Minireview

Bacterial diseases of crabs: A review

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ABSTRACT

Bacterial diseases of crabs are manifested as bacteremias caused by organisms such as *Vibrio*, *Aeromonas*, and a Rhodobacteriales-like organism or tissue and organ tropic organisms such as chitinoclastic bacteria, *Rickettsia* intracellular organisms, Chlamydia-like organism, and *Spiroplasma*. This paper provides general information about bacterial diseases of both marine and freshwater crabs. Some bacteria pathogens such as *Vibrio cholerae* and *Vibrio vulnificus* occur commonly in blue crab haemolymph and should be paid much attention to because they may represent potential health hazards to human beings because they can cause serious diseases when the crab is consumed as raw sea food. With the development of aquaculture, new diseases associated with novel pathogens such as *Spiroplasma* and Rhodobacteriales-like organisms have appeared in commercially exploited crab species in recent years. Many potential approaches to control bacterial diseases of crab will be helpful and practicable in aquaculture.

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1. Introduction

Bacterial diseases of crabs are not common when compared to virus and protozoa diseases. A review of the literature indicates that the majority of studies focus upon bacterial diseases of marine crabs that produce bacteremias or affect the exoskeleton. The genus *Vibrio* is frequently cited, especially *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*, which commonly occur in blue crab, but are also associated with human diseases (Krantz et al., 1969; Tubiash and Krantz, 1970; Sizemore et al., 1975; Davis and Sizemore, 1982; Huq et al., 1986). Shell disease is caused by other *Vibrio* spp. as well as by other chitinolytic or chitinoclastic bacteria which were first described in 1967 (Rosen, 1967). Shell disease continues to remain a topic of significant research (Comely and Ansell, 1989; Vogan et al., 1999, 2001, 2002) as several decapod species of commercial importance are affected. With the development and intensification of aquaculture, new bacterial diseases have recently appeared in commercially exploited crab species. These novel pathogens have inflicted heavy losses on the aquaculture industry. For example, *Spiroplasma* sp. in the Chinese mitten crab, *Eriocheir sinensis*, is a recent discovery and is the first *Spiroplasma* isolated from a crustacean (Wang et al., 2003b, 2004, 2010). In this paper, bacterial diseases of crabs, including the most recent new findings, are discussed. In order to present the information clearly, an outline of bacterial diseases is presented in Table 1.
### Table 1

**Bacteria from different species of crabs, the tissues in which they reside and key references selected by the authors.**

