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A short-review of brown ring disease, a vibriosis affecting clams, *Ruditapes philippinarum* and *Ruditapes decussatus*

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Abstract – Brown ring disease (BRD) in *Ruditapes philippinarum* and *Ruditapes decussatus* is a shell disease caused by *Vibrio tapetis*. This disease has begun in 1987 in clams beds in Brittany (France) and then has spread along the European Atlantic coast. Since about fifteen years, research on BRD has progress a lot and the purpose of this review is to give a short description of BRD in clams in retrospect and in addition of recent and pertinent results. Diagnosis including isolation, biochemical, serological and molecular characterization has been developed to identify and detect the pathogen. Therefore, *Vibrio tapetis*-like strains has been detected in other host specie, in cultured fishes during mortalities, such as the wrasse, *Symphodus melops* in Norway and the halibut, *Hippoglossus hippoglossus* in Scotland. Mechanisms of host-pathogen interactions modulated by environmental factors were studied using in vivo bacterial challenge and in vitro bio-assays. According to these studies, adherence and cytotoxic factors have been yet identified as virulence factors; identification and characterization of virulence genes are in progress. Field and experimental studies confirmed the significant effect of temperature on the development of BRD and on clam defense related activities. A significant increase in temperature over 21 °C may have a preventive effect on the development of the disease.

Key words: Shell disease / Vibriosis / *Vibrio tapetis* / Bivalve mollusc / Immunity / Pathogenicity / Diagnostic methods

Résumé – La maladie de l'anneau brun, une vibriose affectant les palourdes, *Ruditapes philippinarum* et *Ruditapes decussatus* : état des connaissances. La maladie de l'anneau brun (MAB) chez les palourdes, *Ruditapes philippinarum* et *Ruditapes decussatus* est une maladie coquillière provoquée par *Vibrio tapetis*. Cette maladie a commencé en 1987 sur les parcs vénéricoles en Bretagne et s'est propagée par la suite sur toute la façade atlantique européenne. Les recherches sur la MAB ont beaucoup progressé depuis une quinzaine d'année, l'objectif de cette synthèse est donc de présenter une rétrospective complétée de résultats récents et pertinents. Des outils diagnostics, basés sur des méthodes d'isolation, de caractérisation biochimique, sérologique et moléculaire ont été développées afin d'identifier et de détecter le pathogène. Ainsi, des souches de *V. tapetis* ont été isolées chez d'autres espèces hôtes, chez des poissons subissant des épisodes de mortalités comme le labre, *Symphodus melops* en Norvège et le flétan, *Hippoglossus hippoglossus* en Ecosse. Les mécanismes des interactions hôte-pathogène modulés par les facteurs environnementaux ont été étudiés in vivo après stimulations bactériennes et in vitro grâce aux développements de bio-tests. Suite à ces travaux, des facteurs de virulence, tels que des facteurs d'adhérence et de cytotoxicité ont été identifiés ; la caractérisation de ces gènes de virulence est en cours. Les études menées au laboratoire et en milieu naturel confirment l'effet significatif de la température sur le développement de la MAB et sur la réponse immunitaire. En effet, l'exposition des palourdes à des températures supérieures à 21 °C a un effet préventif sur la maladie.

1 Introduction

In spring and summer 1987, mass mortalities of cultured clams, *Ruditapes philippinarum*, occurred in clam bed of Landeda (Brittany) North Finistere, which was the first production site in France (500 t in 1987) (Paillard and Maes 1989; Flassch et al. 1992). Clam mortalities were associated with high prevalence of a characteristic symptom, a brown deposit on the inner surface of the valves (Paillard and Maes 1989). This disease, named brown ring disease (BRD), has decimated completely the cultured clam in Brittany and since today, no intense venerid culture has start again in this site. At the same time, in 1988, natural populations of clams have colonised some mud coast sites along the French Atlantic coast (Bay of Brest, Gulf of Morbihan, Arcachon, etc.). The disease progress slowly in this natural stock and prevalence of BRD reach in maximum 30% depending sites and season. Unfortunately, no legal European procedures have been established concerning transfer of clams at this period and till now the disease had spread in other European countries, in England, Ireland, Spain

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and Italy. Research on BRD has progress a lot of in synergy with different teams around the world (Spain, England, USA, Norway) and the 1994 BRD review is now update (60% of all BRD references have been published since then). Therefore, the purpose of this review is to give a short description of BRD in clams in retrospect and in addition of recent and pertinent results.

1.1 Disease name

This disease was named Brown Ring Disease (BRD) because of the visible typical host signs; an obvious abnormal conchiolin deposit (CD), organic in nature, which generally adheres to the inner shell between the pallial line and the growing, forming the characteristic brown ring (Plate 1, No 1–5) (Paillard and Maes 1989).

