells and *E. tarda* were rather stable (Table 1): They were $10^{4.9}$ cfu/mL and $10^{2.8} - 10^{3.0}$ cfu/mL respectively. The rate of *E. tarda* to total viable cells was fluctuated every time. For example, in a pond, where edwardsiellosis broke out among eel in May, 2003, the percentage showed more than 90%, while that showed less than 0.1% in ponds when examined just after eel had been introduced. Park et al. reported that total viable counts in eel culture ponds in Shizuoka prefecture were $10^{3.8} - 10^{6.1}$ cfu/mL and those of *E. tarda* were $10^{1.2} - 10^{4.8}$ cfu/mL. The former is larger than the present result. Some factors such as rearing period, total weight of eel in a pond and materials of feed might affect the total viable counts in eel culture ponds. In the present greenhouses, concrete ponds were completely drained when...
Table 1. Number of isolates from each source

<table>
<thead>
<tr>
<th>Year of sampling</th>
<th>Log number of E. tarda (cfu/ml)</th>
<th>Log number of total viable cells (cfu/ml)</th>
<th>E. tarda / total viable cells (%)</th>
<th>Number of isolates of E. tarda</th>
<th>Serotype A (%)</th>
<th>Production of siderophore (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>2.8 (1.8–3.8)*</td>
<td>4.0 (3.6–5.0)</td>
<td>18.2 (0.4–92.4)</td>
<td>175</td>
<td>62.3</td>
<td>76.0</td>
</tr>
<tr>
<td>2004</td>
<td>3.0 (&lt;1.0–4.1)</td>
<td>4.0 (3.1–5.0)</td>
<td>21.2 (&lt;0.1–39.8)</td>
<td>195</td>
<td>43.6</td>
<td>57.9</td>
</tr>
</tbody>
</table>

*; range.

Table 2. Serotypes and production of siderophore of E. tarda

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Production of siderophore</td>
<td>A</td>
</tr>
<tr>
<td>Positive</td>
<td>177* (72)**</td>
</tr>
<tr>
<td>Negative</td>
<td>17 (14)</td>
</tr>
<tr>
<td>total</td>
<td>194</td>
</tr>
</tbody>
</table>

*, number of strains; **, %

eel were harvested, washed and then kept dried until next usage. The temperature of pond water was being kept to around 30°C throughout the year, which is approximately 2°C higher than the temperature in Shizuoka prefecture at 1970's. Pond water was well aerated and agitated by paddle aerators. A very small amount of sediments in the culture ponds were accumulated only in settling compartments, which did not allow us to collect enough sediment to examine. These factors could have influences on the generic composition and number of bacteria in the water and supposedly be the reasons for the above mentioned difference between the total viable counts in the present study and those in a previous report\(^1\).

A total of 370 strains of E. tarda (175 strains in 2003 and 195 strains in 2004) was examined for identification of serotype and production of siderophore. Results in 2004 were similar to those in 2003, so they were put together and summarized in Table 2. The number of strains that produced siderophore was significantly larger than that of negative strains (\(\chi^2\) test, \(p < 0.005\)). The number of strains identified to serotype A and an unclassified group were significantly larger than others (\(\chi^2\) test, \(p < 0.005\)). There was a significant relationship between serotype and production of sidrophore; strains that produced siderophore tended to be identified to serotype A, and strains of siderophore-negative strains tended to be unclassified group (\(\chi^2\) test, \(p < 0.005\)).

The present study showed that 52% (194/370) of E. tarda strains isolated from the eel culture ponds were identified to serotype A, and 66% (246/370) of E. tarda produced siderophore. The strains that produced siderophore could not always be identified to serotype A (72%). The rest of these were identified to other serotypes. These might not be in conflict with the result of Park et al.\(^3\), which showed some strains in other serotypes were virulent to fishes. Fourteen percent of the strains that were classified to serotype A did not produce siderophore. These results suggested that virulence of E. tarda could not simply attribute to serotype and production of siderophore.

The fact that more than 90% (177/194) of serotype A strains of E. tarda produced siderophore might support the previous report\(^1\), in which it was suggested that siderophore is necessary for the pathogenicity of E. tarda, but it is not sufficient, and other virulence factors in addition to siderophore could be needed. As the rest (17/194) did not produce siderophore, pathogenicity of these strains against eel remains to be tested.

Park et al.\(^3\) also reported that a serotype composition in E. tarda collected from intestinal contents of cultured eel was almost the same as that in E. tarda collected from pond water; 17% of E. tarda isolates from intestinal contents of eel and 13% of those from pond water were identified to serotype A. In the present study a half of E. tarda isolates form pond water was identified to serotype A. It is suggested that a large number of possibly virulent strains (serotype A) of E. tarda might exist in the intestines of eel in the ponds examined by the present authors. But the eel culturist had only once occurrence of edwardsiellosis in eel during the present monitoring (in May, 2003). The presence of E. tarda in culture ponds no longer means the occurrence of edwardsiellosis in eel. Additional conditions could be needed for outbreak of edwardsiellosis among eel. The disease is still a serious problem in various cultured species in Japan. For the prevention of the disease, many studies regarding pathogenic factors of the bacterium and additional conditions for outbreak of the disease remain to be investigated.

References

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