Bacteria in molluscs: good and bad guys

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The aquaculture of molluscs is seriously affected worldwide by bacterial pathogens that cause high losses in hatcheries as well as in natural beds. The main responsible for the mortality outbreaks is a number of Vibrio species that are considered important pathogens in aquaculture. The pathologies caused by vibrios in bivalves have been described since the 1960s; however, over recent years, successive episodes of high mortality have been recorded due to these microorganisms. The present work provides an updated overview of main diseases and implicated Vibrio species affecting the different life stages of cultured bivalves. With the introduction of new species as the abalone (gastropod) and octopus (cephalopod), new potential pathogens have also appeared, such as members of the genus Pseudomonas or Serratia, among others. On the other hand, in the last years, special attention has been focussed on the use in hatcheries of bacteria with antimicrobial activity to control the composition of microbiota associated to the mollusc larvae in order to avoid pathogens and improve larvae survival. Different taxa such as Phaeobacter or Pseudoalteromonas have been considered as probiotics (extended definition for aquaculture) and tested in laboratory condition and in the field.

Keywords: Bacteria, molluscs, pathogens, Vibrio, probiotics

1. Introduction

One of the main problems in aquaculture of molluscs is the repetitive episodes of mortality, which seriously reduce the production. These outbreaks of disease affect larval and post-larval stages in hatcheries, as well as juvenile and adults cultured in natural environment. In the case of hatcheries, the massive mortalities involve the complete loss of the stocks of production, with serious economic consequences. In most of the cases, the studies have demonstrated that the problems are caused by bacterial pathologies, being the main etiological agents members of genus Vibrio [1, 2]. In relation to the stages cultured in natural beds, despite of the initial attention only to the pathologies caused by parasitic protozoa, in the last years special attention is being paid to diseases with bacterial origin, affecting survival of cultures.

The lack of systematic and rigorous studies on the bacterial populations associated to the mollusc culture and, therefore, the scarce knowledge on the matter, has led to the search of solutions focussed to the complete elimination of the microbiota in the culture water during hatchery stages. The different methods employed, from water treatments to chemotherapy, have proved to be inadequate to avoid the episodes of mortality. The use of probiotic bacteria is the most promising alternative in aquaculture for the development of cultures, obtaining a well-balanced bacterial population with auto-regulation capacity. Moreover, their use avoid the hazards derived from antibiotics and other control measures.

In this chapter, the actual knowledge about this subject is reviewed, detailing the main bacterial pathogens affecting larval and post-larval stages cultured in hatchery, as well as juveniles and adults cultured in natural environment. The characteristic signs of the diseases caused by the different bacterial species are described, as well as their host range and geographical distribution. Besides, the advantages of specific application of probiotics in hatcheries are analyzed, considering the special characteristics of this type of cultures. The need of their utilisation is justified by the disadvantages of the different systems for disease control usually applied, like treatment of water and chemotherapy.

The different bacterial species suggested as potential probiotics are considered, as well as the way to select them, the modes of action, and their possible applications in hatcheries.

2. Bad guys

2.1. Pathogenic bacteria for bivalve larvae

2.1.1. Culture in hatchery. General view

Hatcheries are nowadays the main source of seed for the aquaculture of many bivalve molluscs with high economic value, such as oysters, clams or scallops. Seed obtention by induced lay has been studied from a technical point of view in order to establish the optimum conditions for its performance. The influence of environmental factors including salinity, temperature, culture density, etc, has been the aim of numerous works. However, there is a lack of knowledge on the pathological problems, probably due to the shortage of rigorous systematic studies.
The episodes of high mortality, which lead to the loss of whole production stocks, are of periodic frequency and usually the responsible agent is not determined [3-13], even though they imply an important economic loss and a lack of seed supply. The optimum conditions for bivalve culture also favour the growth of bacteria and the accumulation of their metabolites [7, 14, 15]. The disease process is favoured in many occasions by a larval susceptibility increase due to external stress factors, including bad quality of food or water, organic contamination, etc. In addition, these factors also facilitate the growth of potential pathogenic bacteria [6, 16]. Therefore, in many occasions mortalities can be associated to the overgrowth of opportunist pathogens.