1.2 Etiologic agent

The etiologic agent, isolated in 1990 in Landeda (North Finistere, France), was originally called *Vibrio* Predominant 1 or *Vibrio* P1 (VP1) and named therefore *V. tapetis* (Paillard and Maes 1990; Borrego et al. 1996). Following classical Koch's postulate for pathogen identification, *V. tapetis* was determined as the agent responsible for this disease (Paillard and Maes 1990). *Vibrio tapetis* was characterized as a fermentative, Gram-negative, motile, non sporulating curved rod that is oxidase-positive, with growth inhibited by vibriostatic 0/129. It can be differentiated from other species of *Vibrio* by growth at 4 °C; no growth at temperatures greater than 22 °C and salinities greater than 5% NaCl; no production of arginine dehydrolase, lysine decarboxylase and ornithine decarboxylase; and a positive Voges-Proskauer reaction (Paillard and Maes 1990; Borrego et al. 1996). Antigenic characterisation of *V. tapetis* has shown that all the strains of *V. tapetis* constitute an homogeneous group and that these strains are different in protein and lipopolysaccharides patterns compared to other *Vibrio* species isolated from diseased clams (Castro et al. 1996). Pulse-field gel electrophoresis (FPGE) and ribotyping did not separate the isolates of *V. tapetis* (Castro et al. 1997a). These isolates harbored only one large plasmid of an estimated size of 74.5 kb (Borrego et al. 1996; Castro et al. 1997a). In contrast, a recent study has shown that all *V. tapetis* strains contain between two and four large plasmids sizing approximatively 60 to 100 kb (Le Chevalier et al. 2003). Groups of strains may be distinguished on the basis of their pulse-field electrophoretic profiles and ribotyping and plasmid patterns (Castro et al. 1997a). On the basis of RFLP plasmid patterns, the strain from Norway deviated considerably from the French and British strains (Le Chevalier et al. 2003).

1.3 Host species

BRD affects clams of the genera *Ruditapes*, *Tapes and Venerupis.* It has been detected in natural populations of *R. philippinarum, R. decussatus, V. aurea* and *T. rhomboïdes.* The third ones have been experimentally infected (Paillard and Maes 1989). The most sensitive species is the Manila clam, *R. philipinarum* (Maes and Paillard 1992) and it is the only one to experience BRD-related mortalities. In addition to *R. philippinarum*, *V. tapetis* has been isolated in diseased clams *R. decussatus* and *V. aurea* and also in high concentrations in a carrying species which presented no BRD symptoms, such as cockle, *Cerastoderma edule* (Maes and Paillard 1992). No signs of CD have been observed after *V. tapetis* challenge in different species of oysters, *C. gigas, O. edulis, C. virginica* and in cockle *C. edule* and the hard clam, *Mercenaria mercenaria* (Maes and Paillard 1992; Paillard et al. 1996). In *Pecten maximus*, an increase of CD prevalence has been measured after *V. tapetis* challenge, but the difference was not significant between inoculated and controls ones (Maes and Paillard 1992). Recently, *V. tapetis* strains have been isolated from two cultivated fishes during mortalities, the wrasse, *Symphodus melops* in Norway (Jensen et al. 2003) and the halibut, *Hipoglossus hipoglossus* in Scotland (UK) (Reid et al. 2003a).

2 Diagnostic methods

2.1 Gross observations

The development and recovery of BRD is assessed by scaling macroscopic symptoms according to the classification system established by Paillard and Maes (1994). A least 100 individuals should be examined. The principal symptom is a brown deposit present on the inner shell between the pallial attachment and the edge of the shell. The earliest stages are signaled by the presence of conchiolin spots surrounded by a pale brown halo adhering to the inner shell (first sign of the disease (brown spots) appeared since 3 to 5 days after challenge (Plate 1, No 2). More advanced stages consist of an organic film made up of one or several layers which usually strongly adheres to the prismatic layer (Paillard and Maes 1995b) (Plate 1, No 1 and 3). When the deposit is thick, outer fold of the mantle could present some lesions (desquamation and hypertrophia of the mantle epithelium) (Plate 1, No 4) (Paillard 1992; Paillard et al. 1994). Three stages of shell repair or recovery (SRS) are also proposed (Paillard and Maes 1994) ranged from apposition of calcified concretions (SRS 1) to wide calcified plates partially (SRS 2) or completely (SRS 3) covered the brown deposit. We describe recently a new shell repair stage (SRS 2.5) to be added to the classification of BRD (Paillard 2004). SRS 2.5 corresponds to a near complete recovery from BRD, with only a few small black points persisting despite deposits of wide calcified sheets associated with recovery. By considering the process of conchiolin deposit formation and the process of shell repair together, four phases of the disease are defined. The first phase corresponds to the development of the disease and is characterized only by accumulation of a conchiolin deposit. The second phase is characterized by the two processes occurring simultaneously in a given individual. The phase 2.5 corresponds to the end of the recovery process. The third phase corresponds to the final recovery of the disease when the clams have totally covered the conchiolin deposit.