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Global location</th>
<th>Host</th>
<th>Tissue</th>
<th>Key reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium botulinum</em> type F</td>
<td>York River in Virginia</td>
<td>Blue crab Callinectes sapidus</td>
<td>Haemolymph</td>
<td>Williams-Walls (1968)</td>
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<tr>
<td><em>Vibrio parahaemolyticus</em></td>
<td>Chesapeake Bay</td>
<td></td>
<td></td>
<td>Colwell et al. (1975)</td>
</tr>
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<td><em>Vibrio parahaemolyticus</em></td>
<td>Chesapeake Bay</td>
<td></td>
<td></td>
<td>Krantz et al. (1969)</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>Galveston Bay, Texas</td>
<td>Horseshoe crab, Limulus polyphemus</td>
<td>Haemolymph</td>
<td>Sizemore et al. (1975)</td>
</tr>
<tr>
<td></td>
<td>Porto Novo Coast, Indian</td>
<td>Rock crab, Cancer irroratus; Shore crabs Carcinus maenas</td>
<td>Hepatopancreas</td>
<td>Huq et al. (1986)</td>
</tr>
<tr>
<td></td>
<td>Connecticut USA</td>
<td></td>
<td></td>
<td>Spindler-Barth et al. (1976)</td>
</tr>
<tr>
<td>Chitinoclastic bacteria</td>
<td>New York Bight</td>
<td></td>
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<tr>
<td></td>
<td>Brittany, France</td>
<td>Shore crab, C. pagurus</td>
<td></td>
<td>Morado et al., 1988</td>
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<tr>
<td></td>
<td>Langland Bay, Swansea, UK.</td>
<td></td>
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<td>Gordon (1966), Bakke (1973)</td>
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<td></td>
<td>Mid-Atlantic Bight</td>
<td>Jonath crab, C. borealis</td>
<td>Shell</td>
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<td></td>
<td>South Florida, USA</td>
<td>Stone crab, Menippe mercenaria</td>
<td></td>
<td>Iversen and Beardsley (1976)</td>
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<tr>
<td></td>
<td>South Atlantic Bight</td>
<td>Golden crab, C. fenneri</td>
<td></td>
<td>Wenner et al. (1987)</td>
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<tr>
<td></td>
<td>Eastern Canada</td>
<td>Snow crab Chionoecetes opilio</td>
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<td>Bower et al. (1994)</td>
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<tr>
<td></td>
<td>Southern Gulf of St.Lawrence, Canada</td>
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<td>Benhalima et al. (1998)</td>
</tr>
<tr>
<td><em>Aeromonas trota</em></td>
<td>Brittany France</td>
<td>Edible crab Cancer pagurus</td>
<td>Haemolymph</td>
<td>Baross et al., 1978</td>
</tr>
<tr>
<td></td>
<td>Hangzhou, China</td>
<td>Chinese mitten crab Eriocheir sinensis</td>
<td>Haemolymph</td>
<td>Baross et al., 1978</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Haemolymph, hepatopancreas, muscle connective tissue of hepatopancreas, gut, gills and gonads</td>
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<tr>
<td></td>
<td>Alaska, USA</td>
<td>Blue king crab Paralithodes platypus</td>
<td>Haemolymph</td>
<td>Meyers and Shorts (1990)</td>
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<td></td>
<td>Southeast Alaska, USA</td>
<td>Golden king crabs lithodes aequispina</td>
<td>Haemolymph, connective tissue of hepatopancreas, gut, gills and gonads</td>
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<tr>
<td></td>
<td>Jiangsu, China</td>
<td>Chiones mitten crab Eriocheir sinensis</td>
<td></td>
<td>Wang et al. (2001); Zhang et al. (2002)</td>
</tr>
<tr>
<td><em>Chlamydia-like organism</em></td>
<td>Willapa Bay and northern Puget Sound, Washington, USA</td>
<td>Dungeness crab Cancer magister</td>
<td>Connective tissue and connective tissue cells</td>
<td>Sparks et al. (1985)</td>
</tr>
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<td></td>
<td>Laboratory-reared</td>
<td></td>
<td>Haemocytes and haematopoietic tissue</td>
<td></td>
</tr>
<tr>
<td><em>Spiroplasma</em></td>
<td>Jiangsu, China</td>
<td>Rock crab Cancer irroratus jonah crab Cancer borealis</td>
<td>Connective tissue of hepatopancreas, gut, gills and gonads</td>
<td>Wang et al. (2003), Wang et al. (2004)</td>
</tr>
<tr>
<td><em>Rhodobacteriales-like organism</em></td>
<td>Swansea, Wales, UK</td>
<td>European shore crab, Carcinus maenas</td>
<td>Connective tissue and the blood vessels</td>
<td>Eddy et al. (2007)</td>
</tr>
</tbody>
</table>

### 2. Vibrio and bacteremia

Members of the genus *Vibrio* are ubiquitous throughout the world and are found associated with many marine and freshwater crustaceans. However, the pathological and physiological effects of *Vibrio* infections are best documented in marine crabs where *Vibrio* infections typically cause or produce bacteremias and shell disease. *Vibrio*-caused bacteremias have been reported from the blue crab, *Callinectes sapidus* (Krantz et al., 1969; Tubish and Krantz, 1970; Colwell et al., 1975; Sizemore et al., 1975; Davis and Sizemore, 1982; Sizemore and Davis, 1985; Welsh and Sizemore, 1985), *Callinectes bocourti* (Rivera et al., 1999) and rock crab, *Cancer irroratus* (Newman and Fen, 1982). Under conditions of *Vibrio*-caused bacteremias, a marked reduction in hemocyte numbers and intravascular clotting is observed; the apparent result of an endotoxin in the cell wall of the bacterium (Sritunyalucksana and Söderhäll, 2000).