2.1.2. Vibriosis

In a hatchery, although all larval stages are vulnerable, during the temporary fixation of the larvae on the bottom of the tank they are exposed to a high concentration of potential pathogenic bacteria associated with the tank surface, moribund larvae or organic detritus [17]. Guillard [18] was the first to report evidence of the involvement of a *Vibrio* sp. in the disruption of the velum and internal tissues of the clam larvae *Mercenaria mercenaria*, which produced a mortality of 70% of the population. In the study performed by Tubiash et al. [3], they observed “swarms” of bacteria appearing on the margins of the larvae (Fig. 1), which became progressively denser and after 8 hours, mortality occurred as a result of granular necrosis. This study was the first one to describe the term “bacillary necrosis” affecting numerous bivalves: *Crassostrea virginica*, *Ostrea edulis*, *M. mercenaria*, *Argopecten irradians* and *Teredo navalis*. Typical signs of “bacillary necrosis” included the extension of the velum, motility reduction or erratic movements in circles that appeared after 4-5 hours of exposure to *Vibrio* spp. The species *V. alginolyticus*, *V. tubiashii* and *V. anguillarum* were recognised as the main causal agents of the “bacillary necrosis” in later studies [16, 19]. The disease is characterized by bacterial colonization of the mantle, velum disruption, abnormal swimming, visceral atrophy, and lesions in the organs among other signs. Another characteristic sign of larval vibriosis in hatcheries is the appearance of the phenomenon called “spotting”, defined as an accumulation of moribund larvae agglutinated at the bottom of the tanks [6]. Table 1 provides a summary of the most recent studies on bivalve larvae vibriosis.

![Fig. 1. Detail of the disease signs in experimentally infected larvae: bacterial “swarming” surrounding the larvae. Arrow indicates the bacterial cells. Bar = 200 μm.](image)

Originally, *V. anguillarum*, *V. alginolyticus*, *V. tubiashii* and *V. splendidus* were the recognized agents associated to larval vibriosis. However, other species have been described in the last two decades [13, 20, 21]. This is the case of *V. pectenicida* [21], originally described as a *Vibrio* sp. strain able to produce a high mortality of the scallop larvae *Pecten maximus* after 48 hours of exposure due to the release of bacteria toxins which interrupt the digestive transit and degrade the tissues of the larvae [20]. A number of studies have been conducted with this species to reproduce experimentally the infection and to evaluate the pathogenesis and the interactions with haemocytes [2].

Prado et al. [13] investigated 3 *Vibrio* strains isolated from oyster larvae (*O. edulis*) in three different hatchery outbreaks in Galicia showing a disease compatible with a “bacillary necrosis”. One strain was identified as *V. neptunius*, being the first description of this bacterial species as pathogen of oyster larvae. The other two isolates were classified as *Vibrio* sp., because despite showing similarities to *V. orientalis* and *V. vulnificus* they could not be unequivocally assigned to these species. Further characterization studies showed that one of those *Vibrio* sp. strains constitutes a new species, for which the name *V. ostreicida* was proposed [unpublished results].

The species *V. tubiashii* reported as one of the causative agents of the “bacillary necrosis” [16, 19] has recently been described as a re-emergent pathogen in North America causing a decline of approximately 59% in larvae oyster production [22]. In addition, Hasegawa et al. [23] demonstrated that one of the critical factors in the pathogenicity of *V. tubiashii* in oysters (*Crassostrea gigas*) is the presence of a quorum sensing-regulated metalloprotease (VtpA), which is widespread among *Vibrio* species.