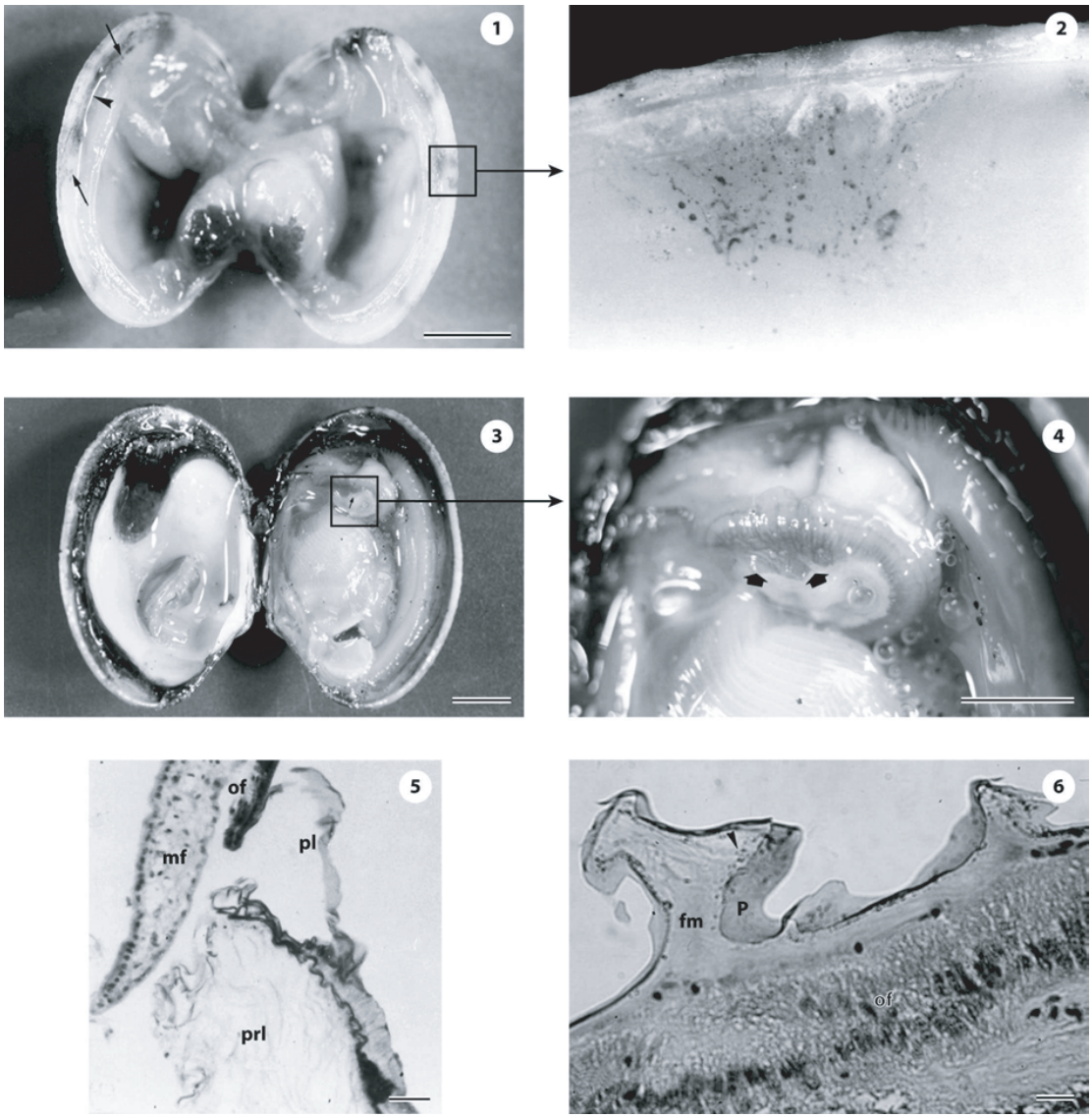

Plate 1. 1: Experimentally *Vibrio tapetis* juvenile infected clams showing characteristic conchiolin deposit (arrows). Experimentally induced brown ring syndrome, two weeks after challenge. Note that both valves develop conchiolin deposit at different stages. Bar = 5 mm.
2: Detail of the shell edge of infected clam showing the first signs of the disease; characteristic brown conchiolin spots adhering strongly to the inner surface of the valves.
3: Naturally diseased clams exhibiting a thick brown ring made up of numerous conchiolin layers. The outer fold of the mantle, in contact with this conchiolin deposit, show a red coloration (arrow); Bar = 0.8 cm.
4: Light micrograph of decalcified section of the edge of the mantle and the shell. The shell egde is composed principally of prismatic layers (prl). The periostracal lamina secreted between the outer fold (of) and the middle fold (mf) is accumulated on the inner surface of the shell and forms a deposit, which was in average 100 μm thick; Bar = 100 μm.
5: Detail of the outer fold of the mantle, in contact with thick conchiolin deposit. Lesions of the mantle (arrows); Bar = 0.4 cm.
6: Light micrograph of the outer fold (of) of the mantle in contact with the periostracal lamina (PL). Numerous bacteria are present within the periostracal lamina. Hematoxylin-eosine coloration; Bar = 10 μm.