The blue crab, *C. sapidus*, soft-shell industry is a major fishery throughout the eastern United States (NOAA Fisheries Office of Sustainable Fisheries, 2010), which leads the top of the species...
rankings by its value. *V. parahaemolyticus* was isolated from lethargic and moribund blue crabs, *C. sapidus*, in commercial tanks during “shedding” of soft crabs in Chesapeake Bay (Krantz et al., 1969; Tubiash and Krantz, 1970). The disease produced a mortality rate in excess of 50% in short-term shedding facilities. Affected crabs were weak and their haemolymph contained many bacteria when examined by phase microscopy. Using biochemical and serological techniques, and computer-based taxonomic analysis, the causative agent was identified as *V. parahaemolyticus*, which has previously been associated with outbreaks of food poisoning in humans. The bacterium is naturally found in the marine environment, occasionally invades marine animals and has been the etiological agent of “shirasu” food poisoning in Japan (Fujino et al., 1953). Furthermore, other common Vibrio human pathogens, such as *V. cholerae* and *V. vulnificus*, have been reported to regularly occur in blue crab haemolymph (Sizemore et al., 1975; Davis and Sizemore, 1982). Two proteolytic strains of *Clostridium botulinum* type F, which were involved in an outbreak of human botulism, have been isolated from the blue crab, *C. sapidus*, from the York River in Virginia (Williams-Walls, 1968). These disease outbreaks in crabs, regardless of degree of infection, should be closely monitored because they may represent potential threats to human health.

Members of the genus *Vibrio* has also been found in healthy blue crabs. Colwell et al. (1975) determined that the haemolymph of healthy blue crabs was not sterile, but instead contained large and variable numbers of viable, aerobic, heterotrophic bacteria. Computer-based taxonomic analysis revealed that the predominant genus was *Vibrio*, but members of the genera *Bacillus*, *Acinetobacter* and *Flavobacterium* were also present. However, Johnson (1976a) has argued that “naturally” infected crab acquired bacterial infections during the stress of capture and transport, not before. Dr. Johnson questioned whether any animal could tolerate the presence of a known pathogen such as *V. parahaemolyticus* within its body fluids and tissues. Indeed, most of the collected crabs were heavily stressed, having been purchased from commercial sources. The effect of commercial capture and handling stresses on the prevalence and levels of infection in blue crab were studied by Sizemore and Davis (1985) and Welsh and Sizemore (1985). They found *Vibrio* spp. to be the predominant bacterium present in heavily infected crabs and were primarily responsible for progressive infections in commercially stressed crabs. As a result, physiological stressors including capture, handling and transport may reduce the effectiveness of host defensive reactions and may lead to increased numbers of bacteria within the haemolymph. As an example, the haemolymph of crabs with missing appendages had significantly higher counts than uninjured crabs (Tubiash et al., 1975).

The *Vibrio* species in the haemolymph of the rock crab, *C. irroratus*, from Connecticut USA, was demonstrated to be pathogenic over a wide range of temperatures (Newman and Feng, 1982). Another blue crab, *C. bocourti*, from a eutrophic system in Puerto Rico, was studied in an effort to quantify total bacterial densities and identify bacterial species in the haemolymph (Rivera et al., 1999). The results showed that *C. bocourti* could tolerate very high densities of bacteria in their hemopox (average = 8.89 × 10^9 cells ml^-1^). However, hemocoelic bacterial infections in green, *Carcinus maenas*, and fiddler, *Uca pugilator*, crabs greatly increased the length of the intermolt stage by three times when compared to uninjured crabs (Spindler-Barth, 1976). But acute hemocoelic bacterial infections can often kill the majority of infected animals in 1–6 days (Johnson, 1976a; Spindler-Barth, 1976).

In addition to an active bacteremia, *Vibrio* bacteria can be found in other tissues, such as gills (Babinchak et al., 1982), gut (Huq et al., 1986; Venkateswaran et al., 1981), hepatopancreas (Johnson, 1983) and exoskeleton (discussion to follow in following section). The research of Babinchak et al. (1982) indicates that the gills of blue crab, *C. sapidus*, provide a protective ecological niche for the growth and physiological activity of heterotrophs. Under experimental feeding conditions, Huq et al. (1986) observed the attachment of *V. cholerae* only to the mucosal surface of the hindgut of the blue crab, *C. sapidus*. This study demonstrated the potential importance of crustaceans in the epidemiology and transmission of cholera in the aquatic environment.