It has been suggested that agents responsible for the development of disease in bivalve larvae seem to act synergistically and infection occurs as a result of stressed larvae [6, 13, 24]. This highlights the importance of maintaining optimum water quality and culture density in a hatchery. The use of antibiotics may be beneficial, although frequent use may lead to the appearance of bacteria resistant strains [25]. One possible measure against bacterial pathogens cultured bivalves could be the genetic selection of resistant larvae populations at the hatcheries [26].
2.1.3. Other bacterial diseases

Members of the genus *Pseudomonas* have been described in some cases as pathogens for bivalve larvae, being in most cases isolated together with representatives of the genus *Vibrio* causing problems in larval cultures. Descriptions have been published from 1959 from different geographic areas and diverse mollusc species, including clams (*M. mercenaria*), oysters (*O. edulis, C. virginica*), and pectinids (*T. gigas, A. purpuratus*) [17, 27-29].

Garland *et al.* [10], studying the mortalities occurred in hatchery of *C. gigas*, obtained two isolates of the genus *Alteromonas*, which resulted pathogenic for the larvae. The genus *Moraxella* has been also occasionally described as pathogenic for *T. gigas* larvae [17]. Finally, the group *Aeromonas – Plesiomonas* comprises representatives isolated from moribund larvae of *T. gigas* [17]. The pathogenicity of an isolate of *Aeromonas hydrophila* for *A. purpuratus* larvae has been experimentally demonstrated [30].

2.2. Diseases of juvenile and adult bivalves

2.2.1. Pathogenic *Vibrio* species

The studies of Tubiash *et al.* [3] associated for the first time moribund adult bivalves (*M. mercenaria, C. virginica, M. edulis* and *M. arenaria*) with the species *V. tubiashii* and *V. alginolyticus*. Further cases of vibriosis in oyster were described by Elston and Leibovitz [8], detecting anomalies in the shell and alterations in the function of the ligament and digestive processes. The classical *Vibrio* infections are Summer Mortality in juvenile oysters and Brown Ring Disease in adult clams. Table 1 lists recent studies of *Vibrio* spp. that have caused pathologies in juvenile and adult bivalves.

In France, Summer Mortality (SM) affects juvenile populations of the Pacific oyster (*C. gigas*) during the warmer months when the water temperature is ≥18ºC and reproduction takes place [31]. This phenomenon has been associated to stress situations, low dissolved oxygen or presence of toxic substances in the sediment [32]. Lipp *et al.* [33] were the first to observe that the oyster haemolymph had high levels of vibrios that were causing death. Later studies identified *V. splendidus* as the causal agent of SM [34-37]. Garnier *et al.* [38] analysed the bacterial populations in the haemolymph of moribund oysters by phenotypic and molecular methods and found that the prevalent species were *V. aestuarianus* (56%), describing the new subspecies *V. aestuarianus* subs. *francensis*, and *V. splendidus* (25%). The pathogenicity of *V. aestuarianus* for the oyster was demonstrated by Labreuche *et al.* [39], that described the immunosuppressive activity of the extracellular products of this species on the haemocyte functions. More recently, Allain *et al.* [40] suggested the possible role of *V. harveyi* as aetiological agent of SM, since it was detected in most samples of affected oysters during the 2008 warm season, and was able to produce mortality in experimental challenges. The most accepted theory today is that the oyster SM cannot be attributed to only one bacterial pathogen or to the oyster Herpesvirus, but to a complex interaction between the physiological and/or genetic state of the host, environmental factors and the presence of various opportunistic infectious *Vibrio* species [2, 39, 41].

