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Microbial diversity and ecology of crustaceans: influencing factors and future perspectives

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Crustaceans such as prawn and shrimp are nutritious seafood and contain high level of protein. However, due to high-water activity and neutral pH, crustaceans are highly susceptible to spoilage microorganisms that are present as normal microbiota. Development stages and culture environment, diet composition and storage temperature were highlighted in this review as factors that contribute to the composition of microbial communities present in prawn and shrimp. However, the use of biofloc technology during culturing/farming could help to reduce the microbial load at pre-harvest level while the use of neutralized oxidized water could be used to further reduce microbial load during post-harvest handling of the seafood. The use of both biofloc technology and neutral electrolyzed oxidizing water would therefore help to preserve the quality and shelf-life of prawn and shrimp.

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Introduction

The fisheries and aquaculture sectors greatly contribute to global food security and nutrition [1,2]. In addition, these two sectors play vital roles in supporting sustainable livelihood of coastal communities and thereby supporting national economic growth [3,4]. The growing global demand for white meat protein sources such as fish and crustacean product, coupled with the dwindling natural resources, has enhanced the rapid growth of the aquaculture sector [5]. Aquaculture is predicted to surpass capture fisheries and contribute 62% of the global seafood demand by 2030 [6]. Therefore, the intensification of the production systems is the prime focus in the aquaculture sector, which is evident by the rapid expansion of the world's indoor and outdoor aquaculture farming industries [7].

Crustaceans are among the highest valued seafood after freshwater fishes, and the global aquaculture production of crustaceans reached 8.4 million ton in 2017 [8]. Aquaculture production increases exponentially once the culture techniques, rearing systems, and diet requirements are developed and optimized. For example, the current production of giant freshwater prawn, *Macrobrachium rosenbergii* was almost 420 000 ton compared to less than 3000 ton over 30 years ago [4,8]. However, it is believed that the microbial ecology and diversity plays an important role for crustaceans' production and quality. The microbial status of seafood after catch is closely related to environmental conditions and the microbiological quality of the culture water [9–12]. This microbial status can provide diverse biological functions including growth, metabolisms, development, and immunity [13]. However, there is a dearth of review articles on the factors that influence microbial diversity and ecology in prawns and shrimps.

This review therefore focuses on the factors that affect the microbial community in crustaceans and future directions on the use of recent technologies such as biofloc technology at pre-harvest and neutralized oxidized water at post-harvest levels to reduce the microbial load and preserve the quality and shelf-life of crustaceans.

Impact of development stages and culture environment (rearing water)

Microbial diversity and ecology in crustaceans and their important influence on health and disease of the host has drawn increasing attention in the last decade. Diverse microbial populations have been associated with prawn and shrimp. These microbial communities can be influenced by various factors, namely life stage, disease, and rearing source of the prawn species [14]. Investigating the gut microbiota of *Penaeus monodon* during the post-larvae, juvenile and adult stages, Cicala *et al.* [15•] showed that the phylum Proteobacteria was significantly predominant in the earliest stages of life, while Actinobacteria significantly increased in adult *P. monodon*. Gainza *et al.* [16] also reported differences in the intestinal microbiota composition between the nursery phase and the harvest phase of *P. vannamei*. Cicala *et al.* [15•] therefore hypothesised that each ontogenetic stage could provide specific conditions for the proliferation of specific microorganisms.

Le *et al.* [17] investigated the microbial diversity of symptomatic (presence of pathological symptoms, such as stunted growth, empty gut, and white aqueous hepatopancreas) and asymptomatic *P. monodon* and found some differences between the two groups of prawn. For instance, the relative abundance of Crenarchaeota was 1.3% and 9.4% in asymptomatic and symptomatic *P. monodon*. The authors also found that bacterial communities of asymptomatic prawn were distinct from those of the rearing-water, while those of symptomatic shrimps and rearing water were clustered together. As a result, they suggested that a disruption on stability of intestinal microbiota might be associated with prawn disease.

The differences in the gut microbial communities of prawn may be attributed to the hosts or habitats [18]. These authors proved that the microbial communities of seawater prawn (*P. monodon*) samples were separated from that of freshwater prawn (*M. nipponense*) which formed a distinct cluster. Landsman *et al.* [19] determined the microbial diversity in the intestinal tract of *Litopenaeus vannamei* raised under two different production systems (indoor and pond) and from a wild population and found some significant differences in the relative abundance of some bacterial taxonomic groups namely the phyla Proteobacteria, Firmicutes, Fusobacteria, Cyanobacteria, and Actinobacteria. The authors concluded that aquaculture practices greatly influence the intestinal bacterial profile of *L. vannamei* and that the bacterial communities of this prawn species could be effectively manipulated using environmental conditions or diet composition.