### 3. Chitinolytic bacteria (other than *Vibrio*) associated with shell disease

Shell disease is common and frequently reported in various species of commercially exploited crabs: the blue crab, *C. sapidus* in New York Bight (Rosen 1967, 1970; Overstreet and Cook 1972; Cook and Lofton, 1973; Sandifer and Eldridge, 1974; Young and Pearce, 1975; Iversen and Beardsley, 1976; Noga et al., 1994); Dungeness crab, *Cancer magister* (Morado et al., 1988); edible crab, *Cancer pagurus* (Gordon, 1966; Bakke, 1973; Leglise, 1976; Ayre and Edwards, 1982; Vogan et al., 1999, 2001, 2002; Powell and Rowley, 2005); rock crab, *C. irroratus* (Young and Pearce, 1975; Sawyer, 1982, 1991); Jonah crab, *Cancer borealis* (Haenner, 1977; stone crab, *Menippe mercenaria* (Iversen and Beardsley, 1976); red crab, *Geryon quinquedens* (Young, 1991; Bullis et al., 1988); golden crab, *Geryon fernerii* (Wenner et al., 1987) and snow crab *Chionoecetes opilio* in eastern Canada (Bower et al., 1994; Benhamma et al., 1998). In general, shell disease of crabs occurs at such a high incidence that this syndrome has been the most studied disease of commercially exploited crabs.

A complex population of chitinolytic or chitinoclastic bacteria are generally associated with shell disease because they possess the enzyme chitinase that is capable of degrading carapace chitin. This degradation generally results in the typical erosion and pigmentation of lesions in the exoskeleton of crabs which are often viewed as box burnts, black-spots on the exoskeleton (Fig. 1). In addition to the external gross signs, this bacterial complex can penetrate the cuticle and establish infections in the blood and cause host tissue damage (Comely and Ansell, 1989; Vogan et al., 1999, 2001, 2002). Histological studies on shell disease of the edible crab, *C. pagurus*, showed that the gills, hepatopancreas and heart of diseased crabs were significantly affected (Vogan et al., 2001). In the gills, epithelial inflammation and necrosis and cuticular erosion in affected crabs leads to the formation of hemocyte plugs termed nodules while inflammation and other irreversible changes were common in the hepatopancreas and heart. The disease resulted in unsuccessful molting (Smolowitz et al., 1992) or septicaemic infections by opportunistic pathogenic bacteria, which entered through the lesion sites (Baross et al., 1978; Vogan et al., 2001).

Initially thought to be restricted to the exterior surfaces of the exoskeleton, recent studies show that shell disease is not a disease caused by a single pathogen and solely restricted to the exoskeleton. Chitinolytic bacteria are members of several genera including *Vibrio*, *Aeromonas*, *Pseudomonas*, *Alteromonas*, *Flavobacterium*, *Spirillum*, *Moraxella*, *Pasteurella* and *Photobacterium* (Getchell, 1989) As a result, a number of bacteria are involved in production of the lesions which may invade the body tissues of crab. The nature of the bacterial complex is not entirely understood, but may result in varying degrees of bacterial colonization, shell erosion and tissue invasion. Host responses differ depending on the nature of the penetrating bacteria and this may ultimately lead to death of the animal (Vogan et al., 2002). Unexpectedly, the severity of shell disease in *C. pagurus* did not cause dramatic changes to the majority of immune parameters tested, including total hemocyte counts (Vogan and Rowley, 2002). Vogan et al. (2002) found that extracellular products (ECP) produced by chitinolytic bacteria from the edible crab (*C. pagurus*) with shell disease could cause rapid death of the crab upon injection. Costa-Ramos and Rowley (2004) examined the nature of the active lethal factor(s) in ECP. They found that...
lipopolysaccharide (LPS), either alone or in combination with other heat-stable factors, was the main virulence factor of *Pseudoalteromonas atlantica* for the crabs.

Degraded environmental conditions are generally considered to be associated with shell disease in crabs because diseased crabs, *C. irroratus*, were often found in waste disposal areas with sewage sludge and dredge spoils in the New York Bight (Young and Pearce, 1975). But Powell and Rowley (2005) detailed an unchanged prevalence of shell disease in the edible crab, *C. pagurus*, 4 years after the decommissioning of a sewage outfall at Langland Bay, UK. and concluded that sewage pollution is probably not a major contributory factor to this disease (Powell and Rowley, 2005). Some researchers have investigated physiological changes that may occur in crabs with shell disease. Noga et al. (1994) reported low serum antibacterial activity coincided with increased prevalence of shell disease in blue crabs, *C. sapidus*, from the Albemarle–Pamlico Estuary, North Carolina, USA. This antibacterial activity may be an important mechanism protecting crabs against shell disease. However, as previously noted, Vogan and Rowley (2002) reported few changes in haemocyte counts and humoral defences in *C. pagurus* affected by shell disease; their results indicate that no dramatic changes in prophenoloxidase and antibacterial activity occurred.