Brown Ring Disease (BRD), caused by *V. tapetis* [42], has been widely studied since it is the main disease with bacterial aetiology in adult clams (*R. philippinarum* and *R. decussatus*) [2, 24]. Susceptibility to *V. tapetis* infections is species-specific, causing greater physiological disturbances and mortality in *R. philippinarum* than in other species of clam (*R. decussatus* and *M. mercenaria*) or in the oyster *C. virginica* [43, 44]. The disease is characterized by the alteration of the calcification process on the inner surface of the valves and the appearance of a characteristic brown deposit consisting of conchiolin between the edge of the shell and the pallial line (Fig. 2a) [42, 45]. BRD is considered one of the main limiting factors in the culture of Manila clam (*R. philippinarum*) in Europe [45-49] and was also recently detected in Manila clams cultured in Korea [50]. Environmental factors (i.e. temperature and salinity) play a role in the development of BRD, which tends to be more frequent in the spring and winter as the optimal growth temperature for *V. tapetis* is 15ºC [2, 24]. Reid *et al.* [51] demonstrated in challenges with Manila clams, that the disease was more severe when performed at 20 ppt salinity than at 40 ppt. The diagnostic of BRD is currently based on the examination of the characteristic brown ring on the inner edge of the shell. For an early diagnosis in the absence of the brown ring, specific PCR detection protocols targeting the 16S rRNA gene have been designed [50, 52, 53]. Biochemical, serological and genetic intraspecific variability within *V. tapetis* had been described as well as three main subgroups that correlate with the host type [54]. This heterogeneity has been further demonstrated with MLSA (multilocus sequence analysis) and 2D-PAGE proteomic analyses [55, 56].

The pathogenic potential of the recently described species *Vibrio celticus*, a component of the microbiota of cultured clam populations in Galicia (NW Spain), has been confirmed in experimental challenges of juvenile clams [57].
Table 1. Experimental infections of bivalve larvae, juvenile and adults with pathogenic *Vibrio* spp.

*Abbreviations: C = Crassostrea, M = Mytilus, O = Ostrea, P = Pecten, R = Ruditapes, BRD = Brown Ring Disease, ECP = Extracellular products, NS = Not specified.

<table>
<thead>
<tr>
<th>Pathogenic species</th>
<th>Origin of the tested strain/s</th>
<th>Host*</th>
<th>Life stage</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. alginolyticus</em></td>
<td>Culture collection</td>
<td><em>M. galloprovincialis</em></td>
<td>Larvae</td>
<td>58</td>
</tr>
<tr>
<td><em>Vibrio</em> sp.</td>
<td>Mortality outbreak</td>
<td><em>C. gigas, O. edulis</em></td>
<td>Larvae</td>
<td>59</td>
</tr>
<tr>
<td><em>V. splendidus</em> biovar II</td>
<td>Mortality outbreaks</td>
<td><em>R. decussatus</em></td>
<td>Larvae</td>
<td>60</td>
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<tr>
<td><em>V. alginolyticus</em></td>
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</tr>
<tr>
<td><em>V. neptunius; Vibrio</em> sp.</td>
<td>Mortality outbreaks</td>
<td><em>O. edulis</em></td>
<td>Larvae</td>
<td>13</td>
</tr>
<tr>
<td><em>V. pectenicida</em></td>
<td>Mortality outbreaks</td>
<td><em>P. maximus</em></td>
<td>Larvae</td>
<td>61</td>
</tr>
<tr>
<td><em>V. splendidus</em>-like</td>
<td>Pacific oyster</td>
<td><em>C. virginica</em></td>
<td>Larvae</td>
<td>62</td>
</tr>
<tr>
<td><em>V. tubiashi</em></td>
<td>Pacific oyster</td>
<td><em>C. gigas</em></td>
<td>Larvae</td>
<td>23</td>
</tr>
<tr>
<td><em>V. tapetis</em></td>
<td>Symptomatic clams</td>
<td><em>R. philippinarum</em></td>
<td>Adults</td>
<td>43, 44, 63</td>
</tr>
<tr>
<td><em>V. splendidus</em></td>
<td>Moribund oysters</td>
<td><em>C. gigas</em></td>
<td>Juveniles</td>
<td>34</td>
</tr>
<tr>
<td><em>V. splendidus</em> biovar II (V. chagasii)</td>
<td>Mortality outbreak</td>
<td><em>C. gigas</em></td>
<td>Juveniles</td>
<td>35</td>
</tr>
<tr>
<td><em>V. splendidus</em>-like</td>
<td>Moribund/ healthy oysters</td>
<td><em>C. gigas</em></td>
<td>Adults</td>
<td>37</td>
</tr>
<tr>
<td><em>V. aestuarianus</em></td>
<td>Moribund/ healthy oysters</td>
<td><em>R. philippinarum</em></td>
<td>Seed</td>
<td>39, 65</td>
</tr>
<tr>
<td><em>V. aestuarianus</em> subsp. francensis</td>
<td>Mortality outbreak</td>
<td><em>C. gigas</em></td>
<td>Adults</td>
<td>38</td>
</tr>
<tr>
<td><em>Vibrio</em> sp.</td>
<td>Pacific oyster</td>
<td><em>C. virginica</em></td>
<td>Juveniles</td>
<td>62</td>
</tr>
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2.2.2. Infections by *Rickettsia*-like organisms (RLO)