2.2 Light and electron microscopy

Decalcified sections of shell and mantle edge, stained with hemalin-eosine allowed localisation of bacteria at the site of infection, within periostracal lamina (Paillard and Maes 1995a) (Plate 1, No 6). Indirect Immunofluorescence (IIF) technique using polyclonal antibodies against *V. tapetis*, could be applied to localise and identify *V. tapetis* in tissue sections, periostracal lamina and conchiolin deposit (Paillard 1992; Paillard and Maes 1995a; Paillard and Maes 1995b; Allam et al. 1996; Allam et al. 2000b). This IIF technique has provided evidence for identification of the bacterium in BRD clams from Spain even if isolation of *V. tapetis* has failed by bacteriological methods (see beneath) (Castro et al. 1997b). Electron microscopy observations allowed to follow the alterations of the periostracal lamina (Paillard and Maes 1995a)

throughout the disease process. An additional layer, rich in vacuoles and cell debris is found between the periostracum and fibrous matrix layer. When the deposit is wider and thicker at the surface of the inner shell, invasion of bacteria can be observed within the layers, scanning electron microscopy (Paillard and Maes 1995b).

2.3 Bacteriological techniques for pathogen isolation

V. tapetis strains have been isolated in BRD-affected clams cultured in France, England, and Galicia using bacteriological methods developped by Maes and Paillard (1992). Briefly, replicate subcultures on differential media are used to select potential *V. tapetis* strains, based on its four key characteristics; non-utilisation of sucrose and mannitol, growth on TCBS (thiosulfate citrate bile sucrose), and failure to grow above 27 °C. The colonies meeting these four requirements were isolated and purified and then subjected to phenotypic, serological and molecular characterization (see beneath). The detection limit is in average 5×10^3 CFU g^{-1} fresh weight or 5×10^3 CFU ml^{-1} pallial fluids (Maes 1992). DNA-DNA hybridisation and sequencing of at least four individual genes (16S rDNA, gyrase, rpoD, recA) must be also performed to confirm their homology with *V. tapetis*. Finally, in vivo injection of the bacterium into the pallial cavity must be carried on to evaluate the capacity of the isolate strain to produce development of clinical signs after four weeks following injection (Paillard and Maes 1990).

2.4 Immunological assay

In different countries (France, England, Spain), polyclonal antibodies against *Vibrio tapetis* reference strain have been successfully used to specifically detect and identify this pathogen in *R. philippinarum* using a slide agglutination test, the indirect immuno-fluorescence technique (IIF), an indirect dot-blot immuno-enzymatic assay and enzyme-linked immunoassay (ELISA) (Maes and Paillard 1992; Paillard 1992; Castro et al. 1995; Allam et al. 1996; Nöel et al. 1996; Castro et al. 1997b; Allam et al. 2000b). Only the dot-blot immuno-enzymatic technique with *V. tapetis* anti-serum showed a weak cross reactivity with some *V. pelagius* and *V. splendidus* strains isolated from diseased clams (Castro et al. 1995). The IIF method can be use to localise the pathogen, but also for detection diagnosis, in particular when bacteriological techniques cited above was not successful to isolate *V. tapetis* (the concentration of the pathogen determined by this method is about 10^3 cells ml^{-1} of pallial cavity). It was the case for BRD clams from the southwestern of Spain, the indirect imunofluorescence technique, provided evidence for the presence of *V. tapetis* in all the BRD-clams analysed although no *V. tapetis* strain has been yet isolated (Castro et al. 1997b). Using ELISA, *V. tapetis* concentrations have been estimated in extrapallial fluids and hemolymph (Nöel et al. 1996; Allam et al. 2002) (The detection limit is in average 5×10^4 CFU ml^{-1} extrapallial fluids and hemolymph).

2.5 Molecular identification

A PCR detection method has recently been developed for *V. tapetis* identification using dot blot hybridization and a species-specific primer (SSP-PCR) (Paillard et al. 2001; Paillard, in press). The detection limit determined by this molecular method is about 10^2 CFU ml^{-1}. This molecular detection allowed to identify *V. tapetis* in larvae, juveniles from hatcheries and in adult from natural and cultured populations (Paillard 2004).

2.6 Diagnostic criteria

Shellfish sample can be consider as being unaffected by BRD under two of the following three conditions (1 and 2 or 1 and 3):

1. No detection of brown deposit after microscopic examination.
2. No *V. tapetis* isolated using the bacteriological method coupled with agglutination test (e.g. *V. tapetis* concentration is inferior to 5×10^3 CFU g^{-1} fresh weight or 5×10^3 CFU ml^{-1} pallial fluids). However, this method is time consuming and may not provide a clear diagnosis because the bacterium grows slowly and is generally non predominant within the total heterotrophic microflora.
3. No detection of *V. tapetis* by immunological assay (indirect immuno-fluorescence, detection limit about 10^3 cells ml^{-1} of pallial cavity; ELISA technique (detection limit is about 5×10^4 CFU ml^{-1} (extrapallial fluids), or by molecular identification (detection limit is about 10^2 CFU ml^{-1} in fluids or tissue homogenates).