### 4. *Aeromonas*

There are a few reports about *Aeromonas* infections causing diseases in crabs. Leglise and Raguenes (1975) isolated a species of *Aeromonas* from the haemolymph of moribund edible crabs, *C. pagurus*, with mortality rates of 50–70%, in commercial ponds in Brittany, France. Experimental inoculations of crabs with the isolated bacterium caused death within 24 h. Wounded crabs immersed in water containing the bacterium died in about 8 days, while non-wounded crabs in the same water remained healthy (Leglise and Raguenes, 1975).

*Aeromonas trota* was isolated from the haemolymph, hepatopancreas and muscle of diseased Chinese mitten crabs, *E. sinensis*, from aquaculture ponds in Zhuantang, Hangzhou, in China (Xu and Xu, 2002). The bacterium was Gram-negative, motile by polar flagella and facultatively anaerobic. Glucose was catabolized with the production of acid and gas. Oxidase and catalase actively were positive but metabolism of esculin, sucrose and salicin were negative. The bacterium was sensitive to ampicillin and carbenicillin. Experimental infection reached the 100% mortality rate. The isolates showed the same morphological, physiological and biochemical characteristics with *A. trota*.

### 5. *Rickettsia* intracellular organisms or *Rickettsia*-like organisms (RLOs)

*Rickettsia* intracellular organisms or *Rickettsia*-like organisms (RLOs) are small, pleomorphic, rod-shaped cocoid prokaryotes containing ribosomes, fibrils and nuclear material, most of which are obligate intracellular Gram-positive organisms (Sparks, 1985). The microorganisms differ from bacteria because they lack a true bacterial wall. Shortly after the turn of the 21st century, *rickettsia* have been identified as causing chronic and acute diseases in insects, birds, man and other animals (Wen, 1999), but their presence in crustaceans is a recent development. In 1970, the first rickettsial disease of a crustacean was reported from a terrestrial isopod from France (Vago et al., 1970). There are some reports of pathogenic *rickettsia*-like organisms (RLOs) causing serious diseases in crustaceans (Frederici et al., 1974; Brock et al., 1986; Keteter et al., 1992; Owens et al., 1992; Bower et al., 1994; Bower, 1996; Nunan et al., 2003). Mass mortality of shrimp has been associated with RLOs (Krol et al., 1991). However, diseases caused by RLOs are rare in crabs and have only been reported from *Carcinus mediterraneus*, (Bonami and Pappalardo, 1980), *Paralithodes platypus* (Johnson, 1984) and *Lithodes aequispina* (Meyers and Shorts, 1990) and *E. sinensis*, a freshwater crab (Wang et al., 2001, 2002; Wang and Gu, 2002; Zhang et al., 2002).

RLOs infect the shore crab, *C. mediterraneus*, and have caused fatal disease in the Útö region on the Mediterranean coast of France (Bonami and Pappalardo, 1980). The organism produced Feulgen-positive microcolonies (10–20 μm in diameter) within intracytoplasmic vacuoles and was widely dispersed in the cytoplasm of connective tissue cells of the hepatopancreas, gut, gills and gonads. Individual RLOs were approximately 2 × 0.7 μm in size and multiplied within host cell cytoplasmic vacuoles. Replication of the microbe eventually caused lysis of infected host cells after which the organisms were released into extracellular spaces. Experimental infection caused death in 15 days.

Johnson (1984) found RLOs in the hepatopancreatic epithelium of an immature female blue king crab, *P. platypus*, from around Kodiak Island, Alaska, USA. The infection was massive and apparently caused necrosis and encapsulation of some hepatopancreatic tubules, arrested ovarian development, and abnormal synchrony of the molt cycle. The *rickettsia*-like organisms measured 0.3 × 0.6–1.0 μm. Lightly Feulgen-positive microcolonies
(10–40 μm diameters) were observed within the cytoplasm of hepatopancreas epithelium.