The presence of intracellular prokaryotes (Fig. 2b) has been described in several marine invertebrates [67-76], being suggested in some occasions an association with mortalities [72]. However, if these infections produce significant alterations in the host has been controversial during decades [71]. Some authors indicated a key role in the appearance of disease and mortality [72, 77-82], whereas other authors suggested that these microorganisms do not cause damages to the host cells or cause just a limited pathology [73, 83].

In 1987, a massive mortality (aprox. 40%) of scallop (*Pecten maximus*) was detected in Brittany (France). The anatomo-pathological study revealed the presence of a bacterial infection in the gills [77]. Intracellular basophilic bacterial colonies of different sizes were observed, in some cases blocking the blood vessels. Koch’s postulates could not be fulfilled, but the authors suggested that the intensity of infection supported the hypothesis that the functionality of gills was compromised [77]. In further studies, the microorganism was purified and characterized, demonstrating its relatedness with the *Rickettsia* group, as well as the production of enzymatic activities, such as catalase or acid phosphatase, which could be related with its pathogenicity [84].

Other cases were subsequently described, also associated with mortalities in clam (*Venerupis rhomboïdes*) in Galicia (Spain) [82], and tropical pearl oyster (*Pinctada maxima*) and Australian oyster (*Crassostrea ariakensis*) in China [80, 85]. In these cases, the only pathogen observed was an intracellular prokaryote RLO, and therefore proposed as responsible of the mortalities. In clam the microorganism was located in the gills, whereas in oysters a more generalized infection was observed, the microorganism being detected in gills, mantle, and digestive tissues.

The currently most accepted hypothesis is that these RLO would be of low virulence and that the degree of the pathological alterations would be related with the infection degree. Then, only massive infections, blocking important physiological functions by mecanic action, would cause a significative pathology.
2.2.3. Juvenile oyster disease (JOD)

Juvenile oyster disease (JOD) was detected for the first time in cultured populations of *Crassostrea virginica* in USA during the 1980s [86]. The disease affects to oyster shorter than 25 mm high, and mortalities may reach 90% of annual production [86, 87]. In affected areas, the disease has annual frequency, when water temperature is ≥ 20ºC [86-88]. The main signs are the growth cessation, irregular edges of the valves and a great buckling of the inferior valve followed a conchiolin deposit in the inner shells [86, 88].

Cohabitation experiments with infected oysters demonstrated the transmissibility of the disease [89, 90], although its etiology was more difficult to evidenced. Its was first suggested that bacteria or parasites occasionally observed in the lesions and/or conchiolin could be the causative agents [91], confirming the bacterial hypothesis some experiments with antibacterial agents which decreased the mortality [92]. Further studies revealed that a new species of α-proteobacteria within the *Roseobacter* branch [92], *Roseovarius crassostreae* [93], showing a strong colonization in all the infected oysters. Koch’s postulates could be than fulfilled [94, 95]. In fact, a change of the disease name to “*Roseovarius* oyster disease (ROD)” was proposed in order to avoid confusion with other diseases of juvenile oysters [95].