For other marine organisms, which exhibiting no BRD symptoms such as other molluscs, fishes, crustaceans, they can be consider as being no infected by *V. tapetis* or being no carrier species under only one of these two conditions above (2 or 3).

2.7 Geographic distribution (Fig. 1)

BRD has been reported in clams along the entire European Atlantic coast and occasionally in Mediterranean and Adriatic seas. BRD was first observed in France in 1987, in Spain in 1994, in England in 1997, in Ireland in 1998. (Paillard and Maes 1994; Castro et al. 1996; Novoa et al. 1998.) In 1990, the disease was reported in an Italian laguna in north Adriatic, but it did not spread and completely disappeared after several months during the summer. In Spain, BRD is observed generally in both *R. decussatus* and *R. philippinarum* inhabiting several rias along the Galician coast of northern Spain where environmental conditions, in particular temperature, are similar to those of the French Atlantic coast. *V. tapetis* has been found in other northern European countries, in Norway and Scotland, but in different host species, in moribund fishes (Fig. 1). Therefore, this disease can be classified as a cold-water disease.

Recently, in Asia, the symptom of BRD has been identified in manila clams, *R. philippinarum* from South Korea (Choi and Paillard, unpubl. data). With the framework of a

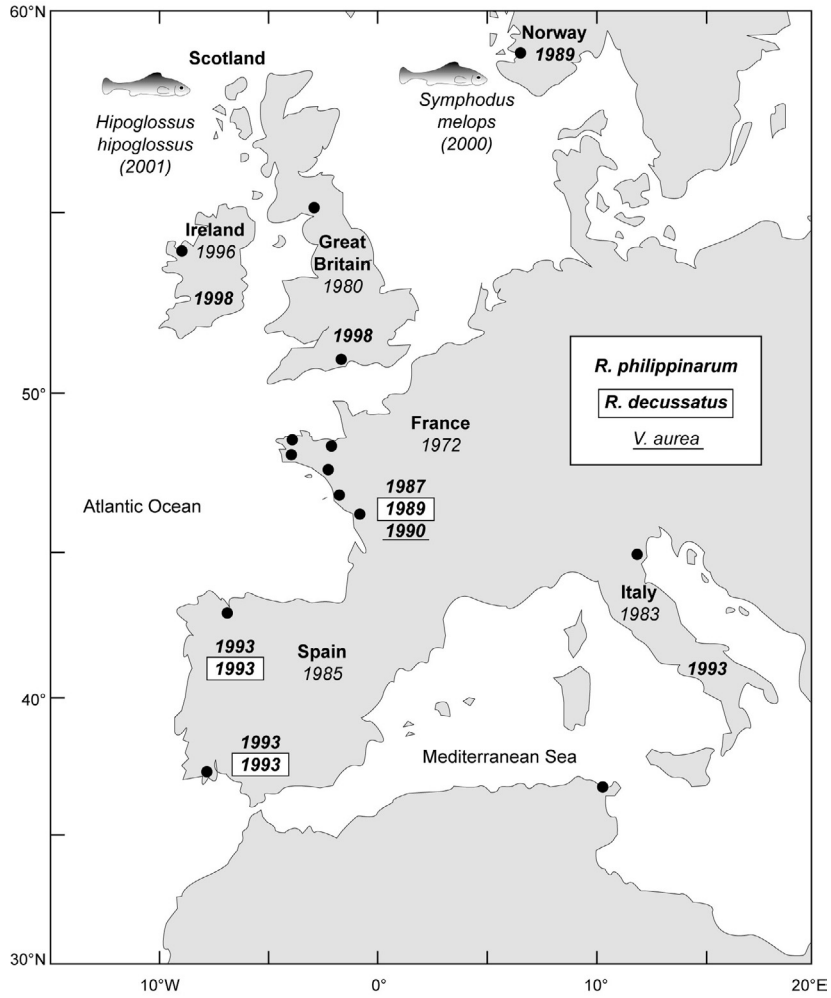

Fig. 1. Geographical distribution of clams in European coast. Detection of Brown Ring Disease in clams and *Vibrio tapetis* in fish. Introduction date of *Ruditapes philippinarum* (beneath the name of the country, italic). Detection date of BRD or *V. tapetis* in different species (italic); for *R. philippinarum* (bold type), for *R. decussatus* (bold type, etiquette), for other clam species (bold type, underline) and for fishes (no bold type, in brackets) (data from Paillard et al. 1994; Allam et al. 2000; Jensen et al. 2003; Reid et al. 2003a).