Infections in blue king crabs *P. platypus* (Johnson, 1984; Meyers and Shorts, 1990), golden king crabs *L. aequispina* (Meyers and Shorts, 1990) and shore crabs *C. mediterraneus* (Bonami and Pappalardo, 1980) were thought to be fatal. Prevalences from aquaculture systems have rarely been reported. In blue crabs, *C. sapidus*, the prevalence of RLO was 2.3% in a Maryland shedding facility, but heavy infections were not fatal (Messick and Kennedy, 1990) and shore crabs *C. irroratus* (Shorts, 1990) and shore crabs *C. sapidus* (Bower, 1996) were associated with little pathology (Messick, 1998). However, the RLO infections in the Chinese mitten crab, *E. sinensis*, are significant due to the high prevalence (30%-90%) in pond systems, and the rapid and high mortality (above 70%) associated with the tremor disease (TD) losses (Yang and Cai, 1998) of cultured crabs in southeast China (Wang et al., 2001, 2002; Zhang et al., 2002). However, the RLOs pathogen in *E. sinensis* was later confirmed to be a spiroplasma rather than rickettsia when 16S rRNA gene analysis was applied (Wang et al., 2004, see Section 7).

6. *Chlamydia*-like organism

*Chlamydia*-like organism (CLOs) differ from rickettsia-like organisms (RLOs) by their special characteristics in multiplication, including small, rigid-walled “elementary bodies”, the infectious forms, that change into larger, thin-walled “initial bodies” dividing by fission and resulting in the formation of daughter cells which eventually reorganize and condense (intermediate bodies) to become elementary bodies (Sparks, 1985; Bower, 1996). High mortalities of Dungeness crabs, *C. magister*, which occurred each winter and spring in Willapa Bay and northern Puget Sound, Washington, USA, were found to be associated with a Chlamydia-like organism (Sparks et al., 1985). The disease appeared to be associated with cold water temperatures. Affected crabs displayed lethargy. The disease was well documented histologically and ultrastructurally. In the histological survey, diseased crabs exhibited systemic infections with a small Gram-negative or basophilic microorganism. Cell necrosis and moderate-to-dense accumulation of hemocytes accompanied the chlamydial infection in some tissues. The agent demonstrated strong affinity for connective tissue and connective tissue cells while only infrequently infecting epithelial cells. TEM observation revealed different developmental stages of CLOs within infected host cells. Microcolonies were membrane-bound and compressed and displaced cytoplasmic organelles. (Sparks et al., 1985).

Other chlamydial infections have been reported in the rock crab (*C. irroratus*) and Jonah crab (*C. borealis*). Louis Leibovitz devoted 6 years (1983–1988) to studying the highly fatal transmissible disease of laboratory-maintained populations of *C. irroratus* and *C. borealis* (Leibovitz, 1988). Primary infections were associated with the haemolymph, and crab hemocytes were filled with fine basophilic chlamydia, which were easily examined by light microscopic observation of fresh and fixed-stained blood smears. The Chlamydia-like organism altered the morphology of infected haemocytes. Ultrastructural examination of infected tissue revealed the presence of typical chlamydial life history stages; reticulate stages (560–475 nm), intermediate (422 nm) and condensing stages, (354 nm) and elementary bodies (214 nm). The crab’s haemopoietic tissues were destroyed as the disease progressed (Leibovitz, 1988).

7. *Spiroplasma*

*Spiroplasma* is a new pathogen in crustaceans that causes serious disease in commercially exploited crustaceans in both freshwater (Wang et al., 2003a,b, 2004, 2005, 2010) and marine (Nunan et al., 2004, 2005) environments. *Spiroplasma* is the only known genus of the family Spiroplasmataceae that is currently placed in the order Entomoplasmatales, class Mollicutes, phylum Firmicutes, field Bacteria or empire Eubacteria (Euzéby, 2005). Members of the genus *Spiroplasma* are unique microbes and are characterized by the absence of a cell wall, a helical shape that is evident during its exponential growth phase and tiny size (one of the smallest prokaryotes in the world). These bacteria can pass through membrane filters with pores 220 nm in diameter and can be cultivated in vitro in artificial medium.

*Spiroplasmas* were first found in 1973 in the United States of America and commonly infect plants and insects (Williamson and Whitcomb, 1975). Historically, *Spiroplasmas* have been associated with insects, ticks and plants (Tully et al., 1977, 1983; Whitcomb and Williamson, 1979; Whitcomb, 1980; Daniels, 1979; Moulder et al., 2002; Gasparich, 2002, 2010). However, a spiroplasma was isolated from the Chinese mitten crab *E. sinensis* associated with highly pathognomonic signs, of tremor disease (TD) in 2003 (Wang et al., 2003a, 2004). This was the first *Spiroplasma* isolated from crustaceans and has begun to change our understanding of the host range of *Spiroplasmas* (Christensen et al., 2005; Regassa and Gasparich, 2006). *E. sinensis* is a commercially important decapod that is widely cultured in China and supports an aquaculture industry that is valued in excess of US$2 billion every year. But from 1994, uncontrolled epizootics have occurred throughout the region; prevalences range from 30 to 90%, and resulting mortalities may exceed 70% and approach 100% (Wei, 1999). Diseased crabs showed weakness and anorexia in the early stage of infection. As the disease progresses, uncontrolled shaking of the pereiopods is observed, which led its description as “tremor disease” by culturists (Fig. 2). The agent was originally thought to be a rickettsia-like organism (RLO) based on morphological and pathological studies (Wang et al., 2002; Zhang et al., 2002).