2.2.4. Nocardiosis

Around 60 years ago, mortalities in Pacific oyster (*C. gigas*) were registered in Japan and USA during the summer months [96, 97]. The presence of pathological alterations in the connective tissue around the digestive tract suggested their infectious nature, probably due to a Gram positive bacterium [98]. It was in 1991, when Friedman and Hedrick isolated a bacterium belonging to the genus *Nocardia* from diseased cultured oysters in Washington (USA) and British Columbia (Canada), naming the disease as Pacific Oyster Nocardiosis (PON) [99]. The phenotypic and genetic characterization of the isolates demonstrated that they constituted a new species for which the name *Nocardia crassostreae* was proposed [100].

The disease, both in natural and experimental infections, shows practically no external signs. In heavy infections, the presence of greenish/yellowish nodules was observed on most tissues, including adductor muscle, gills, heart and mantle [99]. These nodules are formed by haemocytes and Gram positive acid-resistant bacteria.

2.2.5. Other bacterial diseases

Infections by members of the genera *Chlamydia* and *Mycoplasma* have been described in a variety of adult bivalve molluscs, such as oyster, scallop, clam, mussel, and cockle (*Cerastoderma edule*) [67, 76, 79, 101-109].

Most descriptions are based on histological studies observing variable prevalences and, in some cases, pathological alterations in the host tissues related, as in the case of RLO, to the infection intensity [107, 108].

The genus *Cytophaga* has also been related to lesions in *Crassostrea gigas* juveniles. Dungan and Elston [110] have described homogenous bacterial populations associated to the 'resilium' showing a degradation process. The 'resilium' is one of the structural components of the ligament, responsible of the valves opening, and therefore, the degradation of the 'resilium' occasionate problems in the feeding, breathing, and other essential functions, resulting in a general physiologic weakening. In addition, the mechanic barrier of the valves is lost, being easier the colonization of the animal by pathogenic microorganisms.

2.3. Pathogenic bacteria for other molluscs

Abalone are gastropod molluscs of the family *Haliotidae* that inhabit coastal reefs in tropical, subtropical and temperate areas. In the last years, their culture has gained great importance in different countries throughout the world due to their high economic market value. Several infectious diseases have been reported in *Haliotis* spp., mostly bacterial and parasitic [111]. Pathogenic *Vibrio alginolyticus*, *V. harveyi* and *V. parahaemolyticus* have been isolated from diseased
abalones in Japan, Taiwan, China, and France [112-118], causing in some cases mass mortalities. The main clinical signs observed in the affected juvenile and adult individuals were abscessing or ulceration in the mantle, white spots on the foot, general whitening, as well as loss of ability to adhere to surfaces [119].

More recently, *Shewanella alga* and *Klebsiella oxytoca* have been described as causative agents of mass mortalities in post-larvae abalone in China, being their pathogenicity confirmed in experimental infections [120, 121]. In 2008 during an experiment of acclimatization, mortalities were recorded in wild individuals of abalone (*Haliotis tuberculata*) captured to constitute a broodstock for hatching. The phylogenetic analysis of the isolates indicated that they constitute a new genus and two new species within the Family *Oceanospirillaceae*, close to the genera *Neptunomonas* and *Oceanospirillum*, being proposed the names *Nuadamonas halioticida* and *N. abalonii*. Experimental infections in healthy abalone individuals demonstrated their pathogenic potential for this gastropod mollusc [122].

Octopus is a cephalopod mollusc which has also received great attention in the last years due to its interest for Aquaculture. There are some reports describing infectious agents in different species of octopus (*Octopus vulgaris*, *O. joubini*, or *O. briareus*), mainly from cultured animals but also from wild individuals [123-125]. The main signs observed in diseased octopus are skin ulcers, which can derive in deep wounds in head mantle or arms. Different *Vibrio* species, including *V. alginolyticus*, *V. anguillarum*, *V. harveyi* or *V. parahaemolyticus*, *Aeromonas caviae* and *A. hydrophila*, or *Pseudomonas stutzeri* have been associated with the appearance of these lesions in cultured octopus [123, 124]. In addition, Farto and coworkers [125] isolated the species *Vibrio lentus* from gill heart and skin lesions of wild octopus, confirming its pathogenic potential.

