Molecular Immunology 68 (2015) 404–411

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Review

Crustacean hemolymph microbiota: Endemic, tightly controlled, and utilization expectable

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ARTICLE INFO

Article history:
Received 13 April 2015
Received in revised form 15 June 2015
Accepted 16 June 2015
Available online 4 July 2015

Keywords:
Crustacean
Hemolymph microbiota
Shrimp
Antimicrobial peptides
Lectins

ABSTRACT

Increasing number of evidence suggests that the hemolymph of numerous apparently healthy invertebrates is unsterile. Investigation of hemolymph microbiota properties and the homeostasis between host and bacteria is helpful to reveal bacteria pathogenesis, host immunity, and possible utilization in disease control. Crustaceans represent a large family of aquatic animals. Therefore, crustacean fishery is of important economic value worldwide. Research related to crustacean hemolymph microbiota has been performed over the years. In the present study, we conclude currently available information and present a comprehensive analysis regarding homeostasis between host and bacteria. In general, the presence of microbiota in crustacean hemolymph is an endemic event and can be influenced by internal and external factors. Opportunistic bacteria may have generated some changes or mutations under hemolymph stress. Meanwhile, hosts suppress hemolymph microbiota proliferation with the help of some critical antimicrobial peptides and lectins. The hemolymph microbiota may be beneficial for hosts as resistance against external damages. In addition, the hemolymph microbiota may be utilized in aquaculture.

1. Introduction

Metazoan animals harbor a large and diverse microbe population, including beneficial, commensal, and pathogenic microorganisms, in their digestive tract (Dillon and Dillon, 2004; Hooper and Gordon, 2001). In general, the circulatory system of healthy animals, especially vertebrates, is sterile. However, increasing number of studies revealed that the hemolymph of some aquatic invertebrates harbors bacteria. For example, Olafsen reported that healthy Pacific oyster (*Crassostrea gigas*) hemolymph was colonized by some strains of bacteria, which ranged from 1.4×10^2/mL to 5.6×10^2/mL; by contrast, horse mussel (*Modiolus modiolus*) hemolymph harbors the same magnitude of bacteria. Among these microorganisms, *Pseudomonas, Alteromonas, Vibrio*, and *Aeromonas* were the dominant genera (Olafsen et al., 1993). Similarly, microbial community was found in *Anodonta cygnea* hemolymph, which ranged from 1.5×10^2/mL to 6.5×10^2/mL, as well as in genus that is dominated by *Vibrio* and *Aeromonas* (Antunes et al., 2010). Studies on some insects and horseshoe crabs showed that circulating fluids may also be inhabited by some symbiotic bacteria (Brandin and Pistole, 1985; Narita et al., 2007; Scarborough et al., 2005).

Most reports on the presence of hemolymph microbiota involve aquatic invertebrates because the water environment of both fresh and sea water has complex conditions and contains variable and abundant bacterial floras. Microorganisms in such environment may enter the body and hemolymph through certain routes; these microorganisms may survive in vivo through host interaction. Close connection of animals with the water environment results in endemic hemolymph microbiota. Therefore, the presence or absence of bacterial community as well as the properties of bacterial floras are greatly influenced by the living conditions of animals. Nevertheless, the presence of hemolymph microbiota in apparently healthy animals is unique. In addition, such condition provides knowledge to understand the interaction between host and bacteria and to promote exploration of possible utilization and prevention of bacterial communities.

Many crustaceans are important aquaculture animals worldwide. Crustacean fishery is a vital industry in many costal countries and regions because these species are considered high quality food and have great economic value yearly (Bondad-Reantaso et al., 2012). Therefore, numerous research was conducted on crustacean immunity and pathogen epidemiology (Cerenius et al., 2010; Shields, 2012). Currently, the presence of microorganisms

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http://dx.doi.org/10.1016/j.molimm.2015.06.018

in hemolymph has been confirmed in some crustacean species, which showed no clinical syndromes (Kaizu et al., 2011; Ponprateep et al., 2012; Wang et al., 2014). The mechanisms behind homeostasis between host and bacteria were also studied (Fagutao et al., 2009; Kaizu et al., 2011; Ponprateep et al., 2012; Wang et al., 2014). In the current study, we analyzed previous characterizations of crustacean hemolymph microbiota. In addition, we gathered information regarding the mechanisms behind homeostasis maintenance, as well as possible utilization of these bacterial communities in aquaculture.

2. Presence of hemolymph microbiota in crustaceans

2.1. Presence of hemolymph microbiota is an endemic event

The crustacean digestive tract, including gut, stomach, and hepatopancreas, contains a tolerated and relatively constant number of bacteria (Freese and Schink, 2011; Kostanjsek et al., 2004; Kostanjsek et al., 2002; Li et al., 2007; Luis-Villasenor et al., 2013; Tzuc et al., 2014). As determined by our group (unpublished data), bacterial counts that were colonized in the kuruma shrimp (*Marsupenaeus japonicus*) digestive tract were about 10^6/g to 10^7/g tissue for the intestines and the stomach and 10^4/g to 10^5/g tissue for the hepatopancreas (Fig. 1). Based on various studies regarding different species, the presence of relatively high amount of bacteria in crustacean digestive tract is common. However, colonization of indigenous microbiota in crustacean hemolymph is not ubiquitously observed in all animals.

Tubiash et al. found that the hemolymph of more than 80% of 290 wildly blue crabs (*Callinectes sapidus*) from Chincoteague Bay, Virgia was unsterile. The annual mean count of bacterial flora is 1876 for both male and female crabs per mL of hemolymph (Tubiash et al., 1975). Similar phenomenon was observed for red swamp crayfish (*Procambarus clarkii*). More than 40% of apparently healthy animals harbors hemolymph bacteria (Scott and Thune, 1986).