The causative agent is distributed systemically in *E. sinensis* which likely explains the gross signs and behaviour of the disease. Infection in pereiopod skeletal musculature and connective tissue and in the heart and gastrointestinal tract likely results in weakness (lethargy) and anorexia. The presence of the pathogen in the thoracic ganglion and myoneural junctions likely affect nerve transmission leading to the characteristic paroxymal tremors of the pereiopods (Wang et al., 2002).

16S rRNA gene sequence analysis of the agent from diseased crabs led to the molecular identification of *Spiroplasma*. In order to confirm the taxonomic position of the agent, Wang et al. used bacterial universal primers for the 16S rRNA gene to amplify non-host DNA from blood cells, nerves, muscles and connective tissues from affected crabs. In addition, DNA sequencing was also performed on isolates inoculated and isolated from the yolk sac of embryonated chicken eggs (Wang et al., 2003b). PCR amplicons were compared to those in GenBank using the programme BLASTN. The results showed that tremor disease agent (TDA) was not a rickettsia, but rather a spiroplasma belonging to the genus *Spiroplasma* and closely related to *S. mirum* having 98% similarity with the latter. In addition to molecular identity, characteristics such as the absence of a cell wall, helical morphology during exponential growth (Fig. 3), motility, in vitro culture and the ability to pass ultrafilters have been confirmed (Wang et al., 2004). The Chinese mitten crab pathogen has been formally named as *Spiroplasma eriocheiris* sp. nov. (Wang et al., 2010).

Since the initial discovery, other spiroplasma-caused diseases have been identified in crayfish, *Procambarus clarkii*, (Wang et al., 2005) and in shrimp, *Penaeus vannamei* (Nunan et al., 2004) and *Rimicaris exoculata* (Zbinden and Cambon-Bonavita, 2003), and in the sediment where susceptible hosts are encountered (Ding et al., 2007). In Columbia, in cultured *P. vannamei*, *Spiroplasma panca* is responsible for significant mortality (up to 90%) (Nunan et al., 2004, 2005). These studies indicate that spiroplasmas have
a wide distribution in the aquatic environment and may be serious threats to aquatic crustaceans, especially cultured decapods.

Because spiroplasmas are very small and have the ability to pass through 0.22-μm filters, just like viruses, and also have polymorphic features during development, diseases associated with these bacteria are difficult for diagnoses. However, a DNA-based simple, efficient, rapid, sensitive and low-cost method based on a human medicine protocol has been developed for the detection of spiroplasmas. Phylogenetic analysis of the 16S rRNA gene from the bacterium indicated that it is a previously undescribed species of alpha-proteobacterium which could not be cultured on a range of agar-based growth media. The disease appears to be more prevalent during summer months when water temperatures are higher and a Gram-negative bacterium was identified as the putative causative agent. The disease agent (bacteria), the crab and the environment. Crabs are continuously affected by environmental fluctuations and management practices such as handling, crowding, transporting, fluctuating temperatures and poor water quality. All of these factors can impose considerable stress on the homeostatic mechanisms of crab rendering them susceptible to a wide variety of pathogens.