Impact of diet composition

Recently, Guo *et al.* [20] assessed the effect of diet composition, especially that of three C/N ratio levels (CN6, CN10, and CN15) on the intestinal microbiota of *L. vannamei* and found that the increase of C/N ratio input decreased the diversity of the intestinal microbiota. In addition, the increase of C/N ratio led to an increase in the relative abundance of Actinobacteria, Rhodobacteraceae (mainly consist of the genera *Roseobacter* and *Paracoccus*), Alteromonadaceae, and a decrease in that of Cyanobacteria, certain Rhodobacteraceae, Mycoplasmataceae and *Vibrio*. Liu *et al.* [21•] evaluated the effects of a commercial microbial agent (MA) on the bacterial communities of *L. vannamei* and found that the relative abundance of Rhodobacteraceae in shrimp intestine was significantly greater in the MA-treated group than that in the control group with a normal feeding mode. According to Guo *et al.* [20], it would be possible to use appropriate diet composition to increase the relative abundance of potential beneficial bacteria and the accumulation of various bioactive metabolites to suppress the growth of detrimental bacteria.

Impact of storage temperature

Storage temperature could also influence the succession and microbial community present in prawn and shrimp. For example, Parlapani *et al.* [22•] investigated the microbiomes of fresh and chill-stored deep-water rose shrimp (*Parapenaeus longirostris*) and its relation to the volatile organic compounds (VOCs). The study concluded that the initial microbiomes consisted of *Photobacterium*, *Candidatus hepatoplasma*, *Psychrobacter*, *Acinetobacter* and *Delfia*. However, during storage *Psychrobacter* and *Carnobacterium* were dominant while *Psychrobacter* became dominant taxon at the end of the shelf-life of chill-stored shrimps. Study by Don *et al.* [23] identified *Enterobacter* and *Acinetobacter* as the dominant genera in *L. vananmei* stored at room temperature. While *Pseudomonas* and *Aeromonas* were dominants at refrigerated storage and *Aeromonas* and *Enterococcus* at ice storage. Investigation on the spoilage bacteria of Pacific white shrimp was carried out by Yang *et al.* [24] and gave different insight of the dominant genera during storage at ambient temperature and refrigerated storage. The study showed that *Acinetobacter* along with *Psychrobacter*, and *Shewanella* dominated the microbial communities of the refrigerated-storage shrimp, while *Vibrio* was the dominant at 25°C. *Pseudomonas* sp., *Shewanella*, *Flavobacterium*, and *Staphylococcus* were the dominant bacteria in fresh Taihu white prawn (*Exopalaemon modestus*), while lactic acid bacteria, *Pseudomonas* sp., *Shewanella*, and *Flavobacterium* were dominants in the spoiled products [25]. These highlighted studies showed that the microbial communities in prawn and shrimp during storage are dynamic and could be caused by the environments and species.

Future directions

This review shows that different factors contribute to the microbial diversity and ecology of crustaceans. The methods that can reduce the initial microbial load and microbial communities such as *Acinetobacter* along with *Psychrobacter*, and *Shewanella* that can cause spoilage of crustaceans should be investigated and implemented. These could be done during pre-harvest and post-harvest stages of crustacean farming.

Pre-harvest - biofloc technology (BFT)

Biofloc technology (BFT) can be used to reduce spoilage microbial community during crustacean farming. The technology relies on the *in-situ* production of microorganisms to recycle waste nutrients. BFT is gaining attention due to its proven influence on the aquaculture sector's performance and operation [5,11,26]. Microorganisms in BFT help to maintain water quality by recycling the unutilized nitrogen compounds, thus decreasing water treatment costs. Also, the beneficial effects of BFT on the survival, growth, and immune activity of penaeid larvae are well documented [27]. However, knowledge of the microbial composition and their interacting mechanisms within the BFT is still lacking. Uncovering this aspect by understanding the composition and interaction between microorganisms in biofloc could allow better manipulation and optimization of the BFT, which in turn has profound implications for the advancement of the aquaculture sector. Bacteria are efficient bio-degraders. They metabolize organic residues and recycle nutrients efficiently in the form of organic and inorganic matter [9]. These beneficial bacteria (probiotics), microalgae, and other natural biota are naturally found in most aquaculture systems [26] and they exert beneficial effects on the overall health of the host animal and ultimately increase production by improving the host's intestinal equilibrium through improved feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, and increased immune response [9].

Post-harvest - neutral electrolyzed oxidizing water (NEW)

Neutral electrolyzed oxidizing water (NEW) is an emerging technology that involves the use of oxidized water to reduce microbial load in food. It involves dipping and spraying with various concentration of oxidized water on food contact surfaces. The technique can be applied at the post-harvest stage of crustacean farming. However, there is a dearth of knowledge of the use of NEW for shrimp/prawn farming and its effects on microbial community of the shrimp/prawn during farming and post-harvest handling. The information around the best concentration of NEW and its physico-chemical properties (Oxidation-reduction potential - ORP and pH) during farming and post-harvest and are essential to investigate. The knowledge will be useful to the seafood industry to choose the right methods of application of NEW that can help to suppress or reduce the spoilage microbiomes without giving drawbacks for the quality of the products.

Conclusions

The microbial diversity and ecology of crustaceans such as prawn and shrimp are influenced by the development stages, culture environment, diet composition and storage temperature. The initial microbial communities could facilitate the microbial spoilage during the supply chain of prawn and shrimp. The use of biofloc technology at pre-harvest and neutral electrolyzed oxidizing waterpost-harvest levels could reduce the microbial load. The combination of these technologies would therefore help to preserve the quality and shelf-life of prawn and shrimp.

Conflict of interest statement

Nothing declared

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