### 3. Good guys

#### 3.1. Classical methods for control of pathogens

The influence of the environment on the hatchery cultures is enormous. It is important to point out that larvae are released in early ontogenic stages which are highly sensitive to infections. In the case of bivalve larvae the influence is even higher since feeding of these animals is through filtration and therefore, a continuous water flow exist through the organisms [126, 127]. Different water treatments, such as filtration, pasteurization, ozone and UV radiation have been employed in mollusc hatcheries with the aim of eliminating potential pathogens [126]. The use of chemotherapeutic agents has been widespread in mollusc hatcheries, although showing inconsistent results [127]. In addition, drug usage present also other disadvantages including the high economic costs and, more important, the potential appearance of resistences and their transference to human pathogens. In fact, laws are becoming more restrictive for the use of antibiotics, being some of them forbidden for their application to animals for human consumption.

The final objective of all the methods cited above is to eliminate the entire water microbiota within the hatcheries, but this objective does not seem to reasonable, since the bacterial population, or at least part of it, may have a benefitial effect on the larval development. In addition, a lack of microbiota can favour the colonization of the system by non-desirable microorganisms, due to a lack of natural competitors, in an environment with a regular input of organic matter and optimal physical conditions.

Vaccination, an alternative with excellent results in the fish culture [128, 129] is not applicable to molluscs, since these organisms do not possess a real immunologic system.

Taking into account all these facts, it seems clear that different approaches for the control of molluscan bacterial diseases are needed, especially at larval stages.

#### 3.2. Probiotics

Verschuere et al. [126] gave a definition of probiotic appropriated to be used within the field of Aquaculture, taking into account the close interaction of the animal with the environment. According to these authors a probiotic would be a live microbial additive with a beneficial effect on the host, modifying the microbiota associated with the host or the environment, ensuring an optimal use of the feed or improving its nutritional value, improving the host response against the disease, or getting a better quality of its environment.

The desirable characteristics of a probiotic bacteria for aquaculture would include: i) to be isolated from the environment where it will be employed, which means a marine bacterium able to grow in the hatchery environment avoiding, in addition, the risks of introduction of alloctonous organisms in the system; ii) to have benecitial effects on the target host; and iii) to be non pathogenic or toxic, not only for the target host, but also for other live organisms present in the system, such as phytoplankton or other live food.

The majority of studies published on the use of probiotics in aquaculture correspond to works on fish and crustaceans, being scarce those focussed on molluscs. Lodeiros et al. [130] first published a study on the effect of antibiotic-producing marine bacteria on the larval survival of the scallop *Pecten ziczac*. Riguilme and coworkers developed deeper studies on the application of a probiotic bacterium *Pseudoalteromonas haloplanktis* (formerly *Alteromonas haloplanktis*) to *Argopecten purpuratus* larvae [131], determining its spectrum of activity against different bacterial species, including members of the genus *Vibrio*. They performed also a preliminary characterization of the
active compound concluding that it probably is an intracellular component, produced as secondary metabolite, that is secreted during the stationary phase of growth. In vivo experiments with larvae confirmed the protection conferred by the probiotic bacterium to larvae infected by a pathogenic *V. anguillarum* strain. The authors suggested its possible use as profilactic measure against the opportunistic pathogens in aquaculture. Further works of the same group [132] identified *Pseudoomonas* sp. and *Vibrio* sp. strains with a strong antibacterial activity, and studied the use of axenic microalgal cultures (*Isochrysis galbana*) as a way of incorporating the active bacteria to the culture [133]. Finally, they also studied the incorporation of the probiotic bacteria to massive larval cultures [134], inoculating periodically a mixture of antibiotic-producing strains to the system and demonstrating that the larval phase could be completed without adding commercial chemotherapeutic agents. Such treatment managed a modification of the microbiota associated to the larvae, eliminating potential pathogens.

