Analysis of the serological variability of *Vibrio tapetis*, causative agent of BRD in clams.

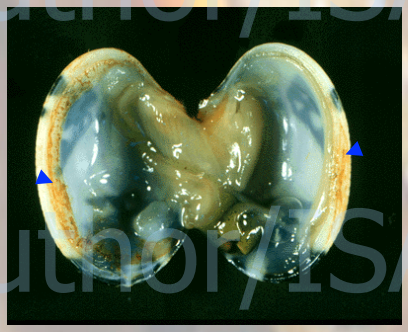
Sabela Balboa, Asmine Bastardo, Alejandra Doce, Juan L. Barja, Alicia E. Toranzo & Jesús L. Romalde

DEPARTAMENTO DE MICROBIOLOGÍA Y PARASITOLOGÍA.
FACULTAD DE BIOLOGÍA.
UNIVERSIDAD DE SANTIAGO DE COMPOSTELA.
SPAIN

Brown Ring Disease

* One of the main pathological problems limiting clam culture in Europe.

* Unique pathology of demonstrated bacterial etiology affecting adult individuals.



Brown Ring Disease

Classical hosts:

Manila clam
(*Ruditapes philippinarum*)

Carpet-shell clam
(*Ruditapes decussatus*)

Brown Ring Disease

Geographical distribution:

France
Italy
England
Portugal
Spain
Ireland
Korea
Japan
Norway

Brown Ring Disease

* Causal agent: *Vibrio tapetis*

* Isolation of new strains is difficult due to the slow growth of the microorganisms in some media usually employed in the microbiological of shellfish (i.e. TCBS).

* Originally described as a homogeneous taxon.

Vibrio tapetis

Other hosts:

Cockle
(Cerastoderma edule)

Pullet carpet-shell clam
(Venerupis aurea)

Corkwing wrasse
(Symphodus melops)

Halibut
(Hippoglossus hippoglossus)

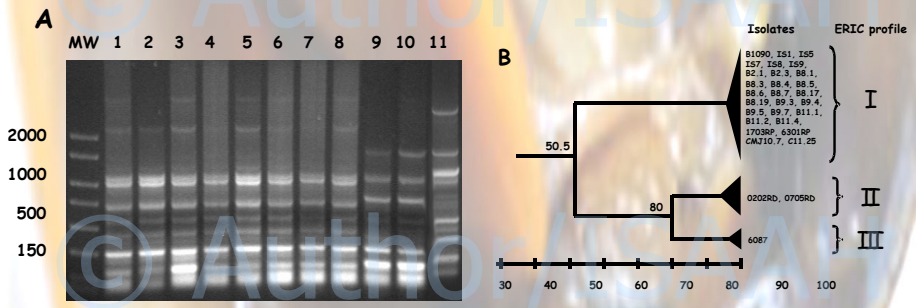
Corb
(*Umbrina cirrosa*)

Wedge Sole
(Dicologoglossa cuneata)

Vibrio tapetis

* The isolation of new strains demonstrated some intraspecific variability (biochemical and genetical):

Three different groups associated with the host.



OBJECTIVES

* To study the antigenic variability within *Vibrio tapetis*

* To establish, if possible, a serotyping scheme for this bacterial species

ISOLATES

Manila clam (Ruditapes philippinarum)	26 strains	Spain 1990, 1994, 2005-07 France 1994 Ireland (UK seed) 2005
Carpet shell clam (R. decussatus)	2 strains	Spain 1994
Pullet carpet-shell clam (Venerupis aurea)	1 strain	France. 1990 (wild)
Cockle (Cerastoderma edule)	1 strain	France. 1990 (wild)
Halibut (Hippoglossus hippoglossus)	1 strain	United Kingdom. 2001
Corb *(Umbrina cirrosa)*	3 strains	España. 2007
Wedge Sole *(Dicologoglossa cuneata)*	4 strains	España. 2007

MATERIAL & METHODS

* Serological Characterization:

  Slide agglutination
  Dot-blot assay

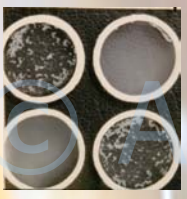
*Sera raised against representatives of the three genetic groups described, raw and cross-absorbed with the heterologous strains.

*Analysis of LPS and total and OMP proteins.

* Western-blot assays.

RESULTS: Slide and Dot Blot

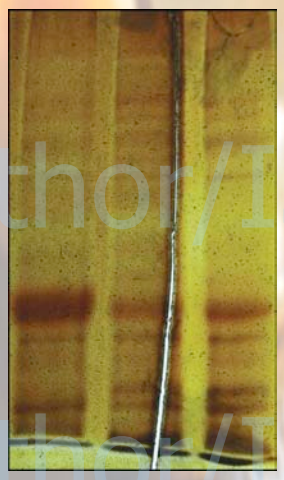
Isolates	B1090^T	0202RD	6087
B1090^T	++	+ (-)	+
R. philippinarum (26 isolates) Spain (1994, 2005-2007) France (1988-1991)	++	+ (-)	+
C. edule (1 isolate)	++	+ (-)	+
V. pullastra (1 isolate)	++	+ (-)	+
0202RD	+	++	+
R. decussatus (1 isolates) Spain 1994	+	++	+
6087	+	+ (-)	+
D. cuneata (4 isolates)	+	+	+
U. cirrosa (3 isolates)	+	++	+
102	-	-	+



RESULTS

Strain	Doble	B1090^T AgO 0202RD	AgO 6087	Doble	0202RD AgO B1090^T	AgO 6087	Doble	6087 AgO B1090^T	AgO 0202RD
B 1090^T	++	++	++	-	-	-	-	-	-
R. philippinarum (26 isolates) Spain (1994, 2005-2007) France (1998-1991)	++	++	++	-	-	-	-	-	-
C. edule (1 isolate)	++	++	++	-	-	-	-	-	-
V. pullastra (1 isolate)	++	++	++	-	-	-	-	-	-
0202 RD	-	-	-	++	++	++	-	-	-
R. decussatus (1 isolates) Spain 1994	-	-	-	++	++	++	-	-	-
Corb (3 isolates)	-	+	+	+	++	++	-	+	-
6087	-	+	+	-	-	-	++	++	++
Sole (3 isolates)	-	+	+	-	+	+	-	+	+
Sole (1 isolate)	-	+	+	-	+	+	-	+	-
102	-	-	-	-	-	-	-	-	-

RESULTS: LPS (silver staining)



B1090 0202RD 6087

RESULTS: LPS (western-blot)

ANTISERA

B1090 0202RD 6087

B1090 0202RD 6087 B1090 0202RD 6087 B1090 0202RD 6087

RESULTS: protein profiles

Whole cell

MW B1090 0202RD 6087

OMP

MW B1090 0202RD 6087

CONCLUDING REMARKS

* All these results demonstrated the antigenic heterogeneity among the isolates of *Vibrio tapetis*.

* The serological groups are coincident with those established previously by genetical procedures.

* The serological groups possess epidemiological significance, since they could be related with the host origin.

Proposition of a serological scheme for *V. tapetis*:

Serotype 01	Manila clam (*R. philippinarum*) Pullet carpet-shell clam (*V. aurea*) Cockle (*C. edule*)
Serotype 02	
Subgroup A	Carpet shell clam (*R. decussatus*)
Subgroup B	Corb (*U. cirrosa*)
Serotype 03	Halibut (*H. hippoglossus*) Wedge sole (*D. cuneata*)

Isolate 102 (Manila clam seed) Non typable