Vibrio aestuarianus, a pathogen of oyster *Crassostrea gigas*? 
Epidemiology study and bacterial characterization

IFREMER PFOM-UMR-PE2M Brest
Matthieu GARNIER - Jean Louis NICOLAS- Céline GARCIA (LGP La Tremblade)
Epidemiological study: MOREST

2001-2003:
Analysis
Dying oysters: 53 (hatchery) and 54 (growout sites)
Healthy oysters: 190 (growout sites)

Hemolymph sampling

Plating on marine agar: concentration and diversity of bacteria

Genotyping of dominant strains (RFLP)

Bay of Veys
Morbihan
Quiberon
La Tremblade
Argenton
Fouras
Arcachon
Sète

Do not disseminate without author authorization
During summer mortalities, hemolymph of dying and healthy oysters contain bacteria. Obviously more in dying bivalves.
Types of bacterial infection in dying oysters

Bacterial species in facilities
- aestu: 67%
- splen: 25%
- pseud: 8%

Bacterial species in all oysters
- aestua: 44%
- splend: 28%
- other: 28%

Bacterial species in oysters infected by one dominant bact
- aestua: 75%
- splend: 25%
**Frequency of vibrios in hemolymph**

![Bar chart showing frequency of vibrios in hemolymph]

*V. aestuarianus* represented 60% of isolates in pure culture and at high concentration in hemolymph in oysters maintained at 9-22°C. When isolated in mixture, strains were more diversified.

*Do not disseminate without author authorization*
**Phenotypic characterization**

- Cells are motile and curve rods
- Colony are less than 1mm diameter, circular white and translucent (easy to recognize)
- Growth Optimum: 20 - 30°C

---

They are phenotypically as diversified as the strains of *V. splendidus* group.
On the contrary of V. splendidus group and phenotypic characters, the cluster of V. splendidus is highly homogenous.
Genotypic characterization: DNA-DNA Hybridization

A species is determined at 70% of homology.

<table>
<thead>
<tr>
<th></th>
<th>Pathogenic Vaestuarianus 01/032</th>
<th>Vaestuarianus ATCC35048</th>
</tr>
</thead>
<tbody>
<tr>
<td>01/140</td>
<td>81</td>
<td>53</td>
</tr>
<tr>
<td>01/032</td>
<td>100</td>
<td>54</td>
</tr>
<tr>
<td>01/151</td>
<td>95</td>
<td>59</td>
</tr>
<tr>
<td>01/064</td>
<td>118</td>
<td>61</td>
</tr>
<tr>
<td>02/041</td>
<td>86</td>
<td>62</td>
</tr>
<tr>
<td>02/093</td>
<td>81</td>
<td>63</td>
</tr>
<tr>
<td>01/031</td>
<td>96</td>
<td>65</td>
</tr>
<tr>
<td>02/114</td>
<td>94</td>
<td>75</td>
</tr>
<tr>
<td>03/015</td>
<td>90</td>
<td>80</td>
</tr>
<tr>
<td>01/308</td>
<td>109</td>
<td>81</td>
</tr>
<tr>
<td>02/103</td>
<td>111</td>
<td>87</td>
</tr>
<tr>
<td>Reference</td>
<td>ATCC35048</td>
<td>100</td>
</tr>
</tbody>
</table>

Only one species but the homology is below 70% with the reference strain.
Virulence of *V. aestuarianus* strains

**Gradient of virulence**

No relation between virulence and other phenotypic characters

This vibrio can invade oyster and be no virulent
Type of infection: septicemia

A to C: Necrosis of muscular fibers of the adductor muscle of *Crassostrea gigas*. Hematoxylin-eosin scale bar = 10 µm

A: Necrosis of muscular fibers (mf)

B: Image of phagocytosis (arrow) of the muscular debris (md)

C: Condensation of muscular fibers (mf) and clear gap (arrow)

D: Epithelium atrophy of digestive diverticules cell atrophy (arrow) of the digestive diverticules (dd).
The development of infection is slower than infection with *V. splendidus*

Koch postulate is verified.
Effect of Temperature

The natural infection is generally triggered at 19°C.

Two different optima but not related with the virulence.
bacterial concentration in hemolymph in apparently healthy oysters

The bacteria concentration reached a maximum just before mortality
Conclusion

*Vibrio aestuarianus* is an ubiquist bacteria phenotypically diversified but with homogenous genotypic characters.

Until now not reported as pathogenic bacterium

It can be considered as pathogen/ opportunist in hatchery even the mortalities are not massive, except in spat where it provoked sudden mortality .

Its role in summer mortalities remains imprecise since not virulent bacteria can invade dying and apparently healthy oysters.

Condition of appearance of infection. Weakness of oyster during maturation phase

19°C was not an absolute limit but favors its growth

Invasive bacteria without direct effect on hemocytes but its Extra Cellular Product attack tissues

- 2001-2005 : frequently found in moribund oysters (MOREST)

  1983 : Shellfish on the west coast (USA) : reference strain

  2002 Sea water of turbot rearing (Spain)

  2004 : Flat oyster(Spain) :