Other bacteria with probiotic potential, including isolates of *Aeromonas media* and *Pseudoalteromonas* sp., were described by Gibson et al. [135] and Longeon et al. [136] to be applied in larval cultures of *C. gigas* and *P. maximus*.

In the last years, different isolates of *Phaeobacter gallaeciensis* (formerly *Roseobacter gallaeciensis*) have received special attention by different research groups, due to their great spectrum of inhibition against pathogenic bacteria from aquaculture systems [127, 137, 138]. Prado et al. [138] in experiments performed in marine water, with phytoplankton cultures and with larval oyster (*O. edulis*) and clam (*R. philippinarum*) cultures confirmed the results obtained *in vitro* on the inhibition of pathogenic vibrios by the *P. gallaeciensis* isolate. In both cases the same pattern of activity was observed, being necesary the introduction of the probiotic isolates in the system previously or simultaneously to the pathogens. These results indicate the potential use of *P. gallaeciensis* as control method in mollusc hatcheries, if its action is allowed before the pathogens reach high concentrations in the system.

**Fig. 3.-** Detection of *in vitro* antibacterial activity of a probiotic isolate of *Phaeobacter gallaeciensis*.

Fixation, irreversible process of adherence to the substrate at the end of the larval phase, is a critical moment during the larval development when important mortalities are usually observed. It is followed by the metamorphosis, when adult structures and benthonic life are acquired. The implication of bacteria in the processes of fixation and metamorphosis of marine invertebrates is well known. In most cases, an induction mediated by bacterial biofilms was observed. Weiner and Colwell [139] demonstrated that biofilms of a pigmented marine bacterium stimulate the larval fixation in *C. virginica*. The bacterium also produces a hydrosoluble exopolysaccharide [140], PAVE (polysaccharide adhesive viscous exopolymer)-like, promoting the adhesion of microorganisms to surfaces [141]. Further characterization of the bacterium identified it as *Alteromonas colwelliana* [142], later reclassified as *Shewanella colwelliana* [143]. Other works demonstrated that the biofilms of *Sh. colwelliana* could also induce the fixation of larvae of *Ostrea edulis*, but not of *Pecten maximus* [144]. *Sh. colwelliana* was successfully employed to induce the fixation of *C. gigas* and *C. virginica* larvae in hatcheries [145].

**4. Concluding remarks**

In this work, a revision of the current knowledge on molluscan bacterial pathology has been performed. It is expected that in a near future, with the diversification of the cultured species of molluscs and the incorporation of the intensive culture of these organisms to new geographic areas, new bacterial species with pathogenic potential emerge. The rapid identification of the new pathogens will be very helpful for their control which has to be based, due to the special characteristics of the culture of juvenile and adult molluscs, in the establishment of adequate preventive measures and the limitation of the movement of affected individuals. The development of molecular methods for diagnosis will be crucial to achieve such objectives. In addition, it will also allow the correct etiology of the diseases, as well as the study of the infection routes and the mode of action of the pathogens.

In the hatcheries, there is a constant entry of bacteria through the different compartments of the system (broodstock, water, phytoplankton, larvae, tanks, etc), which imply multiple routes of incorporation of potential pathogens. The seawater, where the larval cultures are maintained, determine their microbiota. Pathogen colonization can occur through any part of the organism, including the shell, and spread fast and easily. The best way to avoid this fact is the control of the environmental (water) microbiota to maintain a composition beneficial to the larval development and survival, or at...
least, to hamper the proliferation of opportunistic pathogens. Therefore, more studies on the practical utilization of probiosis in mollusc larval cultures are needed in order to know the interaction of probiotics with other live organisms present in the system, to establish their capacity to grow and the effective doses, and to get a method for their conservation and handling in the hatcheries. Their generalized application will allow not only a decrease in the use of antibiotics, with the subsequent beneficial effects on environment and human health, but also a more sustainable aquaculture.

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References