KOSEF-CNRS program, *V. tapetis* isolation is in progress. To date, this disease has never been reported in the country where the species was first introduced (Western USA and Canada).

3 Impact on host

The typical sign of BRD in clams is characterized by an abnormal brown deposit, organic in nature, which generally adheres in the inner face of the valves, generally between the pallial line and the edge of the shell forming a brown ring (Plate 1, No 3) (Paillard and Maes 1989). The clam can recover from the disease by covering the organic deposit by shell deposits, a defense process that has been named nacrezation (Paillard 1992). A classification system based on disease and recovery stages has been established for use in epidemiological and experimental studies (Paillard and Maes 1994). Deformations of the external shell, showing reduced growth, are very often associated with this disease (Paillard 1992). Tissue lesions are not systematically observed in diseased clams. Alterations of the digestive gland and the mantle are detected only in the more advanced stages of the disease (Paillard 1992; Plana and Le Pennec 1991; Plana 1995). Hemolymph parameters, i.e. changes in total and differential hemocyte counts and leucine aminopeptidase activity were altered by experimental challenge with *V. tapetis* (Oubella et al. 1993; Oubella et al. 1994; Oubella et al. 1996). No immune response was observed in *R. philippinarum* that have been challenged with either heat killed *V. tapetis*, *V. anguillarum* or *V. pelagius* (Oubella et al. 1996). Defense parameters in the extrapallial fluid, which is in contact with the site where the pathogen predominates (mantle edge and periostracal lamina), have hemocytes counts and lysozyme activity comparable to those found in the hemolymph (Allam and Paillard 1998). In both compartment, hemocyte counts and lysozyme activity increase in naturally and experimentally BRD-affected clams (Allam 1998; Allam and Auffret 2000; Allam et al. 2000a). BRD is also associated with a significant decrease in glycogen suggesting that mass mortalities could result from the degeneration of metabolic activity (Plana et al. 1996). In winter, when

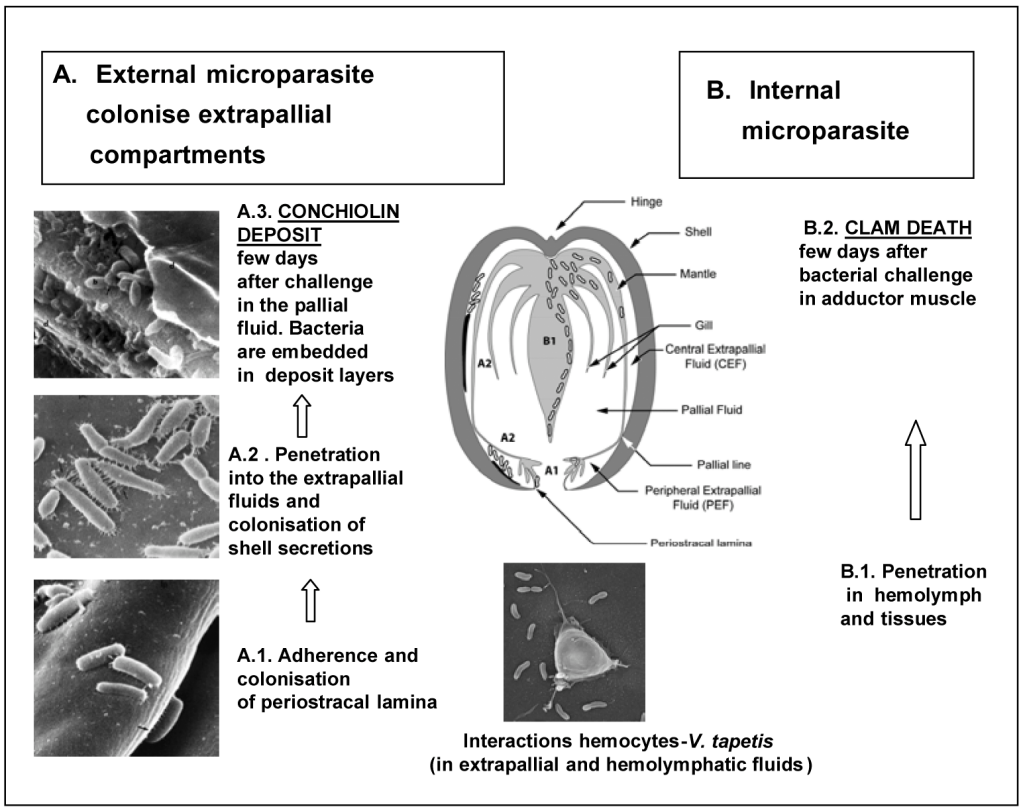

Fig. 2. Vibriosis progress: 3 differents pathways for *V. tapetis* colonisation into clams.
Pathway A: External microparasite inducing conchiolin deposit symptom. Step A.1: Adherence and colonisation of infection site, the periostracal lamina. Step A.2: Penetration and colonisation of the extrapallial compartment (peripheral and/or central). Step A.3: Conchiolin deposit. Step A.1 is an obligatory step for CD development. Step A.2 occurs only in peripheral compartment (2a) or central compartment (2b) or in both (2a and 2b) depending disease stages.
Pathway B: Internal microparasite provoking clam death. Step B.1: Penetration of *V. tapetis* into hemolymph and tissues if some epithelium lesions occur. Step B.2: Clam death.
Pathway A +B: External and internal microparasite. Step B.1 can occur after step A.1 or A.2 or A.3 or directly without these previous steps, depending on the physiological state of clams.

mortalities occurred, the moribund clams generally exhibited higher BRD prevalence compared to the burrowing clams (Paillard 1992).