Farm-reared crustaceans also contain some bacteria in their hemolymph. Based on our study, the mean count in kuruma shrimp hemolymph is about 10^1/mL to 10^3/mL, and these shrimp showed no clinical symptoms (Fig. 1). These bacteria would proliferate greatly when the expression of critical molecules was suppressed. Low but constant count of bacteria was also observed by Fagutao et al. in kuruma shrimp and by Ponprateep et al. in black tiger shrimp (*Penaeus monodon*) (Fagutao et al., 2009; Ponprateep et al., 2012).

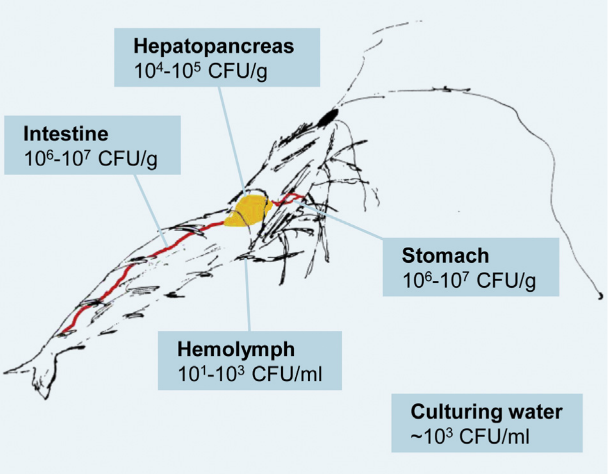

Fig. 1. A typical experiment that determines bacterial counts in different kuruma shrimp (*Marsupenaeus japonicus*) tissues. Shrimp were obtained from a local farm and reared in the lab for a week. Bacterial counts were determined by plating the hemolymph or tissue homogenate to the Luria–Broth plates, which contained 3% NaCl and cultured at 28 °C. Experiments were performed in October 2012. CFU, colony forming unit.

Other studies reported a low ratio of crustacean unsterile hemolymph. For example, only about 14% juvenile white shrimp (*Litopenaeus vannamei*) contained bacteria in hemolymph; such bacterial composition ranged from 2×10^2 to 3×10^3 (Gomez-Gil et al., 1998). The authors claimed that the procedure had been optimized to avoid contamination during the experiment, and to suppose the presence of low bacterial counts is reasonably assumed in shrimp hemolymph. Through lobster (*Homarus americanus*) hemolymph examination, only about 25% animals were found to be unsterile in the circulatory system. On the one hand, these individuals might be in a sterile status. On the other hand, some components used in the selected medium could inhibit growth of some bacterial strains, and this condition might lower the ratio of unsterile hemolymph (Cornick and Steward, 1966).

Although the presence of bacterial communities in healthy crustaceans is different from the general assumption, this finding has been confirmed in different species by independent researchers (Table 1). For these animals, a symbiotic relationship exists with the hemolymph microbiota.

2.2. Species/genera of bacteria identified in healthy crustacean hemolymph

The genus *Vibrio* is usually the dominant bacteria in the hemolymph microbiota of aquatic animals. We isolated a total of 14 strains through several separation processes of bacteria from kuruma shrimp hemolymph; among these strains, 4 was identified as *Vibrio* spp. by 16S rRNA sequencing and represented more than 60% of the total bacterial count (Wang et al., 2014). Six *Vibrio* spp. strains were isolated from juvenile *L. vannamei* hemolymph, including two *Vibrio parahaemolyticus* strains, 1 *Vibrio vulnificus* strain, and another three strains that needed further characterization (Gomez-Gil et al., 1998). *V. parahaemolyticus* is also the dominant species in healthy blue crab hemolymph (Sizemore et al., 1975). In another study performed in *L. vannamei*, *Vibrio navarrensis* was found as the dominant stain instead of *V. parahaemolyticus*; moreover, *V. navarrensis* corresponded to more than half of the total number of isolates (Albuquerque-Costa et al., 2013). Variation of hemolymph bacterial community, even in similar species, further suggested the influence of external endemic conditions. *Vibrio* species are usually abundant in aquatic environments and are major pathogens that cause vibriosis, a serious disease that causes great loss in shrimp aquaculture (De Schryver et al., 2014; Thompson et al., 2004; Wang, 2011). The constant presence of *Vibrio* in healthy crustacean hemolymph might extend the knowledge on *Vibrio* pathogenesis.

In addition to *Vibrio* spp., other strains, which were mostly Gram-negative bacteria, could be also detected in the kuruma shrimp hemolymph. Based on our previous study, several *Pseudoalteromonas* spp. represented about 15% of the total number of isolates in hemolymph. The other isolates were characterized as *Shewanella* spp., *Alteromonas* spp., *Marinomonas* spp., *Tenacibaculum* spp., *Psychromonas* spp., *Neptunomonas* spp., *Enterobacter* spp., and *Shewanella* spp. (Wang et al., 2014). The high amount of Gram-negative bacteria in the hemolymph microbiota was consistent with that in the marine environment where the shrimp lived in (Anwar and Choi, 2014).

On the contrary, the red swamp crayfish (*P. clarkii*) hemolymph hosted more kinds of Gram-positive bacteria. The bacterial flora consisted of several Gram-positive bacterial strains, including *Bacillus* and *Corynebacterium*, aside from the crayfish true pathogen, *Aeromonas* and *Vibrio* (Scott and Thune, 1986). Bacterial strains in