9. Potential approaches to control bacterial diseases of crab

Generally, diseases are the consequence of interactions of the disease agent (bacteria), the crab and the environment. Crabs are continuously affected by environmental fluctuations and management practices such as handling, crowding, transporting, fluctuating temperatures and poor water quality. All of these factors can impose considerable stress on the homeostatic mechanisms of crab rendering them susceptible to a wide variety of pathogens (Iwanaga and Lee, 2005). The crustacean host defence system has been well characterized and summarized by Smith (Smith et al., 2003). Circulating hemocytes play an extremely important role in the defence reactions by phagocytosis, hemocyte clumping, the production of reactive oxygen metabolites and the release of microbicidal proteins (Smith and Chisholm, 1992, 2001; Smith et al., 2003). In addition, gills serve another important role in the immune response (Johnson, 1976a,b; White et al., 1985; Martin et al., 2003). Johnson (1976) provided histological evidence that nodule formation in response to stress-induced bacteremia in blue crabs might have adverse effects on gill function, including distortion of gill lamellae and disruption of haemolymph flow. In C. maenas injected with bacteria, Smith and Ratcliffe (1980a) observed the formation of compact hemocyte clumps or diffuse hemocyte networks that appeared to occlude the lumen of the lamellar sinus, leading several authors, including White et al. (1985) and Martin et al. (2000), to suggest that hemocyte aggregates nodules could interfere with respiration and ion regulation. Burnett found that Vibrio campbellii interfered with the respiratory function of the gills of the Atlantic blue crab C. sapidus. He concluded that a healthy blue crab can eliminate most invading bacteria, unless the respiratory function of the gills is impaired (Burnett et al., 2006). Immune stimulation can be achieved by different kinds of microorganisms or compounds. Live bacteria, killed bacteria (bacteria or bacterial antigen), glucans, peptidoglycans and lipopolysaccharides (LPS) are thought to act as ‘immunostimulants’ because of their known effects on the crustacean immune system (Teunissen et al., 1998; Alabi et al., 2000; Vici et al., 2000; Sung et al., 1994, 1996; Song et al., 1997; Chang et al., 2000; Takahashi et al., 2000). These compounds have been considered for use in crustacean aquaculture in order to boost immune responses.
reactivity and promote immunoprotection in crustaceans (Nogami et al., 1997). There is no single compound or strategy that can provide a solution to the problem of disease and for long lasting protection in both adults and juveniles within aquatic cultural systems. So suitable strategies and approaches to disease control will be required which take into account the three key factors: disease agent (bacteria), the host and the environment. A suite of techniques has been recommended by Valerie Smith including the manipulation of the rearing environment, addition of probiotics as a matter of routine during culture, and the use of immunostimulants and other compounds during vulnerable growth phases (Smith et al., 2003). Another potential approach for the control of bacterial pathogenic bacteria is to make use of a bacterial predator *Bdellovibrio*. *Bdellovibrio* can attack other Gram-negative cells, penetrate their periplasm, multiply in the periplasmic space and finally burst the cell envelope to start the cycle anew (Jurkevitch, 2000). This lytic action can rapidly reduce bacterial populations therefore this microorganism can be used for effective biological control or as a therapeutic agent. There is also an opportunity for evaluation of the therapeutic potential of these predatory bacteria because the complete genome sequence of *Bdellovibrio bacteriovorus* HD100 has been determined (Sokkett and Lambert, 2004). Recent experimental data showed the ability of *B. bacteriovorus* to impact *Escherichia coli* and *Pseudomonas fluorescens* biofilms in a flow cell system (Kadouri and O'Toole, 2005). There was a report on the occurrence of *Bdellovibrios* in the blue crab, *C. sapidus*, from the Chesapeake Bay and adjacent Atlantic Ocean coastal waters (Kelley and Williams, 1992). This was the first time an animal species has been implicated as a natural reservoir for the bacterial predators. We can screen out the *Bdellovibrio*, which is effective even against bacteria that have multiple resistances to antibiotics and set up an effective therapeutic method (Mah and O'Toole, 2001). With greater knowledge of *Bdellovibrio*, the applications of using *Bdellovibrio* as a biological control of diseases in aquaculture will become more extensive in the near future.

Diagnoses and prevention of spiroplasma disease of freshwater culture crustaceans in China have been established recently (Wang et al., 2009; Liang et al., 2009). ELISA for the rapid field diagnoses of the spiroplasma pathogen was developed and the whole test procedure was rapid, being completed within 3 h (Wang et al., 2009) without any equipment. Based on the susceptibility test results, oxytetracycline (OTC) was screened out to be the most effective at inhibiting spiroplasma. Treatment experiments showed that the best concentration of OTC for use against spiroplasma disease was 40 mg OTC kg⁻¹ crab weight and the safe concentration was 82.5 mg OTC kg⁻¹ crab weight (Feng et al., 2010). This result suggests that OTC has potential as a highly effective inhibitor of spiroplasma pathogens in aquatic animals and has been proven to be a potent, safe and low-cost cure for spiroplasma disease (Liang et al., 2009; Feng et al., 2010).

References


References