3.1 *Vibrio tapetis* pathogenicity

In the clam, *R. philippinarum*, the majority of *V. tapetis* cells are localized at the mantle surface, in the extrapallial fluids, and on and within the periostracal lamina (Plate 1, No 6, Fig. 2) (Paillard and Maes 1995a; Allam et al. 1996). Adherence factors such as pili have been identified on the bacteria and allowed the colonisation and proliferation of *V. tapetis* in the peripheral compartement (periostracal lamina and extrapallial fluids) (Fig. 2) (Paillard 1992; Paillard and Maes 1995a). All the *V. tapetis* strain showed higher adhesion capability towards the clam hemocytes and mantle cells as compared to several fish cell lines (Lopez-Cortes et al. 1999). These finding suggests the existence of a host and tissue specificity Lopez-Cortes et al. (1999). Two different types of experimental challenge have been established to determinate in vivo pathogenicity of *V. tapetis* strains. The first one consist in the experimental reproduction of CD symptom and is performed by inoculation of *V. tapetis* suspension in pallial cavity (5×10^7 CFU ind.$^{-1}$ for a clam length in average 15−20 mm, T °C: 14 °C) (Plate 1, No 1 and 2, Fig. 2A). After four weeks, the prevalence of CD reached from 60 to 95%, depending of *V. tapetis* strains and challenge years (Paillard and Maes 1990; Maes 1992; Paillard 2004). The second type of in vivo challenge consists in the experimental reproduction of mortalities and the inoculation is performed directly into the posterior adductor muscle of healthy *R. philippinarum.* This challenge causes no conchiolin deposit, but mortalities of up to 100% occurred in twelve days (5×10^7 CFU ind.$^{-1}$ for a clam length in average 15−20 mm, T °C: 14 °C) (Allam et al. 2002b) (Fig. 2B). Experimental studies after *V. tapetis* challenge into the pallial cavity and the adductor muscle have allowed to distinguish differences between *V. tapetis* strains in term of their capacity of producing BRD signs and mortalities (Paillard 2004; Choquet et al. 2004; Novoa et al. 1998). Toxins such as hemolysin and exo-enzymes such as esterase and chymotrypsin have been detected in extracellular products (Borrego et al. 1996). *In vitro*, *V. tapetis* has a cytotoxic effect on clam hemocytes that results in the loss of filopods and cell

rounding (Lane and Birkbeck 2000; Choquet et al. 2003). This cytotoxic activity can be measured using a bioassay developed by Lane and Birkbeck (1999), modified for flow cytometry by Choquet et al. (2003). According to this bio-assay, Choquet and collaborators have demonstrated that there are differences of pathogenicity among the different isolates. Identification and characterisation of the genes involved in *V. tapetis* cytotoxicity are in progress.

3.2 Vibriosis progress (Fig. 2)

The attachment of *V. tapetis* to the periostracal lamina can be the initial event in the BRD process (Fig. 2, step A.1.). Depending on the host immune response, and its physiological conditions, the colonisation process of the pathogen would be successful or not. In favourable conditions for the pathogen, *V. tapetis* colonisation provokes some alterations and rupture of the periostracal lamina which allows the penetration of bacteria into the extrapallial fluids (Fig. 2, step A.2). In these "pseudo-internal" compartement, the bacteria colonise shell secretions and progressively are embedded into the conchiolin deposit layers. During development of the disease, the deposit becomes thicker and wider and invaded by numerous bacteria (Fig. 2, step A.3). If some tissue lesions occurred, such as gill alterations observed in winter (Fig. 2, step B.1), and mantle lesions associated with high developed conchiolin deposit (Fig. 2, pathway A+B), *V. tapetis* could penetrate and proliferate into tissues and finally could provoked the clam death (Fig. 2, step B.2) (Paillard 1992; Allam et al. 2002b).

3.3 Host susceptibility

V. tapetis induces low impact in *R. decussatus*. Experimental field study and laboratory experiments have shown that BRD prevalence in *R. decussatus* reached 20% to 40% compared to *R. philippinarum* in similar conditions (60 to 100%) (Paillard et al. 1997; Allam et al. 2001). No mortalities were observed following injection of *V. tapetis* directly into the posterior adductor muscle of this native specie, *R. decussatus* (Allam et al. 2002). Susceptibility of United States population (US) of *R. philippinarum* is clearly different from French (FR) *R. philippinarum* populations (Allam et al. 2001). US clams developed fewer and lighter BRD symptoms (50% compared to 100% in FR *R. philippinarum*) and showed better recovery than the FR populations. Low BRD development and recovery in *R. decussatus* and US *R. philippinarum* may be associated with high number of phagocytic cells in the extrapallial fluids, the site of infection (Allam et al. 2001).

3.4 Associated environmental conditions

V. tapetis is very sensitive to high temperatures. It does not grow and survive at temperatures exceeding 27 °C and is rapidly killed at 30 °C in sea water (Maes 1992; Paillard et al. 1997). Field and experimental studies confirmed the significant effect of temperature on the development of BRD and on clam defense related activities (Paillard et al. 1997; Allam 1998; Paillard et al. 2000; Allam et al. 2002a; Reid et al. 2003b; Paillard et al. 2004). Salinity could affect also the BRD prevalence and the immune defense of the clam, *R. philippinarum*. Experimental studies have shown that lower salinity could increase BRD prevalence (Reid et al. 2003b). Along the French Atlantic coast, the Loire River appears to be a natural boundary. Sites located south of the Loire exhibit much lower prevalences (0 to 3%) than the sites to the north (20 to 60%) (Paillard et al. 1997). In France, in all sites, BRD is more prevalent in culture *vs* wild clams.

Significance. BRD causes mass mortalities of cultured juvenile and adult Manila clams, especially in winter and spring, when maximum prevalence occurs. Several physiological parameters such as growth, weight and condition index are also affected. Diseased clams are significantly smaller, lighter and have a condition index significantly lower than healthy ones (Paillard 1992). It is clear that the clams are weakened by BRD and therefore more sensitive to various causes of mortality such as environmental stress, pollution and secondary infections but also less resistant to cultural and commercially practices (seed, high density, storage, travel, exchange) (Paillard 1992; Flassch et al. 1992). Therefore, this disease has a real impact on fisheries and aquaculture of clams. Since 1987, BRD has caused mass mortalities on various cultured clam beds along the west coast of France essentially stopped clam culture in the northern sites of Brittany.

4 Methods of BRD control

Reducing density of clams may be beneficial (Paillard 1992). Also, the type of substrate in clam beds significantly affects the disease. Laboratory studies indicated that the most probable transmission route is by means of direct contact with infected clams (Martinez-Manzanares et al. 1998) and by feces (Maes 1992). In order to control BRD epizootics, different chemotherapeutic agents were tested such as Romeiod, Agroseptil, Chloramine T and Furazolidone. Administration of 10 mg L^{-1} for 3 days of Furazolidone seemed to give 100% protection against *V. tapetis* (Noël et al. 1992). Regarding the use of antibiotics, some restrictions must be taken into considerations; Furazolidone is reported to have mutagen and carcinogen effects on higher animals even if it is rapidly photodegraded and remaining in the sediment only for a short time, and then metabolized in a component which exhibits no mutagenicity and no antibacterial activity (Samuelsen et al. 1991; Samuelsen et al. 1991). Thermic preventive treatments have been also investigated. The observation that the disease is absent in areas with high summer temperatures is supported by experimental evidence that showed a recovery process occurring at temperatures over 21 °C (Paillard et al. 1997; Paillard et al. in press). Allam et al. (2001, 2002) indicated that these temperatures increased potential cellular defense mechanisms, especially phagocytosis of *V. tapetis* by haemocytes in the extrapallial fluid. Temperatures above 21 °C also reduced the growth of *V. tapetis in vitro* (Maes 1992; Paillard et al. 1997; Paillard et al. 2004). A significant increase in temperature may have a preventive effect on the development of the disease. To take advantage of this effect, French hatchery operators

produce larval and juvenile clams in warm-water sites along the country's south Atlantic coast.

5 Comments

The organic deposit which characterizes BRD in *R. philippinarum* and *R. decussatus* has been reported in several other bivalve species (*V. aurea, T. rhomboides, Mercenaria mercenaria, Dosinia exoleta, Pecten maximus, Crassostrea virginica*) (Maes 1992; Bricelj et al. 1992) and recently been found in gasteropods such as abalone (*Haliotis tuberculata, H. rubra, H. leviagata H. scalaris*) (Sherperd and Huchette 1997; Sherperd and Triantafillos 1997; Marshall and Day 2001; Huchette et al. 2002). The deposit is a defense reaction and it is not exclusively due to *V. tapetis*; some others parasites (fungi, annelids and trematodes) are also well known to disturb the pallial edge by shell boring, by irritating the epithelium, or by living within mantle tissue (Paillard et al. 1994; Paillard et al. 1996). Shell disease have been also described also in the gold lip pearl oyster, *Pinctada maxima* and in *Pinctada margaratifera* (Perkins 1996; Cuif and Dauphin 1996). Because of the non specificity of the symptom, the BRD must be always associated with *V. tapetis* detection.

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